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ORIGINAL ARTICLE

Epithelial atypia in biopsies performed for microcalcifications. Practical considerations about 2,833 serially sectioned surgical biopsies with a long follow-up

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Abstract This study analyzes the occurrence of epithelial atypia in 2,833 serially sectioned surgical breast biopsies (SB) performed for microcalcifications (median number of blocks per SB:26) and the occurrence of subsequent cancer after an initial diagnosis of epithelial atypia (median follow-up 160 months). Epithelial atypia (flat epithelial atypia, atypical ductal hyperplasia, and lobular neoplasia) were found in 971 SB, with and without a concomitant cancer in 301 (31%) and 670 (69%) SB, respectively. Thus, isolated epithelial atypia were found in 670 out of the 2,833 SB (23%). Concomitant cancers corresponded to ductal carcinomas in situ and micro-invasive (77%), invasive ductal carcinomas not otherwise specified (15%), invasive lobular carcinomas (4%), and tubular carcinomas (4%). Fifteen out of the 443 patients with isolated epithelial atypia developed a subsequent ipsilateral (n=14) and contralateral (n=1) invasive cancer. The high slide rating might explain the high percentages of epithelial atypia and concomitant cancers and the low percentage of subsequent cancer after a diagnosis of epithelial atypia as a single lesion. Epithelial atypia could be more a risk marker of concomitant than subsequent cancer.

Keywords Breast · Epithelial atypia · Lobular neoplasia · Atypical ductal hyperplasia · Cancer

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Introduction

Breast biopsies for infraclinical lesions are more frequent with mammographic screening programs, but the distribution of the corresponding histological lesions and their associations are still imprecise. Difficulties encountered in following up patients without cancer account for the fact that the clinical significance of certain non-malignant lesions and the management of patients are still debated. Moreover, the problem of surgical biopsy sampling has never been fully investigated and has added additional confusion in appreciating the distribution and clinical significance of such lesions. In 1981, breast epithelial atypia were hardly mentioned and not clearly defined in the World Health Organization/International Union Against Cancer (WHO/UICC) histologic classification of breast tumors [73]. The histologic classification of noncancerous lesions has been mainly based on studies analyzing for each lesion the associated risk of subsequent cancer. These studies were initiated by the survival studies of Dupont and

Page [15, 46] based on lesions discovered by palpation before the era of mammography. Thereafter, further studies [6, 9, 17, 24, 31, 51, 62, 67] substantiated these results, which were ratified in 2003 by the new American Joint Committee on Cancer (AJCC)/UICC classification of breast tumors [70]. Schematically, this classification differentiates benign epithelial lesions (usual ductal hyperplasia and other lesions) from atypical lesions of ductal or lobular type. Although this historical classification is challenged by a new classification [68], it remains the most widely used in practice. Interestingly, the occurrence of epithelial atypia was low in Page's study [46] and has increased with mammographic screening programs [60, 66] and with the development of percutaneous large core needle biopsy (CNB) methods using stereotactic mammography or ultrasound guidance. At present, CNB is frequently used for the initial evaluation of clinically occult breast lesions, thus generating dilemma for the subsequent management of certain noncancerous lesions. At our institution, surgical biopsies (SB) have always been managed in the same way, and most patients with atypical and malignant lesions have been followed. The objectives of our work were to analyze the occurrence of epithelial atypia and their association with a concomitant cancer in a large series of SB performed for microcalcifications without a palpable tumor and to assess the subsequent cancer probability in the group of patients with an initial diagnosis of epithelial atypia. Finally, we provide some practical considerations for the management of patients with epithelial atypia in this era of mammographic screening and CNB.

Materials and methods

Selection of patients

At Institut Bergonié, from January 1975 to December 2002, 3,166 breast biopsies for diagnostic purposes, 2,833 SB and 333 CNB, were performed for microcalcifications without any palpable mass in 2,708 patients (mean age 51.8 years, range 19.7–81 years). Among them, 248 (9%) had several biopsies in the same or contralateral breast. Since 1998, microcalcifications have been classified according to the classifications of the American College of Radiology [2]. SB for diagnostic purposes were defined before 1998 by the absence of a preoperative diagnosis based on the clinical–mammographic–cytologic triplet and by the absence of a positive frozen section and, since 1998, by the presence of epithelial atypia on CNB. Excluded from this study were 132 cancers and 139 non-atypical benign lesions diagnosed on CNB as well as 49 re-excisions performed elsewhere than in our center. Thus, 2,833 SB in 2,375 patients were available for analysis, among which 13 corresponded to re-excision after a CNB with epithelial atypia. Since 1989, needle localization, intraoperative specimen radiography, and post-excisional biopsy mammography have been performed in most cases.

Surgical biopsies and tissue sampling: serial macroscopic sectioning

SB was removed in one fragment and measured more than 3 cm in 94% of the cases (mean size 60 mm, 5–250 mm). For SB margin assessment, either the surface of the specimen was inked or the surgeon during the same operation removed additional tissue in the remaining cavity after excision of the specimen (surgical margins). After fixation in Holland Bouin, SB and margin specimens were serially sectioned in their entirety into numbered slices every 2 mm [12]. In most cases (89%), careful macroscopic examination of the specimen failed to reveal any lesion. Each numbered slice was put in as many numbered separate cassettes as necessary and paraffin-embedded in sequence. The median number of blocks per SB was 26 (from 2 to 180) and 8 (from 1 to 44) for surgical margins. Each block was examined on one hematoxylin–eosin–safran stained slide.

Classification of lesions and review of slides

Since 1975, all patients have been prospectively included in our clinical, histologic, and biologic database by senior pathologists (IM, GMG, IS, JMC). For each SB and each lesion, we prospectively entered in our pathologic database morphological descriptive criteria by using 65 pathological items for noncancerous lesions and 181 for cancers. Definitions and terminologies given in the literature were used to report columnar cell lesions (CCL), non-atypical ductal hyperplasia, atypical ductal hyperplasia, ductal carcinoma in situ (DCIS), and lobular carcinoma in situ (LCIS) [1, 4, 7, 10, 17, 19, 21–28, 38, 43–45, 47, 49, 50, 56, 59, 61, 69, 72, 74, 75]. The interest of our database was to collect morphological descriptive criteria of nearly all the breast lesions without labeling them. In fact, labels and definitions of breast lesions have varied throughout the past 30 years, while neither lesions nor their corresponding descriptive criteria (i.e., size, type, architecture, cellular and nuclear features, etc...) have changed. The only changes during this period were the definitions and/or the names given to these lesions. As we have listed for each lesion all the corresponding descriptive criteria among the 236 available items, we have been able to reclassify each lesion according to the "new" criteria recommended by referent authorities for a new definition, by selecting in our database the "new" correspondent descriptive criteria corresponding to this new definition. Consequently, this provided a

homogeneous approach to the pathological lesions at the time of our study. For example, low-grade DCIS≤2 mm have been reclassified as atypical ductal hyperplasia/ductal intraepithelial neoplasia (ADH/DIN) 1B (n=30) according to the new AJCC/UICC classification of breast tumors [70], and lesions that we used to term before 1997 [71] as clinging carcinoma of the monomorphic type [4] have been reclassified as flat epithelial atypia (FEA)/DIN 1A or columnar cell change (CCC) with atypia (n=84) [61]. About half of these 114 cases have been systematically reviewed by one (IM) or two senior pathologists (IM and GMG or IS), and there was a complete concordance between the second review and the initial descriptive criteria listed in the database. Similarly, lesions that we used to name LCIS before 1997 have been renamed lobular neoplasia (LN) since 1997, corresponding either to atypical lobular hyperplasia (ALH) or to LCIS. On the contrary, all the cases with micropapillary lesions were reviewed (n=155) because there was no item corresponding to precise descriptive criteria of micropapillations (number, topography around the duct, type).

Atypical ductal hyperplasia: definition and sizing

Among the group of ADH/DIN 1B, we individualized two morphologic types of ADH. Neither had any high-grade cytological atypia or necrosis.

ADH "mimicking DCIS" (Fig. 1). In this type, architectural atypia were qualitatively insufficient to allow a diagnosis of DCIS, therefore this "mimicking" DCIS lesion was classified as ADH whatever its size. Tufts and short micropapillations formed by cells had a broad base and were cohesive. There was no polarization of cells, i.e., no true cribriform spaces. Pseudo-cribriform patterns comprised irregular or relatively round microlumina with incomplete polarization of surrounding epithelial cells. Cellular bridges were wavy without any cellular polarization. Cells corresponded either to columnar cells with uniform ovoid to elongated nuclei or to cells with a slight increase in the nuclear/cytoplasmic ratio with more or less distinct cell borders and round or ovoid nuclei. These cells were sometimes admixed in the same lesion displaying a morphological gradient, but there was no regular arrangeent. Nuclear chromatin was evenly dispersed, homogeneous, or slightly marginated, and nucleoli were inconspicuous. Apical snouts, intraluminal secretions, and psammoma-type calcifications were frequently present.

ADH corresponding to "mini DCIS" (Fig. 2). In this type, architectural and cytologic atypia corresponded to a low-grade DCIS but were quantitatively insufficient to allow a diagnosis of DCIS, therefore this "mini" DCIS lesion was classified as ADH when ≤2 mm. Tufts and short micropapillations had a tight base, were present on over all the periphery of the duct, and were non-cohesive with small

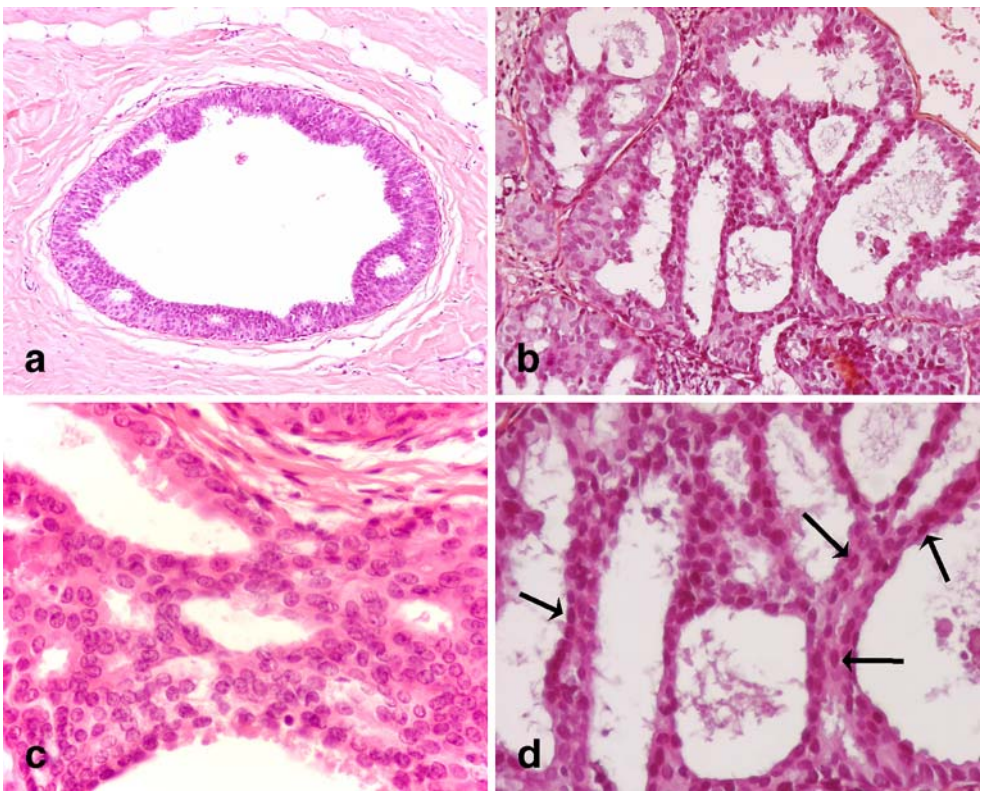

Fig. 1 a–d. ADH "mimicking DCIS." **a** Tufts and short micropapillations with a broad base. **b** Pseudo-cribriform spaces. **c** Microlumen with incomplete polarization of surrounding epithelial cells. **d** Cellular bridges without cellular polarization. Cells are parallel to the axes (*arrows*)

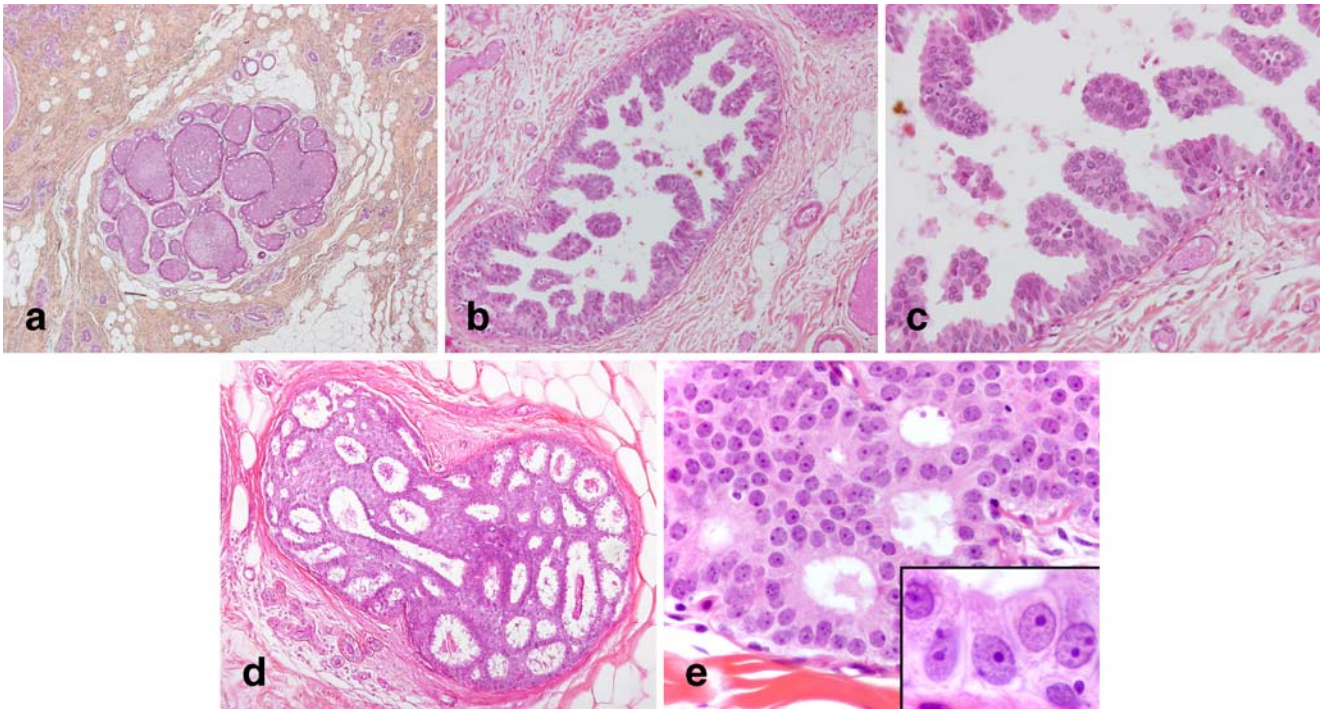

Fig. 2 **a**–**e**. ADH corresponding to "mini DCIS." **a** A solid mini DCIS focus measuring less than 2 mm in one TDLU. **b** Tufts and short micropapillations over the entire periphery of the duct with small free papillary tufts in the lumen. **c** Short micropapillations with a tight base. **d** True cribriform spaces. **e** Microlumen with complete polarization of surrounding epithelial cells

free papillary tufts in the lumen. There were true cribriform patterns with a polar organization of cells around glandular spaces and/or variants of cribriform patterns (i.e., trabecular bars, cartwheel formations, and Roman bridges, Fig. 3) with polarized cells arranged perpendicular to the axes. Some solid areas with regular arrangement of cells were also present. Cells were often small, monomorphous, sometimes without a columnar change, with a distinct cytoplasmic membrane and a spaced regular round nucleus with uniformly dispersed chromatin without prominent nucleoli. Intraluminal secretions and calcifications (amorphous or psammoma-type) were also frequently present (Fig. 4). When one mini DCIS focus was found in one partially or completely involved duct/ductular cross-sections in one terminal ductal lobular unit (TDLU), it was classified as ADH when it measured ≤2 mm and as DCIS when it measured >2 mm. When there were several foci of "mini" DCIS in close duct/ductular cross-sections in the same TDLU or in TDLUs located in the same field at low power magnification (2.5), the lesion was classified as ADH when its size, i.e., its largest diameter, was ≤2 mm and as DCIS when >2 mm. When there were several foci of "mini" DCIS in distant duct/ductular cross-sections in the same TDLU or in close TDLUs, the size of each focus was assessed separately. FEA, rare and scattered single micropapillations, and cribriform variants

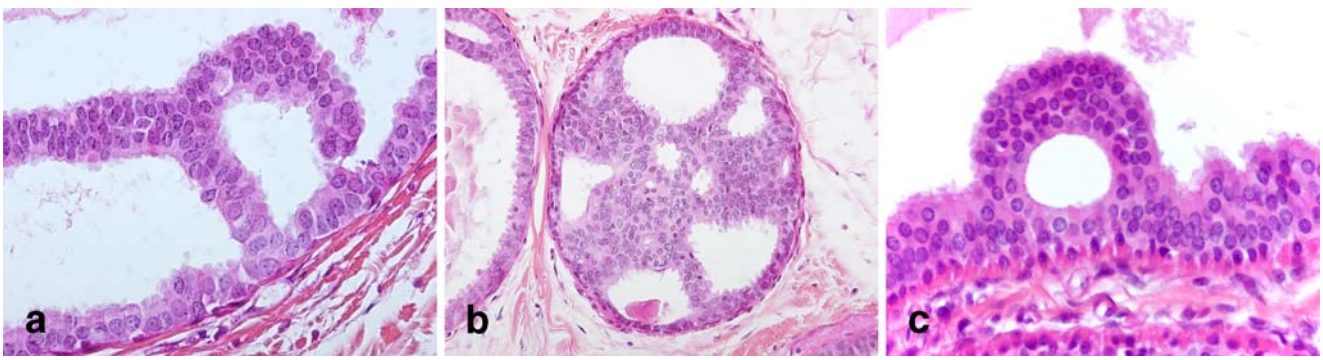

Fig. 3 **a**–**c**. Variants of cribriform patterns. Polarized cells arranged perpendicular to the axes. **a** Trabecular bars. **b** Cartwheel formations. **c** Roman bridges

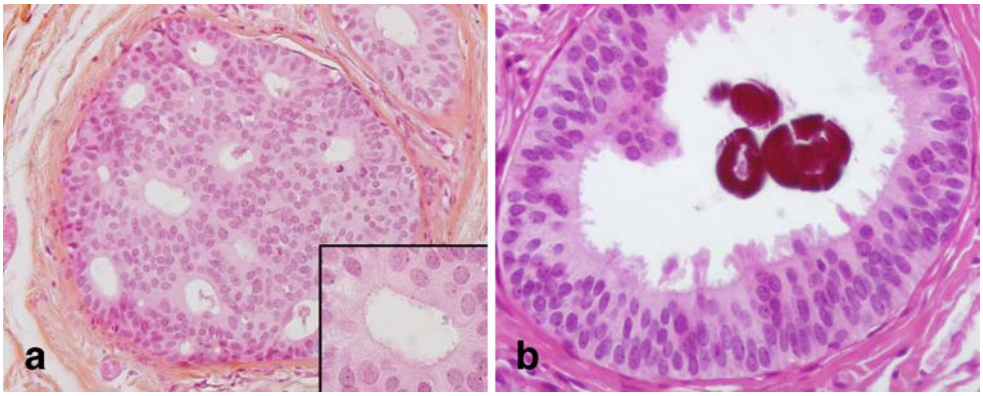

Fig. 4 **a** and **b**. **a** Mild cytologic atypia. **b** Columnar cells with uniform ovoid nuclei, intraluminal calcifications

were not taken into consideration for sizing, even if located in the same TDLU.

FEA were present either as a single lesion or in association with ADH in the same TDLU and since 1997 have been included in the ADH group. The distinction of FEA from columnar change without atypia was based on the criteria given by the WHO for the definition of FEA. Furthermore, columnar change without atypia was characterized by one or two layers of columnar cells without nuclear atypia, i.e., no increase in the nuclear/cytoplasmic ratio, no prominent nucleoli. Nevertheless, some cases of columnar change with progesterone impregnation, especially in the second part of the cycle, might display a lobular distension with a secretory material and large nuclei with prominent nucleoli. In such cases, myoepithelial cells displayed the same alterations with clarified cytoplasms, thus facilitating the diagnosis. The distinction of ADH mimicking DCIS from usual hyperplasia (UDH) was based on morphological criteria. Architectural pattern and cytologic criteria of usual ductal hyperplasia were easy to identify in most cases. UDH corresponded to a proliferation of epithelial cells in solid or fenestrated areas without any polarization of surrounding cells. Cells were haphazardly arranged with overlapping nuclei or were parallel with characteristic streamings. They were elongated or pseudo epithelioid, but there was no columnar metaplasia. Cytoplasms were more or less abundant with indistinct borders. Nuclei had irregular size and shape and sometimes contained a prominent eosinophilic inclusion. In some rare cases, immunohistochemical staining with cytokeratin 5/6 [41] was used and was negative in ADH mimicking DCIS and strongly positive in UDH. In some lesions, differential diagnosis between ADH and low-grade DCIS was all the more difficult because there were intermediate and intricated morphological aspects in the same TDLU. In practice, diagnosis of micropapillary lesions was often difficult. Extensive micropapillary lesions were classified as DCIS when quantitative and qualitative criteria were simultaneously present, i.e., lesion sizing more than 2 mm corresponding to micropapillations with a tight base over the entire periphery of the ducts. Additional sections could be useful for demonstrating more or less qualitative or quantitative diagnostic criteria. When malignancy remained equivocal, the case was classified as ADH. When a concomitant cancer was diagnosed, histologic size was assessed, and in DCIS, the percentage of blocks with cancer ("positive blocks") was specified [13]. Presence and topography of microcalcifications were also assessed. Lastly, when FEA and/or "mimicking" DCIS foci were found on excision margins of a SB with DCIS, a further surgical resection was not performed.

Follow-up of patients with epithelial atypia as a single lesion

There were 443 patients with epithelial atypia in one or several SB, without any previous or synchronous carcinoma in the same or contralateral breast and treated by biopsy alone (median follow-up 160 months, 7 to 315). Only 28/443 (6%) were lost to follow-up. Among the 415 other patients, 180 were monitored at our institute and 235 outside by correspondent specialists working in close relationship with our institute. All patients received a clinical examination and mammography once a year. When a new biopsy was necessary, it was performed at our institute.

Statistical analyses

Comparison of clinical and histologic characteristics was conducted by using the chi-square test. For women with epithelial atypia, the probability of developing in situ or invasive cancer was calculated from the date of the first biopsy to the earliest event: breast cancer (ipsi- or contralateral), death, or last contact (last consultation for the group monitored at our institute and checkpoint date, i.e., 1 March 2004, for the others). Probabilities were calculated according to the Kaplan–Meier method (SPSSv11).

Results

Occurrence of epithelial atypia in the 2,833 surgical biopsies

Epithelial atypia were recorded in 971/2,833 SB (34%). They were found with and without a concomitant cancer in 301/971 (31%) and 670/971 (69%) of the cases, respectively. Thus, isolated epithelial atypia were found in 23% of the cases (670 out of the 2,833 SB). Calcifications were present at histologic examination in 98.6% of SB with cancer and were located in benign, cancerous, and both lesions in 10, 39, and 51% of the cases, respectively. In several cases, cancerous foci without any microcalcifications were located at points distant from those with calcifications detected by needle localization.

Types of epithelial atypia

Among the 971 SB with epithelial atypia, there were 101 SB with FEA as a single lesion (11%), 342 (35%) with ADH, 223 (23%) with LN, and 305 (31%) with ADH and LN. Thus, ADH was encountered in 647/971 SB (66%).

Types of cancers associated with epithelial atypia

Cancers associated with epithelial atypia corresponded to DCIS and micro-invasive carcinoma (DCIS-MI) in 233 cases (77%). Among invasive carcinomas (n=68), there were 13 (9%) lobular and 11 (6%) tubular carcinomas (Table 1). Cancers were small (≤5 mm in 46% of invasive carcinomas, fewer than half of the blocks positive in 76% of DCIS). They were non-high grade in 78 and 67% of DCIS and invasive carcinoma, respectively. In most cases, ADH and cancer were situated close to each other. FEA alone were less frequently associated with a concomitant cancer than ADH and/or LN ($p=5\times10^{-4}$).

Cancers without epithelial atypia (malignancy alone)

There were 821 malignant SB without epithelial atypia [590 micro-invasive carcinomas, 206 infiltrating ductal carcinomas (IDC), and 25 infiltrating lobular carcinomas (ILC)].

Subsequent cancer in patients with an initial diagnosis of epithelial atypia as a single lesion

At 5 and 10 years, the probabilities of developing invasive breast cancer in the group of 443 patients with epithelial atypia were 2.8% [95%CI=1.4 to 5.5] and 5.5% [95%CI= 3.3 to 9.9], respectively (Fig. 5). Among the 18 subsequent carcinomas, 15 were invasive (11 IDC and 4 ILC), and 3 corresponded to DCIS. Most subsequent carcinomas were encountered in the homolateral breast (n=14) and before 10 years (n=16). Seven carcinomas occurred in the group of patients with an initial diagnosis of LN, in the same (n= 5) or contralateral (n=2) breast. They corresponded to infiltrating ductal (n=6) or lobular (n=1) carcinomas. The interval of development was 4, 5, 6 (n=2), 9, and 12 (n=2) years. Seven carcinomas occurred in the group of patients with an initial diagnosis of ADH, in the same (n=3) or contralateral (n=4) breast. They corresponded to DCIS (n= 2) and to infiltrating ductal (n=4) or lobular (n=1) carcinomas. The interval of development was 1, 2, 3 (n=3), 9, and 12 years. Four carcinomas occurred in the group of patients with an initial diagnosis of ADH associated with LN, in the same (n=3) or contralateral (n=1) breast. They corresponded to DCIS (n=1) and to infiltrating ductal (n=2) or lobular (n=1) carcinomas. In the four cases, the interval of development was 4 years. There was no subsequent carcinoma in the group of patients with FEA.

Table 1 Types of concomitant cancers (n=301) in the 971 surgical biopsies with epithelial atypia

Epithelial atypia	FEA (n=101)		ADH (n=342)		LN (n=223)		ADH+LN (n=305)	
	No. of cases (%)		No. of cases (%)		No. of cases (%)		No. of cases (%)	
Without cancer	84	83	220	64	139	62	227	74
With cancer	17	17	122	36	84	38	78	26
DCIS/DCIS-MI	12	12	103	30	58	26	60	20
IDC/NOS	–	–	16	4.7	17	8	11	3
ILC	1	1	1	0.3	6	3	5	2
TC	4	4	2	1	3	1	2	1

FEA Flat epithelial atypia; *ADH* atypical ductal hyperplasia; *LN* lobular neoplasia; *DCIS* ductal carcinoma in situ; *DCIS-MI* DCIS with micro-invasion; *IDC* infiltrating ductal carcinoma; *ILC* infiltrating lobular carcinoma; *TC* tubular carcinoma

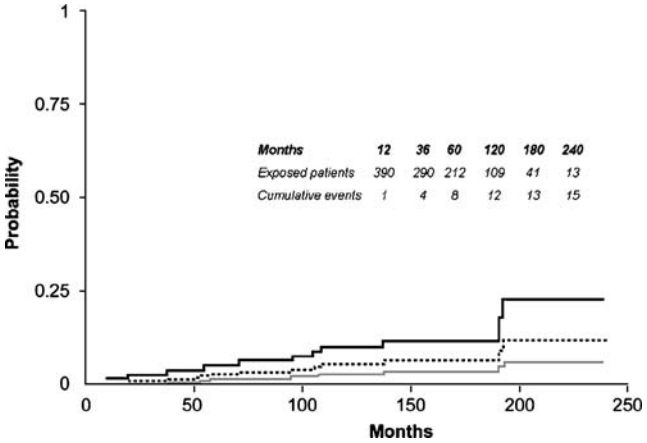

Fig. 5 Probability of developing subsequent invasive breast cancer in the group of 415 patients with epithelial atypia (*dotted line*: confidence interval 95%)

Discussion

Application of the WHO classification: practical considerations

For a long time, DCIS was diagnosed even if the characteristic features were found in only one ductal space [6]. Thereafter, some authors introduced quantitative criteria for distinguishing between ADH and DCIS [46, 67], while others [23] rejected them. More recently, Rosen [57–59] and Schnitt and Vincent-Salomon [61] described CCL comprising CCC and columnar cell hyperplasia (CCH) with and without atypia. Nasser [40] challenged this classification based on columnar shape and limited the group of lesions to proliferations characterized by a low-grade atypicality, "atypical columnar cell lesions," (ACCL) rather by a columnar cell configuration. In the WHO classification, ADH includes various not clearly defined types of lesions (Table 2). On one hand, there are lesions with arcades, moundings, and micropapillary formations, but without any true cribriform/complex architectural patterns [34]. This type of ADH corresponds to the CCH with atypia of Schnitt and Vincent-Salomon [61] termed category 3 in Simpson's study [51], to the definition of ADH by Koerner [34] and to ADH "mimicking" DCIS in our study. On the other hand, there are lesions displaying architectural and cytologic atypia. This type of ADH corresponds to the complex architectural pattern with cytologic and architectural atypia of Schnitt and Vincent-Salomon [61] termed category 5 in Simpson's study [64], to the definition of "microfocus of DCIS" by Koerner [34] and to "mini DCIS" in our study. In Simpson's study, the ADH/category 5 contained chromosomal changes and the same total mean number of changes to that observed in DCIS/DIN IC, unlike the other CCL. Lastly, because there is still no consensus for measuring ADH, there is no clear-cut distinction between ADH and DCIS, and the cut-off at 2 or 3 [52] mm or at two completely involved spaces [70] seems arbitrary. While awaiting a definitive molecular classification, the simplest attitude could be recommended in routine

Table 2 Terminologies used for intraductal proliferative lesions with low-grade cytologic atypia, so-called atypical columnar cell lesions

Spectrum of lesions	1, 3–5 Layers	No polarization*	With polarization	
	Occasional mounding	Mounding, arcades	Cribriform spaces and their variants	
	No or rare arcades and micropapillary formations	Cohesive micropapillary tufts with a broad base	Non-cohesive micropapillary tufts with a tight base	
[70]	Flat epithelial atypia/DIN 1A	ADH/DIN 1B≤2 mm; or in two spaces		DCIS/DIN 1C
[59, 61]	Columnar cell hyperplasia with atypia		ADH if not extensive	DCIS if extensive
			=	
	Columnar cell change (CCC) with cytologic atypia		Complex structures with architectural and cytologic atypia	
[34]	Columnar cell lesions + ADH		Microscopic focus of DCIS	DCIS
Institut Bergonié	Ex-clinging carcinoma of monomorphic type	ADH "mimicking" DCIS	ADH corresponding to "mini" DCIS≤2 mm	DCIS
[64]	CCC with cytologic atypia Or CCH with cytologic atypia	CCH with architectural atypia	CCH with architectural atypia And Cytologic atypia	DCIS
Regrouping?	DIN 1A	ADH/DIN 1B Not measured	ADH/DIN 1C Measured: ≤3 mm	DCIS/DIN 1C >3 mm

*Or incomplete polarization

practice. Only mini foci of ADH obviously similar to low-grade DCIS foci could be measured and classified as ADH when equal to or less than 2–3 mm [52] and as DCIS when more than 3 mm. Although the mode of measurement in our study is not under consensus, it is simple and can been routinely applied.

Occurrence of epithelial atypia and their association with a concomitant cancer: practical considerations

In our study, the proportion of epithelial atypia is high (23%), a result difficult to compare to others in the literature, as the methodologies used by teams are different. In the Page and Dupont case-control studies [15, 46], ADH and ALH were found in 2.1 and 1.6% of the cases, respectively. In mammographic screening programs, epithelial atypia and cancers increase as the number of biopsies performed for microcalcifications increases, especially as ACR4/ACR5 lesions are more often excised than ACR3. However, as underlined by Page [46], "the most direct relationship of epithelial atypia incidence is to slide rating." The number of slides per SB in our study (median 26) was higher than in the other studies on benign breast lesions: 1–5 in 93% of the cases in the study of Page et al. [46] (n=283), 3 (range 1–25, n=674) in the study of Shaaban et al. [62], and a mean of 1.6 slides per cm of tissue (n=199) in the study of Tavassoli and Norris [67]. In a recent study conducted in the south west of France in women aged between 50 and 75 with mammographically detected non-palpable breast lesions, a similar proportion of atypical lesions were found when biopsies were serially sectioned [39]. Furthermore, this high slide rating allowed the detection of small concomitant cancers in the vicinity of epithelial atypia in 31% of our cases, with a skew towards low-grade lesions (high proportions of DCIS and low-grade invasive carcinomas, especially tubular carcinomas). Our results strengthen the hypothesis that FEA and ADH are risk markers of low-grade cancers. This has been confirmed by the study of Simpson et al. [64] on molecular genetic profiles of CCL. In some of them, there are both a morphological and a molecular continuum in the degree of proliferation and atypia, supporting the hypothesis that "CCL are a non-obligate, intermediary step in the development of some forms of low grade in situ and invasive carcinoma." The association of epithelial atypia with a concomitant cancer in nearly one third of the cases in our study parallels previous findings concerning the frequency of cancers found in SB performed for atypia in CNB. Thus, approximately 30% [20, 29] and 15 to 21% [5, 8, 14, 18, 35, 45, 53, 63, 76] of excisions after CNB with ADH and LN, respectively, were proven to have cancer. Consequently, excision is recommended [45] for all patients in whom ADH is identified on CNB and may be justified in patients with FEA, as they are included in the spectrum of ACCL. Excision remains a controversial issue in patients with LN. Some authors have advocated it [3, 18, 33, 63], while others have rebutted it [55], especially when LN is an incidental non-extensive finding [48] with no radiologic–pathologic discordance [18] and without any synchronous mass lesion [37]. SB corresponding to re-excision should be processed in its entirety by serial macroscopic sectioning [32, 65]. When pathologic examination is exclusively focused on mammographic calcifications, the risk is to underestimate the DCIS size/extension because cancerous foci without any calcification (10% in our study vs 6% in Owing'study) [42] may be located at points distant from those with benign breast tissue containing calcifications.

Subsequent cancer after an initial diagnostic of epithelial atypia as a single lesion: practical considerations

In the literature, 4 to 22% (average interval 8.3 years follow-up) [6, 42, 67] and 15 to 20% [16, 30, 56] of patients developed invasive carcinomas after a diagnosis of ADH and LN, respectively. The risk of developing cancer increases with extended follow-up, but many cancers after a diagnosis of LN have a good prognosis and a low mortality [36]. These results are difficult to compare to ours because the methodologies are different. The low probabilities of subsequent invasive cancer in our study could be due to the high slide rating, allowing the detection of small concomitant cancer that might have been missed with a low slide rating and inadequate patient management [11, 54].

In conclusion, when epithelial atypia are present, they are associated in nearly one third of the cases with a concomitant close cancer and are found as isolated lesions in nearly 23% of SB performed for microcalcifications. In practice, ADH should be more clearly defined with simple guidelines for measuring lesions. When malignancy remains equivocal and/or when sizing is difficult, it is better to classify the lesion as ADH. Epithelial atypia could be more a "risk factor" of a concomitant geographically small close cancer than a risk marker for a subsequent cancer, as they form part of a spectrum of lesions [64].

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ORIGINAL ARTICLE

Ki-67 expression in primary breast carcinomas and their axillary lymph node metastases: clinical implications

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Abstract Proliferative activity of tumour cells assessed by immunohistochemical Ki-67 expression is one of several prognostic indicators in breast cancer. The major objective of this study was to investigate the prognostic impact of Ki-67 proliferative activity in the axillary lymph node metastases and in the matched primary breast carcinoma from 194 patients. There was a statistically significant up-regulation of Ki-67 protein in the metastatic deposit compared to where the primary tumour was found (p=0.001). A low Ki-67 index in both the primary and the metastatic tumours was a favorable prognostic factor. A high index in both primary and metastatic lesion and an up-regulation from a low index in the primary tumour to a high index in the metastatic deposit represented an unfavorable prognostic factor. Multivariate analysis showed that Ki-67 expression in the metastases was a superior independent prognostic factor of clinical outcomes compared to that in the primary tumours. Ki-67 expression in ≥10% of carcinoma cells in the primary tumours and ≥15% in the nodal metastases seems to be optimal cut-off levels. Ki-67 is of value as an independent prognostic factor in breast cancer.

Keywords Cell proliferation · Ki67 · Invasive breast cancer · Prognostic factors

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Introduction

The proliferative capacity of breast cancer is an important prognostic factor and can be evaluated by a variety of methods such as number of mitoses per 10 high power field (HPF), thymidine labeling index, bromodeoxyuridine labeling, S-phase fraction, and Ki-67 /MIB-1 antigen, Ki-S1 antigen, and proliferating cell nuclear antigen proliferative index [4, 11, 17, 24]. Determination of the proliferative index by means of immunohistochemistry (IHC) represents an easy and reliable method of assessing tumour cell proliferation in breast cancer [1, 23].

Ki-67 is a labile, nonhistone nuclear protein that is tightly linked to the cell cycle and is expressed in all continuously cycling cells of mid-G_1, S, and G_2 phase and in mitosis, but not in quiescent or resting cells in the G_0 and early G_1 phase [12]. As this protein is present in all proliferating cells (normal and tumour), it may serve as a marker to evaluate the growth fraction of a given cell population [7]. The functional significance of Ki-67 protein in cell cycle regulation still remains unknown [23]. Previous studies have shown controversial results concerning Ki-67 as an independent prognostic factor [18, 19, 25, 31]. To our knowledge, no study has investigated the relationship of Ki-67 expression in the metastatic lesions with clinical outcomes in breast cancer.

We have assessed the associations between Ki-67 immunoreactivity and other established clinicohistopathological parameters and the prognostic significance of Ki-67 both in primary breast carcinomas and in their corresponding axillary lymph node metastases (ALNM). We have also compared Ki-67 immunoreactivity in the primary tumours with the corresponding metastases and discuss the clinical implications of our findings.

Materials and methods

Patients

The study comprised 194 consecutive breast cancer cases that, from May 2000 to August 2004, underwent mastectomy or breast conserving surgery with complete axillary dissection at Ullevaal University Hospital for primary breast carcinomas with ALNM. Patients, 191, received postoperative adjuvant therapy according to national adjuvant treatment guidelines (http://www.nbcg.net/filer/79.ppt). Follow-up included clinical examination at 6–12 months intervals with annual mammography. Paraffin sections from the primary tumours and their corresponding metastases were retrieved from the archives. Data registered included age, tumour size, histological subtype, and grade, estrogen/progesterone receptor (ER/PgR) status, size of the largest metastatic focus and number of lymph nodes with metastasis. The characteristics of the study population and their specimens are summarized in Table 1. The median number of lymph nodes with metastasis was one (range : 1–27). The histological subtype and grade of the primary tumours had been evaluated according to the criteria of the World Health Organization [28] and the Elston and Ellis system [10].

Immunohistochemistry

Paraffin sections were immersed in Tris/ethylenediaminetetraacetic acid (EDTA) pH9 (HIER) in microwave oven (2.5 min at 750 W and 15 min at 160 W) for antigen retrieval. The IHC staining of Ki-67 was performed with the Ventana ES automated immunostaining system (Ventana Medical Systems, Inc., Tucson Arizona, USA), using mouse monoclonal antibodies with Ki-67 (Clone MIB-1; dilution 1:75; DAKO Norden A/S, Glostrup, Denmark).

The histological sections were reviewed to ensure representative tumour tissue for IHC from both primary tumour and its corresponding metastatic deposit. IHC analysis was carried out simultaneously on the primary mammary tumours and their corresponding metastases. The number of positively stained nuclei of all cases was assessed by one observer (DP). Another 35 randomly chosen cases were examined by TS to assess the inter-observer variation. Ki-67 positive nuclei were counted in several random areas including the periphery. At least 100 nuclei were counted in each area using a ×40 objective magnification. The results were expressed as percentages of positively stained nuclei over the total number of nuclei counted. The mean of the percentages of positively stained nuclei obtained in all areas was used for defining high/low Ki-67 index. A cell was considered positive if there was a clearly detectable brown colour in the nucleus. Cytoplasmic staining was considered nonspecific and was interpreted as negative. For high/low Ki-67 indices, four different cut-off values, 5, 10, 15 and 20%, were set and tested.

Statistical analysis

The results were analyzed using the statistical software SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). The comparison between the percentage of cells expressing Ki-67 in the primary tumour and ALNM was evaluated for statistical significance using Student's *t* test. The correlation between Ki-67 protein at the four different cut-off points and histopathological parameters was assessed using the χ^2 test and linear-by-linear test. For the interobserver agreement, the kappa (κ) statistic [15] was used. (Value of κ; poor to fair agreement: <0.4, moderate: 0.4–0.6, substantial: 0.6–0.8, almost perfect: >0.8). The clinical end points in the survival analysis were: disease/relapse free survival (DFS),

Table 1 Summary of the characteristics of the study population (N=194) and their specimens

Characteristics	Values
Age (years)	
Median (range)	54 (22–82)
Primary tumour type	
Invasive ductal carcinoma	172 (88.7%)
Invasive lobular carcinoma	22 (11.3%)
Primary tumour grade	
1	42 (21.6%)
2	112 (57.7%)
3	40 (20.6%)
Primary tumour size (cm)	
<1	29 (14.9%)
1–2	96 (49.5%)
>2	69 (35.6%)
Estrogen receptor status	
Positive	164 (84.5%)
Negative	30 (15.5%)
Progesterone receptor status	
Positive	143 (73.7%)
Negative	51 (26.3%)
Size of the largest axillary lymph node metastatic focus (mm)	
0.2–2.0	33 (17.0%)
2.0–5.0	47 (24.2%)
5.0–10.0	63 (32.5%)
>10.0	51 (26.3%)
Number of removed axillary lymph nodes	
Median (range)	13 (2–29)
Number of lymph nodes with metastasis	
1	99 (51.0%)
2	43 (22.2%)
≥3	52 (26.8%)
Total number of relapse (local/systemic)	28 (4/24)

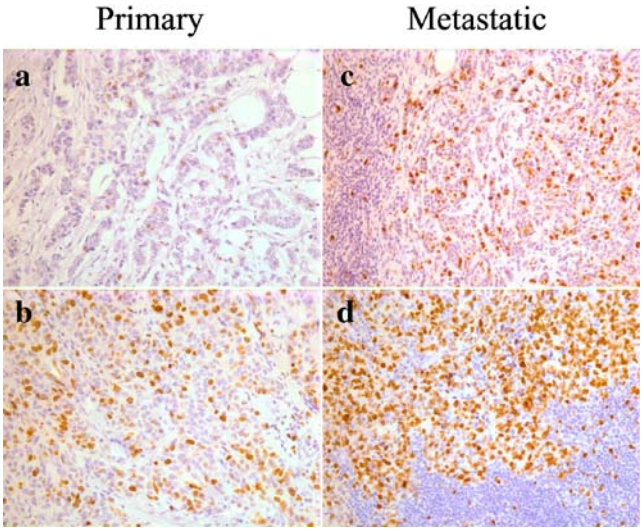

Fig. 1 Expression of Ki-67 protein in two primary tumours and their corresponding axillary lymph node metastases. (IHC, magnification ×200). Tumour 1 reveals a low number of positively stained nuclei (Ki-67 negativity) in the primary tumour (**a**), whereas the number of positively Ki-67 stained nuclei in the axillary lymph nodal metastases is significantly increased (**c**) (up-regulation). In tumour 2, both the primary (**b**) and metastatic carcinoma cells (**d**) show Ki-67 positivity, but the metastatic lesion reveals a higher number of Ki-67 positive cells than the primary tumour

distant disease free survival (DDFS), and breast cancer specific survival (BCSS). Kaplan–Meier survival curves were constructed, and the survival rates were compared using the log-rank test. Uni- and multivariate analyses of the independent prognostic factors were performed using the Cox proportional hazards regression model (stepwise backward elimination). A p-value of <0.05 was considered statistically significant.

Results

Method validation

The interobserver reproducibility was tested in 35 randomly chosen patients according to Ki-67 cut-off values and the tumour sites (i.e., in the primary tumours and in the ALNM). The agreements between the two observers were in the sufficient or good agreement range, irrespective of Ki-67 cut-off values and the tumour sites (k>0.6).

Expression of ki-67 in the primary tumours and their corresponding ALNM

There was a significant difference in the number of positively stained cells between the primary tumours and the corresponding ALNM (p=0.001) for Ki-67 immunoreactivity (Fig. 1). The mean percentage of positively cells was 11.4% in the primary breast tumours and 18.1% in the metastases (11.4±17.9 and 18.1±20.7%, respectively).

Correlation of ki-67 expression in primary and metastatic tumour with other histopathological parameters

Ki-67 expression in the primary tumours and the metastases correlated with a high histological grade (all Ki-67 cut-off values, p<0.001) and with a negative ER/PgR status (p=0.05~p<0.001). No correlation was found with age, cancer type, tumour size or number of lymph nodes involved. All cut-off values for Ki-67 expression in the ALNM correlated with the size of the metastases. The relationships between Ki-67 IHC staining and histopathological parameters are detailed in Table 2.

Table 2 Correlation between the expression of Ki67 and histopathological parameters

Parameters	5% Ki67 P + vs −	5% Ki67 M + vs −	10% Ki67 P + vs −	10% Ki67 M + vs −	15% Ki67 P + vs −	15% Ki67 M + vs −	20% Ki67 P + vs −	20% Ki67 M + vs −
Age (<55, ≥55)	NS	NS	NS	NS	NS	NS	NS	NS
Cancer type (IDC, ILC)	NS	NS	NS	NS	NS	NS	p=0.029[a]	NS
Grade (1, 2, 3)	p<0.001[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]
Primary tumour size (<1, 1–2, >2)	NS	NS	p=0.013	NS	NS	NS	NS	NS
ER (+, −)	p=0.05[a]	p=0.019[a]	p=0.001[a]	p=0.003[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]
PgR (+, −)	p<0.001[a]	NS	p<0.001[a]	p=0.029[a]	p<0.001[a]	p=0.011[a]	p<0.001[a]	p<0.001[a]
Number of nodal involvement (1, 2, ≥3)	NS	p=0.016[b]	NS	NS	p=0.035[b]	NS	NS	NS
Size of largest metastases (0.2–2, 2–5, 5–10, >10)	p=0.027[b]	p<0.001[b]	NS	p=0.002[b]	p=0.045[b]	p=0.002[b]	NS	p=0.007[b]

P Primary tumour, *M* metastatic tumour, *%* cut-off value for defining low/high Ki-67 index, *NS* nonsignificant
[a] Chi-square test
[b] Linear-by-linear test

Prognostic value of ki-67 in primary and metastatic tumours

All 194 patients were included in the analyses for DFS, DDFS and BCSS. Twenty-eight patients had been diagnosed with recurrence within a median observation time of 40 months after primary surgery (range 8–73 months). A local relapse without distant metastasis was observed in 4 of 28 patients, while distant metastases occurred in 24. Nine had died of breast cancer.

Uni- and multivariate analyses

Age, cancer type, hormone status, histology grade, primary tumour size, size of largest metastatic focus and Ki-67 expression were all tested as prognostic factors in uni- and multivariate analyses. Hormone status, histology grade, primary tumour size and Ki-67 with 10 and 20% cut-off points both in the primary breast tumours and the nodal metastases were significantly correlated with both DFS and DDFS in univariate analyses. Ki-67 with 5 and 15% cut-off points in the primary tumours did not reveal association with DDFS. Multivariate Cox regression analyses based on four different Ki-67 cut-off values (Table 3) showed that primary tumour size and Ki-67 for all cut-off points in ALNM were significant prognostic factors for DFS. The same prognostic factors were found for DFS with exception of Ki-67 with 5% cut-off point where only histological grade and primary tumour size were independent prognostic factors. No independent prognostic factor was found for BCSS in uni- or multivariate analysis.

Survival analyses according to different cut-off values

Kaplan–Meier survival analyses based on four different Ki-67 cut-off points showed significantly reduced DFS and DDFS in patients with high Ki-67 index in the ALNM and in the primary tumours compared to those with low Ki-67 index ($p=0.036 \sim p<0.001$). In the primary tumours, the survival curves for Ki-67 with 5% and 15% cut-off points showed no significant difference in DDFS. The DDFS curves for Ki-67 with all four cut-off points are shown in Fig. 2. A high Ki-67 expression with cut-off points of 15 and 20% in the metastases was significantly associated with a shorter BCSS ($p=0.048$ and 0.037, respectively), whereas Ki-67 expression with the other cut-off values showed no difference in survival.

Survival analyses according to the combined results of both Ki-67 expressions in primary and in metastatic tumours

Most cases had a similar Ki-67 expression in the primary tumour (10% cut-off) and its corresponding metastasis

Table 3 The prognostic factors (multivariate analyses, stepwise backward elimination)

Variables	HR	DFS 95% CI	*p*-value	HR	DDFS 95% CI	*p*-value	HR	BCSS 95% CI	*p*-value
Ki67 with 5% cut-off value									
Age (<55 vs ≥55)		NS			NS			NS	
Cancer type (IDC vs ILC)		NS			NS			NS	
Hormone receptor status[a] (− vs +)	0.4	0.2–0.9	0.025		NS			NS	
Histology grade ($G_{1–2}$ vs G_3)		NS		2.5	1.1–5.7	0.034		NS	
Tumour size (T_1 vs $T_{2–3}$)	3.5	1.6–7.6	0.001	4.0	1.7–9.5	0.002		NS	
Size of largest metastatic focus (0.2–2 mm vs > 2 mm)		NS			NS			NS	
Ki67 in primary tumours (− vs +)		NS			NS			NS	
Ki67 in metastatic tumours (− vs +)	4.0	1.2–13.5	0.026		NS			NS	
Ki67 with 10% cut-off value									
Tumour size (T_1 vs $T_{2–3}$)	3.7	1.7–8.1	0.001	4.8	2.0–11.5	<0.001		NS	
Ki67 in metastatic tumours (− vs +)	5.9	2.0–17.4	0.001	5.8	2.0–17.2	0.001		NS	
Ki67 with 15% cut-off value									
Tumour size (T_1 vs $T_{2–3}$)	3.6	1.7–7.8	0.001	4.7	2.0–11.0	<0.001		NS	
Ki67 in metastatic tumours (− vs +)	4.1	1.6–10.3	0.003	5.1	1.9–13.6	0.001		NS	
Ki67 with 20% cut-off value									
Tumour size (T_1 vs $T_{2–3}$)	3.2	1.5–6.9	0.003	4.2	1.8–10.0	0.001		NS	
Ki67 in metastatic tumours (− vs +)	4.4	2.0–9.8	<0.001	5.1	2.1–12.5	<0.001		NS	

From Ki-67 with 10% cut-off value, the significant prognostic factor(s) were simply enrolled in the table.

DFS Disease free survival, *DDFS* distant disease free survival, *BCSS* breast cancer specific survival, *HR* hazard ratio, *CI* confidence interval, *IDC* invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *NS* nonsignificant

[a] Hormone receptor status: positive—ER and/or PgR positive; negative—ER and PgR negative

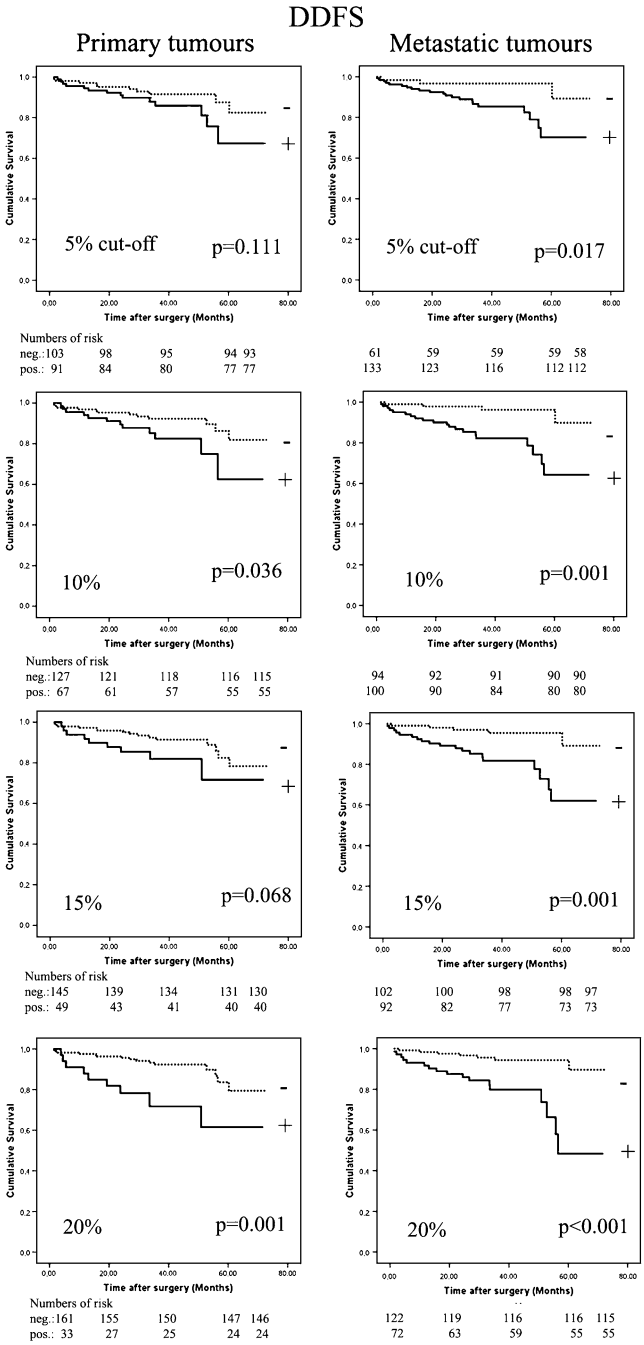

Fig. 2 Kaplan–Meier survival analyses based on four different Ki67 cut-off values show significantly shorter distant disease-free survival (DDFS) in patients with high Ki67 index in the nodal metastases and in the primary tumours compared to those with a low Ki67 index. No difference was found in 5 and 15% cut-off points in the primary tumours

(15% cut-off), either low (P−/M−: n=86, 44.3%) or high (P+/M+: n=53, 27.3%). A different Ki-67 expression between primary lesion and corresponding metastasis was detected in 55 cases (P+/M−: n=14, 7.2% and P−/M+: n=41, 21.1%, respectively). The correlation between the four groups and clinical outcome is detailed in Table 4. There was a highly significant difference in DFS and DDFS between P−/M- and the other groups; DFS: P−/M− vs P+/M− (p=0.002), P−/M− vs P−/M+ (p<0.001) and P−/M− vs P+/M+ (p<0,001), DDFS: P−/M− vs P+/M− (p=0.02), P−/M− vs P−/M+ (p=0.001) and P−/M− vs P+/M+(p=0,001). There was no difference among the three groups that had a high Ki-67 index either in the primary tumour or in the metastasis (i.e., P+/M−, P−/M+, and P+/M+), whereas a significant difference in BCSS was found between P−/M− and P−/M+ (p=0.006). The survival curves are shown in Fig. 3.

Survival analyses according to the presence of high Ki-67 expressions either in the primary or in the metastatic lesion compared to histological grade

The distribution of the four groups (i.e., P−/M−, P+/M−, P−/M+, and P+/M+) according to histological grade is shown in Table 4. Two groups were created according to the presence or absence of high Ki-67 index irrespective of the tumour sites (i.e., P−/M− vs the other group with P+ or/and M+: P+/M−, P−/M+ and P+/M+ were regarded as one group) and analyzed for the survival rates. Grade 2 P−/M− cases had a significantly better DFS than the other group. No significant differences in DDFS and BCSS were shown, but the difference in DDFS showed a borderline significance (p=0.057). No statistics were computed for grade 1 and grade 3 cases because they were all censored.

Discussion

Cell proliferation rate is a major determinant of the biologic behaviour of invasive breast carcinoma [25]. Ki-67 protein expression is strictly correlated to cell proliferation and to the active phases of the cell cycle [23]. Assessment of Ki-67 index represents an easy and reliable method for evaluating cell proliferative activity in breast cancer [1]. In the present study, we have assessed Ki-67 expression and compared the proliferative activity of cells in the primary breast carcinoma and their corresponding ALNM. We have detected significantly higher Ki-67 activity in the ALNM compared to the primary tumours (p=0.001). The finding corresponds with the result of Buxant et al. [5] who reported that Ki-67 expression was significantly increased in the ALNM. There was a significant difference in clinical outcome (DFS, DDFS and BCSS) between the groups with similar Ki-67 expression levels in primary and metastatic tumours and the groups with different expression levels. The group with similar expression was composed mainly of cases with P−/M− (73%, 72/98). As expected, this turned out to be a group with favorable prognosis (Fig. 3). Cases showing different Ki-67 expression in the primary tumour and the corresponding metastasis and cases with a high

Table 4 The high (+) or low (−) Ki-67 expression in primary (P) and metastatic tumours (M) and correlation to histological grade and clinical outcome

Groups	Number of patients (%)	Number of patients within histological grade			Number of any relapse (%)	Number of systemic relapse (%)	Number of breast cancer death (%)
		1	2	3			
P−/M−	86 (44.3)	35	48	3	3 (10.7)	3 (12.5)	1 (11.1)
P+/M−	14 (7.2)	2	10	2	2 (7.1)	2 (8.3)	1 (11.1)
P−/M+	41 (21.1)	2	28	11	10 (35.7)	9 (37.5)	5 (55.6)
P+/M+	53 (27.3)	3	26	24	13 (46.5)	10 (41.7)	2 (22.2)

expression in both lesions had an unfavorable prognosis. This finding supports Buxant et al.'s supposition [5] that a primary tumour is composed of multiple cell populations and that only those with the most aggressive potential (like a high proliferative index) are the most likely to escape from the primary tumour and establish itself as a lymph node metastasis.

Previous studies have reported significant associations between high Ki-67 index and clinicohistopathological parameters, such as age [18, 22], tumour size [18, 30, 32], histologic grade [2, 3, 18, 27, 32], ER/PgR status [6, 8, 18, 27, 30, 32] and lymph node status [32]. We found a statistically significant correlation of high Ki-67 expression in metastatic breast cancer with increasing histologic grade, negative ER/PgR status, size of largest metastatic focus, but not with age, cancer type, tumour size and the number of nodes involved. The high correlation between Ki-67 and grade is not surprising, as Ki-67 expression and mitotic rate are strongly interrelated [9, 18, 27]. In agreement with our findings, Ding et al. [9] reported that most ERα positive tumours were negative for the proliferation marker, Ki67. They suggested that such an inverse association between ERα expression and Ki-67 expression might be due to their different tumourigenic features; i.e., ERα positive tumours are more likely to be well differentiated, while Ki-67 positive breast tumours are frequently poorly differentiated [9].

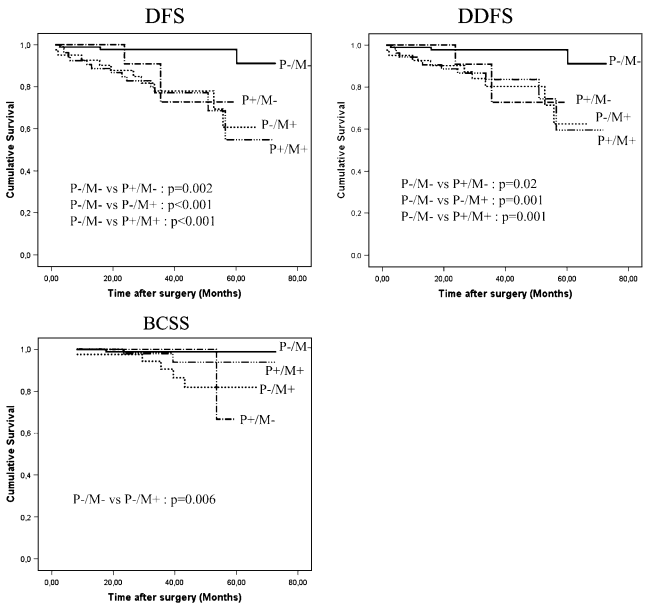

Fig. 3 The survival analyses (DFS, DDFS and BCSS) of all patients (n=194) according to the combined Ki67 status [high index (+) or low index (−)] in the primary tumours (P) and their nodal metastases (M). A significant difference in DFS and DDFS between P−/M− and the other groups was shown. No significant difference in DFS and DDFS among the combined groups (P+/M−, P−/M+ and P+/M+) was detected. In BCSS, the only survival difference between P−/M− and P−/M+ groups was significant

As there is no universally established standard Ki-67 cut-off value, we decided to treat Ki-67 expression as categorical variables by using four different Ki-67 cut-off points between 5 and 20% in 5% steps to be able to define a cut-off point for high/low proliferation rate in relation to clinical outcome.

Previous studies [3, 13, 14, 16, 21, 29] have shown statistically significant associations between Ki-67 immunoreactivity and clinical outcomes/survival. In our study, the associations between Ki-67 cut-off values and the predictabilities of DFS and DDFS were highly significant in all Ki-67 cut-off values both in the primary tumours and the metastases, except for Ki-67 expression of 5 and 15% cut-off in the primary tumours in predicting DDFS. The lack of significance in the two latter is probably due to too few cases in the group. BCSS correlated with Ki-67 expression at cut-off points of 15 and 20% in the ALNM. Ki-67 expression in more than 15% of tumour cells in the metastatic tumours pinpoint patients with a worse survival. Thus, it seems biologically "correct" to set the optimal "lower limit" of Ki-67 cut-off point to distinguish between a clinically relevant high and low proliferative activity at 15% in ALNM. In multivariate analysis, the Ki-67 expression in the metastatic tumours was a potential independent prognostic factor in predicting shorter DFS and DDFS. No association was found in BCSS, but the number of events with breast specific death (9/194) in the median follow-up of 40 months was low. A longer follow-up is probably required to see an eventual relation to BCSS.

Based on the statistical analysis, a cut-off of 10% seems to be optimal for Ki-67 expression in primary tumours. Ki-67 cut-off values have varied from one study to another. Some have used median values [14, 26], while others have chosen an arbitrary value (e.g., 10, 20% and so on) [9, 20, 25, 33]. Intriguingly, all the studies [14, 20, 26, 33] have shown a statistical correlation with clinical outcome irrespective of cut-off points. The reason might be explained by the fact that they all had chosen cut-off values that were found to be within the optimal range for evaluation of Ki-67 in the primary tumours.

On the basis of our results, we suggest that Ki-67 expression in ≥10% of carcinoma cells in the primary tumours and ≥15% in the ALNM should be used as cut-off levels for a clinically relevant prognostic difference. Evaluation of the Ki-67 index in the ALNM would pick up P−/M+ in addition to P+ cases. This would be in agreement with the finding that the level of Ki-67 expression in ALNM is a superior independent prognostic factor of clinical outcomes in metastatic breast cancer compared to that of Ki-67 expression in primary tumour.

The group with low Ki-67 index in both primary and metastatic tumour cells (P−/M−) is a favourable prognostic group albeit node-positive. Histological grade 2, P−/M− cases revealed significantly better survival than the other groups. The number of histological grade 1 P−/M− was probably too small to reach statistical significance. Histological grade 2 (and probably grade 1) tumours with a low Ki-67 expression in both the primary lesion and the corresponding metastasis (P−/M−) could be regarded as a low-risk group despite their ALNM and possibly with less need of adjuvant chemotherapy. However, further studies are necessary to confirm the association between low Ki-67 index (P−/M−) within grade 1 and 2 tumours and a good clinical outcome before such a conclusion might be drawn.

In conclusion, Ki-67 index has a potential to be used as an additional marker in metastatic breast cancer to select a high-risk (P+/M+, P−/M+ and P+/M−) and low-risk group (P−/M−) for distant metastasis that again might have consequences for adjuvant therapy strategies. Our results indicate that evaluation of Ki-67 expression in the axillary lymph node metastases is superior to evaluation of Ki-67 expression in primary breast carcinomas for predicting survival.

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ORIGINAL ARTICLE

Comparison of automated silver enhanced in situ hybridisation (SISH) and fluorescence ISH (FISH) for the validation of *HER2* gene status in breast carcinoma according to the guidelines of the American Society of Clinical Oncology and the College of American Pathologists

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Abstract HER2 is an important tumour marker in breast cancer. However, there is controversy regarding which method reliably measures HER2 status. This study evaluates the concordance between *HER2* gene amplification in invasive breast cancer determined by fluorescence in situ hybridisation (FISH) and a new silver enhanced in situ hybridisation (SISH) technique. Ninety-nine cases were analysed by direct-labelled manual FISH (PathVysion®, Abbott/Vysis) and bright field automated SISH (INFORM®, Ventana). For comparison, all specimens were stained by immunohistochemistry (Dako-HercepTest™ and Ventana-PATHWAY®4B5). Evaluation was performed by five pathologists following the algorithms of the manufacturers and the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. Concordance was calculated and the value of κ statistics estimated. Overall concordance between FISH and SISH was 96.0% (κ=0.754, 95%CI). Discrepancies were mostly seen in tumours with intra-tumoural heterogeneity of HER2 amplification. In conclusion, *HER2* gene copy status can be reliably determined by SISH. The 96% concordance with FISH fulfils the ASCO/CAP requirement of greater than 95% concordance for amplified vs non-amplified cases. There was a low inter-observer variability in the interpretation of SISH, suggesting that SISH is equally reliable in determining *HER2* amplification as FISH. Because SISH combines bright field microscopy with molecular analysis and full automation, it appears to be particularly suited for routine application in surgical pathology.

Keywords HER2/neu · Silver enhanced in situ hybridisation (SISH) · Fluorescence in situ hybridisation (FISH) · Breast carcinoma · Diagnostic test

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Introduction

Identifying tumours which over-express the human epidermal growth receptor 2 gene (ERBB2; also referred to as HER2/neu) has become an established guide for treating breast cancer with targeted therapeutics [3, 15, 23], e.g. the humanised anti-HER2 monoclonal antibody trastuzumab (Herceptin® Genentech, South San Francisco, CA) [4, 20]. It may be equally important in the selection of patients responsive to a small molecule dual HER1/HER2 tyrosine kinase inhibitor lapatinib (Tykerb, GlaxoSmithKline, Philadelphia, PA), which in combination with capecitabine improves clinical outcome in advanced disease [10]. Without doubt, the therapeutic relevance of the ERBB2 status mandates highly reliable and robust assays. However, prospective sub-studies from 2 adjuvant randomised trastuzumab trials have shown that approximately 20% of HER2/neu protein assays proved incorrect when the same specimens were re-evaluated in a high volume, central laboratory [13, 19]. More recently, a consensus statement has labelled the high rate of inaccuracy of ERBB2 testing unacceptable [22]. Clearly, a reliable, reproducible and easy to perform procedure for assessing the ERBB2 status is of utmost importance because any specific antibody-based therapy can be effective only with the target present on the cancer cells to be attacked.

Amplification of the *ERBB2* gene appears to be the primary mechanism underlying over-expression of its gene product [2]. Basically, there exist two approaches to determine the ERBB2 status: detection of the protein and/or of the genomic status as provided by immunohistochemistry (IHC) and in situ hybridisation (ISH), respectively. Both have advantages and disadvantages; quantification or semi-quantification of IHC reactions is generally difficult and suffers from a great inter-observer and inter-laboratory variability whereas ISH enables the pathologist to perform a more robust quantification of the genomic alteration. However, the most widely used technology of fluorescence in situ hybridisation (FISH) requires specialised microscopic equipment and suffers from fading of fluorochromes, which comprises long-term stability. In addition, the inter-observer variability is comparably high due to the possibility of misinterpretation of signal vs noise. This means that FISH requires a learning curve and only persons experienced in FISH interpretation should read the slides [7]. Despite many attempts by the international pathology community to improve the accuracy of HER2 testing in routine practice, high inter-observer and inter-laboratory variability remains an issue with both IHC and FISH [1, 8, 11, 24].

A newly introduced fully automated method—silver enhanced in situ hybridisation (SISH)—may overcome some of the disadvantages mentioned above since it allows the quantification of the number of centromeric chromosome 17 and HER2-specific signals by conventional bright field light microscopy [1].

In the present study, the *HER2* gene amplification status of 100 consecutive breast cancer cases was examined in parallel by FISH and SISH. For validation and comparison of the genetic assays (FISH vs SISH) and to determine inter-observer variability, a panel of five pathologists (MD, IE, HH, HK, HM) read and scored the slides following the guidelines of the American Society of Clinical Oncology and the College of American Pathologists [22]. The results of two HER2/neu IHC assays were used for discrepancy analyses.

Materials and methods

Clinical material Diagnoses and tumour characteristics are listed in Table 1.

Slide preparations One hundred consecutive breast cancer cases diagnosed and treated surgically during the years 2005 and 2006 at the Interdisciplinary Breast Cancer Centre of the Charité-University Hospital Berlin were selected for the study. All cases were formalin-fixed, paraffin-embedded and processed in the routine diagnostic laboratory of the institute according to standardised protocols. The time of fixation in 4% neutral buffered formalin ranged between 6 and 24 h. All specimens were excisional biopsies with an extended part of invasive breast carcinoma.

Table 1 Clinicopathological characteristics of the study cohort

Tumour data	Number of patients
Histology	
Ductal	75
Lobular	16
Medullary	2
Mucinous	4
Tubular	2
Metaplastic	1
pT status	
pT1	32
pT2	42
pT3	16
pT4	8
Missing	2
pN status	
pN0	47
pN1	16
pN2	11
pN3	8
Missing	18
Histological grade	
G1	14
G2	58
G3	25
Missing	3

The slides were prepared from archived paraffin blocks and were processed in parallel for H&E, FISH (PathVysion® HER-2 DNA Probe Kit, Abbott/Vysis), SISH (INFORM® *HER2* DNA Probe and ultraView™ SISH Detection Kit, Ventana) and IHC (HercepTest™, Dako and PATHWAY® anti-HER2/neu (4B5) Rabbit Monoclonal Antibody, Ventana). Before ISH and IHC staining, each case was checked to confirm the presence of invasive tumour tissue by three pathologists (MD, GL, AD). All procedures were performed at room temperature.

Staining procedures

Silver enhanced in situ hybridisation

Automated SISH of consecutive slides from the same paraffin blocks as for H&E were stained according to the manufacturer's protocols for the INFORM HER2 DNA and chromosome 17 probes. Both probes were labelled with dinitrophenol (DNP) and optimally formulated for use with the ultraView SISH Detection Kit and accessory reagents on Ventana's Benchmark® series of automated slide stainers. The *HER2* DNA probe was denatured at 95°C for 12 min and hybridisation was performed at 52°C for 2 h. After hybridisation, appropriate stringency washes (3 times at 72°C) were performed. The chromosome 17 probe was denatured at 95°C for 12 min and hybridisation was performed at 44°C for 2 h. After hybridisation, appropriate stringency washes (3 times at 59°C) were performed. The HER2 and chromosome 17 DNP-labelled probes were visualised using the rabbit anti-DNP primary antibody and the ultraView SISH Detection Kit. The detection kit contains a goat anti-rabbit antibody conjugated to horseradish peroxidase (HRP) utilised as the chromogenic enzyme. The chemistry of the SISH reaction, briefly described, is driven by the sequential addition of silver A (silver acetate), silver B (hydroquinone) and silver C (H_2O_2). Here, the silver ions (Ag+) are reduced by hydroquinone to metallic silver atoms (Ag). This reaction is fueled by the substrate for HRP, hydrogen peroxide (silver C). The silver precipitation is deposited in the nuclei and a single copy of the *HER2* gene is visualised as a black dot. The specimen is then counterstained with Ventana Hematoxilin II for interpretation by light microscopy.

Fluorescence in situ hybridisation

For FISH, consecutive sections from the same blocks used for SISH and IHC were cut at 6 μm thickness and mounted on SuperFrost +/+ slides. Deparaffinising, pre-treatment and protease digestion procedures followed the ABBOTT/Vysis PathVysion HER-2 DNA Probe Kit protocol (PathVysion CE Package Insert, Revision I, 2002) with additional monitoring of the progress of proteinase digestion by propidium iodide staining. Probe mixes were hybridised at 37°C between 14 and 18 h. After hybridisations, slides were washed in 2X SSC/0.3% NP-40 at 72°C for 30 min, air dried and counterstained with DAPI. A minimum of 20 nuclei of invasive tumour cells were scored using LEICA epifluorescence microscopes equipped with ×100 oil immersion objective and DAPI/Spectrum Green/Orange single and triple bandpass filters (AHF).

Immunohistochemistry

IHC was performed following routine procedures as described in detail by Fritzsche et al. [9]. For automated staining, Ventana's Benchmark® XT was used.

Teaching and evaluation

A 1-h consensus meeting was conducted to teach the elements and characteristics observed with the SISH assay and the algorithm for the assessment of *HER2* gene amplification status. The scoring algorithm and morphological details defined therein were clearly described and accepted by the panelists. A teaching set of 10 specially selected cases were read together using a multi-head microscope. The teaching set contained specimens that were negative, equivocal and positive for *HER2* gene amplification and examples of polysomy and inadequate staining. After this introduction, a blinded test set of six cases was given to the participants and evaluated independently. The results were compiled and then discussed in the group.

After achieving consensus, all 100 cases were independently examined in a blinded fashion by 5 pathologists (MD, IE, HH, HK, HM) during a 2-day meeting; each participant read 500 slides (100 cases by H&E, chromosome 17 SISH, *HER2* SISH, Herceptest IHC and PATHWAY 4B5 IHC). In detail, each participant first read the H&E and SISH slides together and recorded the results. In a second independent review, the IHC slides were read in a blinded fashion. The FISH reaction was determined separately and independently by MD and KK.

Scoring criteria and algorithms

SISH Per American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, *HER2* gene amplification status was classified applying the following criteria:

- Negative for *HER2* gene amplification was defined as a *HER2*/Chr17 ratio less than 1.8.
- Equivocal for *HER2* gene amplification was defined as a *HER2*/Chr17 ratio between 1.8 and 2.2.

– Positive for *HER2* gene amplification was defined as a *HER2*/Chr17 ratio greater than 2.2.

The detailed evaluation was performed following the instructions provided by the manufacturer (Ventana INFORM *HER2* DNA Probe CE Package Insert 16034EN Revision 1 [2007], for more details see http://www.her2sish.com).

FISH Per ASCO/CAP guidelines, *HER2* gene amplification status was classified applying the following criteria:

– Negative for *HER2* gene amplification was defined as less than 4.0 *HER2* gene copies per nucleus or a *HER2*/Chr17 ratio less than 1.8: no staining or weak, incomplete membrane staining in any proportion of tumour cells.
– Equivocal for *HER2* gene amplification was defined as a *HER2*/Chr17 ratio between 1.8 and 2.2.
– Positive for *HER2* gene amplification was defined as more than 6 *HER2* gene copies per nucleus or as a *HER2*/Chr17 ratio greater than 2.2 in on average of 60 cells.

The detailed evaluation was performed following the instructions provided by the manufacturer (ABBOTT/Vysis PathVysion CE Package Insert Revision I (2002), for more details see http://www.vysis.com/PathVysionHER2DNAProbeKit_35793.asp).

IHC Per ASCO/CAP guidelines, HER2 protein expression status was classified applying the following criteria:

– Negative for HER2 protein is an IHC staining of 0 or 1+: no staining or weak, incomplete membrane staining in any proportion of tumour cells.
– Equivocal for HER2 protein is an IHC staining of 2+: complete membrane staining that is either non-uniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells.
– Positive for HER2 protein is an IHC staining of 3+: uniform intense membrane staining of >30% of invasive tumour cells.

The protein analysis was performed to assist discrepancy resolution.

Statistics

Consensus among the panelists was defined as the agreement among three or more members representing either a simple or greater majority. Only samples that yielded informative results at both techniques were included in the concordance analyses. Consensus FISH results reported by MD and KK were used for the comparison of FISH to SISH.

Concordance data (or agreement) between consensus FISH and SISH methods and between consensus FISH and individual observers were determined and the κ statistics calculated using Cohen's κ [12].

Results

Case evaluation

From the 100 cases entering the study, 99 were dual-evaluable (informative) for SISH and FISH (details see Table 2). One case was excluded due to an unusually high polysomy of chromosome 17 (will be described separately). Typical examples of the SISH and FISH staining are shown in Figs. 1 and 2.

Comparison of SISH vs FISH *HER2* gene amplification status

There was agreement in 95 of 99 cases representing a concordance of 96.0% (κ=0.754, 95%CI; 0.518–0.993). Representative concordant cases are illustrated in Figs. 1 and 2.

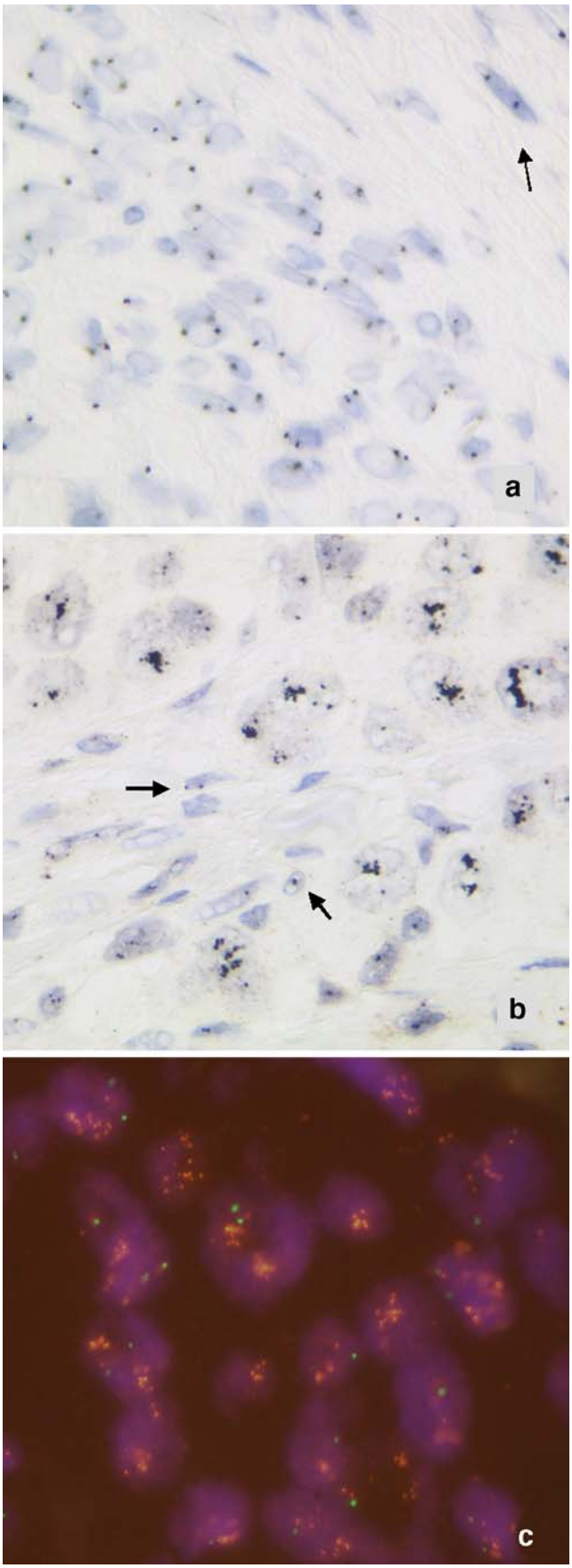
Concordance with consensus among the pathologists regarding amplified vs non-amplified cases: As per ASCO/CAP guidelines, the *HER2* gene amplification status judged by each individual relative to the consensus SISH results gave the following: among the pathologists a high overall agreement was achieved where 3 were nearly equivalent (concordance with consensus 94.4–95.2%, κ=0.767, 95% CI; 0.568–0.965), 1 tended to underscore (concordance of 92.6%, κ=0.630, 95%CI; 0.345–0.915) and 1 tended to over-score (concordance of 92.8%, κ=0.770, 95%CI; 0.592–0.947, respectively).

There were four discrepant cases, two of them representing FISH positive, SISH negative and two the converse. A case by case analysis revealed the following:

Case 1. The SISH consensus ratio of 2.25 gave a positive result for *HER2* gene amplification. The FISH analysis yielded a ratio of 2.0 forcing the

Table 2 Detailed demonstration of FISH vs SISH *HER2* gene amplification status obtained from 99 consecutively examined invasive breast cancer cases

HER2 gene amplification status		FISH			
		Positive	Equivocal	Negative	Total
Consensus SISH	Positive	9	0	2	11
	Equivocal	0	3	0	3
	Negative	2	0	83	85
	Total	11	3	85	99



◀ **Fig. 1** Typical example of an amplified case as demonstrated by SISH. **a** Chr17—2 signals in fibroblasts (*arrow*) and max. 3 signals in tumours cells, **b** same case HER2—clusters of HER2 signals in tumour cells. Note the fibroblasts (*arrows*) as internal control, i.e. 2 HER2 signals. **c** FISH Chr17 shown as 2 or 1 *green* signal and >6 HER2 signals

mathematical decision of equivocal for *HER2* gene amplification due to definition but was called negative by the observer. The consensus HER2 protein expression staining analysis was 1+. Thus, the final diagnosis reported in the pathology report would be negative for *HER2* gene amplification.

Case 2. The SISH consensus ratio of 2.56 gave a positive result for *HER2* gene amplification. The FISH analysis yielded a ratio of 1.0 giving a negative result for *HER2* gene amplification. Re-reading of the SISH slides revealed heterogeneity in the signal distribution and explains the discrepancy. The consensus HER2 protein expression staining analysis was 1+. Thus, the final diagnosis reported in the pathology report would be negative for *HER2* gene amplification.

Case 3. The SISH consensus ratio of 1.44 gave a negative result for *HER2* gene amplification. The FISH analysis yielded a ratio of 3.0 giving a positive result for *HER2* gene amplification. Re-reading of FISH revealed some areas of signal heterogeneity and areas difficult to interpret explaining the discrepancy. The consensus HER2 protein expression staining analysis was 1+. Thus, the final diagnosis reported in the pathology report would be *HER2* negative.

Case 4. The SISH consensus ratio of 1.06 gave a negative result for *HER2* gene amplification. During re-reading of the case, it became obvious that there was a discrepant interpretation of what is a single signal or a cluster (should be counted as six signals due to the manufacturer's instruction). The FISH analysis yielded a ratio of 2.2 giving a positive result for *HER2* gene amplification. Re-reading of FISH revealed some areas of signal heterogeneity explaining the discrepancy. The consensus HER2 protein expression staining analysis was 2+. Thus, the final diagnosis reported in the pathology report would be equivocal for *HER2* gene amplification. Regarding therapy, these cases may well be handled as amplified.

Discussion

Pathomorphological detection of tissue-based biomarkers identified as target molecules for humanised antibodies

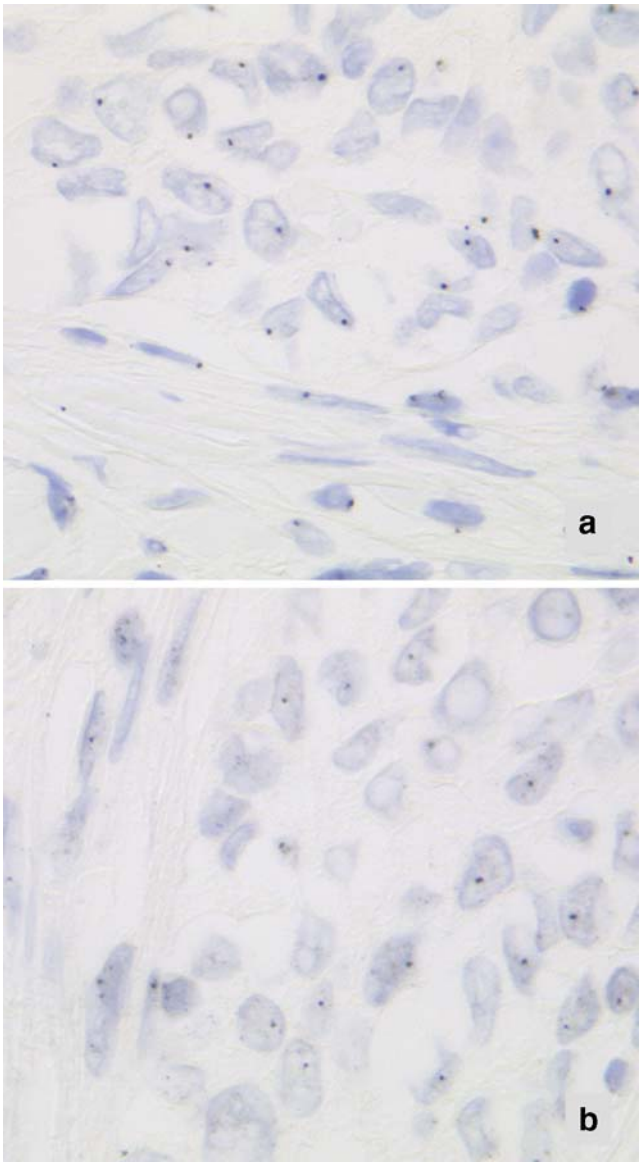


Fig. 2 Typical example of a non-amplified case as demonstrated by SISH. **a** Chr17—2 signals, **b** same cells HER2—2 signals. Note the fibroblasts as internal control, i.e. 2 HER2 signals

such as trastuzumab or cetuximab represents an essential tool in medical progress towards more personalised cancer therapies [5, 6]. The ideal procedure of target detection apart from being reliable and reproducible should lend itself to automated processing. This study compares a novel methodology, the SISH, fulfilling these requirements with a high concordance with the current imperfect gold standard, FISH.

The ASCO/CAP guidelines recommend the ERBB2/HER2 status to be optionally determined by IHC or FISH/CISH [22]. In most laboratories, IHC is done first. In case the primary immunohistochemical result is equivocal on the protein level, such as an IHC score of 2+, faint staining or other artefacts, an additional test is strongly recommended to clarify the HER2 status on the genomic level diagnosing amplification or non-amplification. Most often, this additional genomic testing is accomplished by FISH. However, this approach is not optimal because it is time-consuming, laborious, non-automated and expensive. Further exclusive use of FISH as the primary method for determining *ERBB2*/*HER2* gene status may also be problematic due to specialised instrumentation, special training, high costs and other disadvantages in the diagnostic workflow [14]. Previous methods of bright field in situ hybridisation have not found wide acceptance in routine pathology laboratories because they require manual processing and overnight hybridisation.

An alternative may be the newly developed SISH technology because it consists of a 6-h automated protocol and yields a stable and discrete chromogenic reaction product. Without the need for specialised microscope equipments, the pathologist can integrate this testing within the "normal" workflow. Due to the stable chromogenic signal, a cell-by-cell discussion using a screen or a multi-head microscope is facilitated. This possibility is of great advantage because it enables a continuous educational process and, in difficult cases, consensus can be reached more easily. Thus, SISH could readily be integrated into the surgical pathology workflow for routine assessment of ERBB2/HER2 status in breast carcinoma.

A major prerequisite for the acceptance of SISH is that it has to yield a high concordance with the current imperfect gold standard (FISH) and should exhibit low inter-observer variability. The current study proved that this is the case; the rate of cases that were negative vs equivocal vs positive for *HER2* gene amplification were very similar when detected by SISH or FISH (concordance 96.0%). This concordance then is compliant with the ASCO/CAP requirements of greater than 95% concordance among amplified/non-amplified cases. Only 4 out of 99 cases proved discordant among the panelists. Also the inter-observer concordance of 5 pathologists was unexpectedly high (concordance with consensus between 92.8% and 95.2%). This high percentage of scoring reproducibility was surprising because four of the five panelists did SISH interpretation for the first time. This clearly illustrates that the SISH signals are relatively straightforward to interpret.

Image analysis can be an effective tool for achieving consistent interpretation [16, 21]. The constant black signal of SISH will help to develop computer-based tools for automated signal detection and, by this, may contribute to a better reproducibility of *HER2* evaluation and a reduction of interpretive variation. However, image analysis procedures would need further validation before implementation.

Further independent international external quality assurance studies to demonstrate accuracy of SISH-HER2 testing should be initiated. To standardise inter-laboratory tests,

cell cultures with defined protein and gene alterations may be included [17, 18]. This would facilitate proficiency testing as made mandatory by the ASCO/CAP guideline [22] before the introduction of new assays.

In summary, the challenge to standardise, facilitate and speed up the diagnostic process above the gold standard of today is met by the newly introduced SISH technology. Thereby it appears to contribute to the progress of diagnostic pathology.

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ORIGINAL ARTICLE

Clinicopathological significance of WT1 expression in ovarian cancer: a possible accelerator of tumor progression in serous adenocarcinoma

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Abstract Recently, oncogenic potential of the WT1 gene has been proposed in some human solid tumors and leukemias. Although previous studies have shown the frequent expression of the WT1 protein in ovarian serous adenocarcinomas (OSAs), its clinicopathologic significance is still unclear. We immunohistochemically examined the expression status of WT1 in 119 OSAs and analyzed the correlation of the intensity of WT1 immunoreactivity with the level of WT1 mRNA expression by quantitative real-time polymerase chain reaction, clinicopathologic variables, expression of p53, Bcl-2, and Ki-67 labeling index (LI). Of 119 OSAs, nuclear WT1 immunoreactivity was positive in 99 (83%), of which 44 (44%) and 55 (56%) exhibited high and low WT1 immunoreactivities, respectively. The quantitative WT1 mRNA levels were significantly correlated with the intensity of WT1 immunoreactivity ($P<0.05$). In comparison with WT1-negative OSAs, the WT1-positive OSAs showed a higher grade ($P=0.007$), advanced stage ($P=0.018$), and higher Ki-67 LI ($P<0.001$). Additionally, high WT1 immunoreactivity was correlated with a higher grade ($P=0.003$), Ki-67 LI ($P=0.012$), Bcl-2 expression ($P=0.003$), and poorer patient outcome (5-year survival, 36.5 vs 63.8%, $P=0.008$ by log-rank test). The WT1 protein may be an accelerator of the progression of OSA.

Keywords Ovarian carcinoma · Ovarian serous adenocarcinoma · Prognostic factor · Tumor progression · WT1

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Introduction

The WT1, located on chromosome 11p13, was at first identified as a gene responsible for the development of Wilms' tumor [10]. Although the role of the product of the WT1 gene was initially described as transcriptional regulation, recent researchers have demonstrated its multifunctional roles sometimes with contradictory features, including being an activator vs a repressor of transcription, transcription in the nucleus vs RNA metabolism and

translational regulation in the cytoplasm, and even being an oncogene vs a tumor supressor gene [12].

Although the *WT1* gene was primarily considered to be a tumor-suppressor gene, recent studies have demonstrated an overexpression of the wild-type *WT1* gene in leukemias [15] and various solid neoplasms such as those of the breast [16, 19], lung, digestive tract [25, 26], pancreas [23], bone, and soft tissues [33, 37]. Moreover, several reports indicating an oncogenic, rather than a tumor-supressor, role of the wild-type *WT1* gene have been accumulated. For example, high expression levels of WT1 messenger ribonucleic acid (mRNA) are correlated with poor prognosis in leukemias [15], breast cancer [19], and soft tissue sarcomas [33]. Treatment with WT1 antisense oligomers specifically causes growth inhibition in leukemic blast cells [2, 39] and in cell lines derived from cancers of the lung, stomach, colon, and breast [24, 41]. These findings indicate that the *WT1* gene product is a potent target of immunotherapy because *WT1* gene expression is restricted to a limited set of normal tissues including the gonad, uterus, kidney, and mesothelium [4, 27].

Ovarian carcinoma behaves more aggressively and has a worse prognosis than any other cancer involving the female genital tract [28]. Because of the paucity of specific early symptoms, up to 70% of patients present with advanced disease [28]. Of the various histological subtypes of ovarian carcinoma, ovarian serous adenocarcinoma (OSA) is the most common and usually shows aggressive behavior and secondary resistance to current adjuvant chemotherapies.

It is a generally accepted idea that the expression of a single or multiple specific oncogenes and subsequent activation of cell proliferation contribute to neoplastic transformation of cells, tumorigenicity, and tumor progression. Such oncogenes and oncogene products may be of a clinical utility as predictive or prognostic factors and as therapeutic targets. So far, it has been shown that the incidence of WT1-positive tumors was highest in OSA among the various histological types of ovarian carcinoma [1, 13]. However, there is limited evidence for the clinicopathologic significance of WT1 in ovarian carcinomas [13, 21].

In the present study, to evaluate the clinicopathologic significance of the WT1 expression in OSA, we analyzed WT1 protein expression status by immunohistochemistry (IHC) applied to a tissue microarray (TMA) containing 119 specimens of OSA. Tumors showing a distinctly intense immunoreaction were further tested for their expression levels of WT1 mRNA by real-time, quantitative, reverse transcriptase polymerase chain reaction (RT-PCR) analysis. We demonstrated that these features of OSA were correlated with clinicopathologic parameters, expression of other molecular markers, and patient outcome.

Materials and methods

Patients and tissue samples

This study was performed with the approval of the Internal Review Board on ethical issues. All patients involved gave their informed consent to participate. We reviewed the clinicopathologic records of 119 patients who underwent initial surgery followed by platinum-based chemotherapies for primary ovarian OSA at the Department of Obstetrics and Gynecology, National Defense Medical College Hospital, Tokorozawa, Japan, between 1987 and 2004. All 119 patients underwent surgical staging according to the International Federation of Gynecology and Obstetrics (FIGO) system. Radical cytoreductive surgery was performed for patients with advanced disease. The chemotherapeutic regimens comprised cyclophosphamide, doxorubicin, and cisplatin for 74 patients, paclitaxel and carboplatin for 32, docetaxel and carboplatin for 10, cyclophosphamide and cisplatin for one, cyclophosphamide and carboplatin for one, and irinotecan and cisplatin for one. All pathology specimens were reviewed at our institution, and the tumors were classified histologically as OSAs according to the World Health Organization criteria [34]. The clinicopathologic details, such as patient age, FIGO clinical stage, tumor histologic grade, residual tumor after initial surgery, and overall patient survival were assessed for all 119 patients.

Histologic grading was performed by reference to the system proposed by Shimizu et al. [31] and Silverberg [32] (Table 1). Clinical response to chemotherapy was evaluated by ultrasonography or computed tomography and classified as complete response (CR), partial response (PR), stable disease, or progressive disease in accordance with the new Response Evaluation Criteria for Solid Tumours [35].

Table 1 Scoring system used for grading

Score	Criteria
A. Predominant architectural pattern	
1	Tubular and/or cystic
2	Papillary
3	Solid
B. Cytologic atypia	
1	Slight
2	Moderate
3	Marked
C. Mitotic Figures/10 high-power fields[a]	
1	0–9
2	10–24
3	≥25

Sum of the scores for A, B and C: 3–5=Grade 1; 6, 7=Grade 2; 8, 9=Grade 3

[a] Counted in the most active region at 10× 40× using an Olympus Optiphot microscope (field area 0.345 mm^2)

Tumor size was determined as the product of the maximum diameter and the length perpendicular to the maximum diameter. Clinical response to chemotherapies was assessed for the 63 patients who had residual tumors 2 cm in size or larger, after initial surgery.Tumors that showed CR or PR to first-line chemotherapies were considered to be chemosensitive.

Follow-up was calculated from the date of initial definitive surgery to the date of either last follow-up or death. The average follow-up period after initial surgery was 46.4 months, ranging between 2 and 227 months. Forty-two of the 119 patients died because of OSA spread, and two patients died of other causes.

Tissue microarray and immunohistochemistry

To construct TMA blocks, we selected formalin-fixed paraffin-embedded tissue blocks containing the areas where histologic grading had been performed, from the 119 OSA cases. Two core specimens 2.0 mm in diameter were taken from a tissue block of each case and transferred to recipient blocks using a Tissue Microarrayer (Beecher Instruments, Silver Spring, MD).

These TMA blocks were cut into 4-μm-thick sections, mounted on silane-coated glass slides, and subjected to IHC using the following primary monoclonal antibodies and dilutions: anti-WT1 (clone 6F-H2, 1:50, Dako, Grostrup, Denmark), anti-p53 (clone DO7, 1:100, Dako), anti-Bcl-2 (clone 124, 1:50, Dako), and anti-Ki-67 (clone MIB-1, 1:50, Dako).

These tissue sections were deparaffinized, subjected to antigen retrieval by digestion with proteinase K (ready to use, Dako) for 10 min for WT1, or by autoclaving in sodium citrate buffer (pH 6.0) for 15 min at 121°C for p53, Bcl-2, and Ki-67, then being allowed to cool at room temperature. Endogenous peroxidase was blocked using 5% hydrogen peroxide. Nonspecific staining was blocked in 2% normal swine serum. The slides were incubated with primary antibodies at 4°C overnight and then reacted with a dextran polymer reagent combined with secondary antibodies and peroxidase (Envision Plus; Dako) for 1 h at room temperature. Specific antigen–antibody reactions were visualized with 0.2% diaminobenzidine tetrahydrochloride and hydrogen peroxide. Counterstaining was performed using Mayer's hematoxylin. As positive controls, we used leukemic cell line K562 for WT1, a breast cancer with nuclear p53 immunoreactivity, and a lymph node showing reactive hyperplasia positive for Bcl-2 and Ki-67. As negative controls, the primary antibodies were omitted from each reaction process.

A case showing any degree of nuclear immunoreactivity for WT1 in more than 10% of the core tissue was considered to be the WT1-positive case. The intensity of WT1 expression was classified as 0 (nonstaining), 1+ (weak), 2+ (moderate), or 3+ (intensive; Fig. 1). Cases showing heterogeneous intensity of WT1 immunoreactivity were classified according to the highest degree of immunoreactivity of the WT1-positive cells in each core when the area with such immunoreactivity occupied more than 10% of the core tissues. If the part with the highest degree of immunoreactivity occupied less than 10% of area, the intensity of the largest area was adopted. Tumors scored as 1+ were defined as a low-level immunoreactivity group and those with a score of 2+ or 3+ as a high-level immunoreactivity group. Based on the literature, p53 and Bcl-2 were regarded as positive if more than 10% of tumor cells showed nuclear immunoreactivity for p53 and cytoplasmic immunoreactivity for Bcl-2 [3, 29, 36]. The Ki-67 labeling index (LI) was determined using the formula: Ki-67 LI (%)=100× number of tumor cells with nuclear Ki-67 immunoreactivity/ number of tumor cells with and without nuclear Ki-67 immunoreactivity. In total, 400 cell nuclei were counted twice independently, and both sets of data were combined.

RNA extraction and reverse transcription

To compare the intensity of WT1 immunoreactivity with its level of messenger ribonucleic acid (mRNA) expression, we selected a total of 21 OSA samples based on the pattern of their WT1 immunoreactivity—seven different samples each with negative, low-level, and high-level immunoreactivity—and prepared them for mRNA expression analysis. As controls for WT1 mRNA expression, the K562 leukemic cell line and five samples of non-neoplastic ovarian tissue obtained from separate patients who had undergone radical surgery for uterine cancers confined to the uterus were used [15]. All these non-neoplastic ovarian

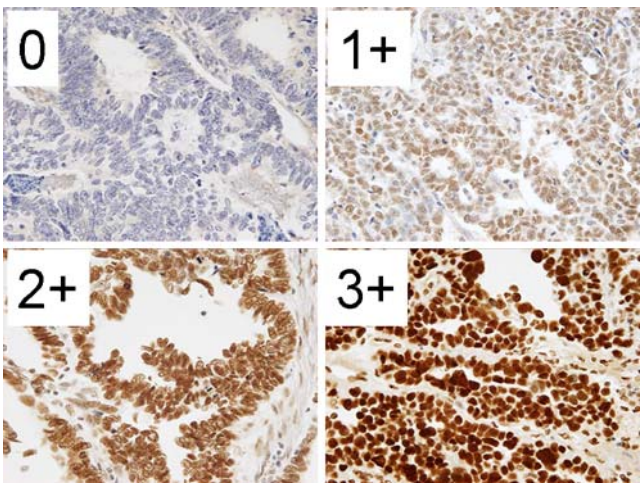

Fig. 1 Nuclear immunoreactivity of WT1 in ovarian serous adenocarcinomas (OSAs). OSA with negative WT1 (*0*), OSA with low-level WT1 immunoreactivity (*1+*), OSA with high-level WT1 immunoreactivity (*2+*), and OSA with high-level WT1 immunoreactivity (*3+*). Original magnification, ×400

tissues contained the ovarian surface epithelia, which are reportedly immunoreactive for the WT1 [30], on their peritoneal surface. All tissue samples were snap-frozen immediately after resection and stored until use in liquid nitrogen.

Total RNA was isolated from the tissues and cell line using RNeasy Mini (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Real-time PCR amplification of synthesized complementary deoxyribonucleic acid (cDNA) was performed in a total volume of 50 μl of reaction mixture containing 2 μl cDNA, corresponding to 100 ng of total RNA, 0.4 pmol/μl of each primer, and 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) using an ABI Prism 7700 Sequence Detection System (Applied Biosystems). We used TaqMan, a set of primers, and a probe (assay ID: Hs01103751_m1) for WT1 cDNA amplification. Cycling conditions were 95°C for 10 min, followed by 40 repeats of 95°C for 0.5 min and 63°C for 1 min. Standard curves for quantification of WT1 and β-actin (TaqMan β-actin Control Reagents: no. 401846) were constructed from the results of simultaneous amplification of serial dilutions of the cDNA from K562 cells, in which the expression level of WT1 mRNA was defined as 1.0, as described previously [15]. WT1 mRNA expression levels in the 21 samples of OSA and five non-neoplastic ovaries were determined according to the standard curves. To normalize the difference in RNA degeneration and in RNA loading for RT-PCR in individual samples, the level of *WT1* gene expression divided by the level of β-actin gene expression were defined as the relative WT1 mRNA expression level in each sample.

Statistical analysis

Statistical analyses were performed using the StatMate III software (ATMS, Tokyo, Japan). Comparisons between parameters were computed by the Chi-squared test or by the Student's *t* test or the Mann–Whitney test for unpaired data. For survival analysis, Kaplan–Meier curves were drawn, and differences between the curves were calculated by the log-rank test. Independent prognostic significance was computed by the Cox proportional hazards general linear model for disease-specific survival. Differences at $P<0.05$ were considered to be statistically significant.

Results

Correlation of nuclear WT1 immunoreaction with the corresponding mRNA expression level

Of the 119 OSAs, 99 (83.2%) were found to be WT1 positive and the remaining 20 (16.8%) were considered to be WT1 negative. Of the 99 WT1-positive cases, 55 (55.6%) were classified as having low-level immunoreactivity (1+) and 44 (44.4%) as having high-level immunoreactivity (2+, 24 cases; 3+, 20 cases).

The relative WT1 mRNA expression levels in the groups showing different intensities of immunoreactivity were quantified using real-time PCR assay and represented relative to the level in the human leukemia cell line K562 (defined as 1.0; Fig. 2). The median levels of mRNA expression in the WT1-negative, low-level, and high-level immunoreactivity groups were 0.66, 3.15, and 8.17, respectively. The WT1 mRNA expression level in the WT1-immunonegative group was statistically different both from that in the group with low-level WT1 immunoreactivity ($P=0.009$; Mann–Whitney *U* test) and from the group with high-level WT1 immunoreactivity ($P=0.0027$; Mann–Whitney *U* test). The mRNA expression levels were also significantly different between the low-level and high-level immunoreactivity groups ($P=0.017$; Welch's *t* test) and between the non-neoplastic ovarian tissues and the WT1 high-level immunoreactivity group ($P=0.028$; Mann–Whitney *U* test). WT1 mRNA expression in the immunohistochemically WT1-negative group was significantly lower than that in the non-neoplastic ovarian tissues (median, 0.66 vs 3.21, $P=0.028$; Mann–Whitney *U* test). There was no significant difference between non-neoplastic

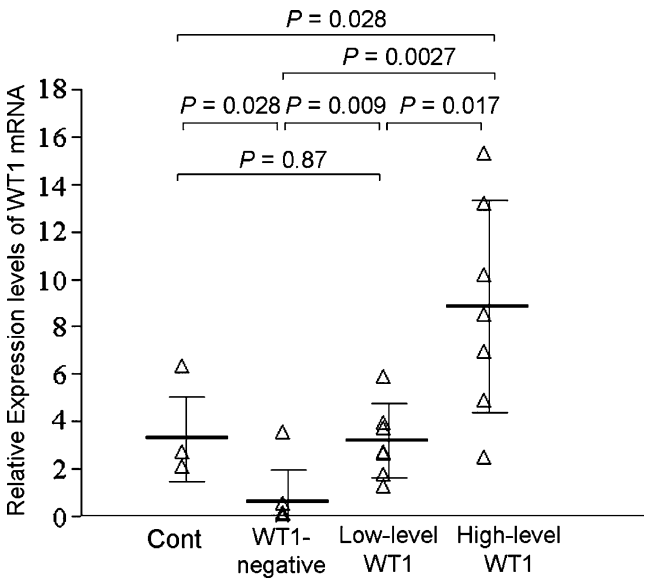

Fig. 2 Relative levels of WT1 mRNA expression in ovarian serous adenocarcinomas (OSAs) with distinct levels of immunoreactivity and in normal ovarian tissues. WT1 mRNA expression levels were calculated by comparison with the WT1 expression level in the K562 cell line. There was a significant difference in the mean relative expression level of WT1 mRNA between control ovarian tissues and WT1-negative OSAs ($P=0.028$), between the WT1-immunonegative group and the group with low-level WT1 immunoreactivity ($P=0.0088$), between the WT1-immunonegative group and the group with high-level WT1 immunoreactivity ($P=0.0027$), between the low-level and the high-level immunoreactivity groups ($P=0.017$), and between control ovarian tissues and the high-level immunoreactivity group ($P=0.028$). *Cont* Normal ovarian tissue samples

ovarian tissues and the low-level WT1 immunoreactivity group (median, 3.21 vs 3.15, P=0.871; Mann–Whitney U test).

Correlation of nuclear WT1 immunoreactivity with clinicopathologic factors

Eight (40%) of the 20 cases in the WT1-negative group were in FIGO stage I to II, compared with only 13 (13.1%) of the 99 WT1-positive cases (P=0.019; Table 2). Moreover, the WT1-positive group showed a higher proportion of histologically high-grade tumors (P=0.0072) and a higher Ki-67 LI (mean, 51.0 vs 29.3, P<0.0001) than the WT1-negative group. With regard to patient age, residual tumors (2 cm in size or more), the proportion of chemosensitive tumors, and expression of Bcl-2 and p53, there were no significant differences between the WT1-negative and WT1-positive groups. Although not statistically significant, patient outcome in the WT1-negative group tended to be better than that in the WT1-positive group (5-year survival, 73.6 vs 52.0%, P=0.387; log-rank test; Fig. 3).

As well as the comparison between the WT1-negative and WT1-positive groups, the group showing a high level of WT1 immunoreactivity had a higher histological grade (P=0.0032) and a higher Ki-67 LI (mean, 57.3 vs 46.0, P=0.0121) than the low-level immunoreactivity group (Table 3). Moreover, the high-level immunoreactivity group showed a higher frequency of Bcl-2 expression than the low-level immunoreactivity group (52.3 vs 23.6%, P=0.0032). There was no significant difference in age, FIGO stage distribution, residual tumors (2 cm in size or more), proportion of chemosensitive tumors, or p53 expression status between these two groups. Patient outcome was significantly poorer in the high-level WT1 immunoreactivity group than in the low-level WT1 immunoreactivity group (5-year survival, 36.5 vs 63.8%, P=0.0082; log-rank test; Fig. 3).

Table 2 Comparison of clinicopathologic factors and WT1 immunoreactivity in ovarian serous adenocarcinomas

Factor	Total	Number of cases (%)		P value
		WT1 negative (n=20)	WT1 positive (n=99)	
Age (year, median)		53.4	56.7	0.242
FIGO[a] stage				0.019
I	14	6 (43)	8 (57)	
II	7	2 (29)	5 (71)	
III	71	7 (10)	64 (90)	
IV	27	5 (19)	22 (81)	
Residual tumor				0.298
0 cm	21	6 (29)	15 (71)	
<2 cm	30	5 (17)	25 (83)	
≥2 cm	65	9 (14)	56 (86)	
Details unknown	3	0 (0)	3 (100)	
Response to chemotherapies				0.517
CR/PR[b]	41	5 (12)	36 (88)	
SD/PD[b]	22	4 (18)	18 (82)	
NE[c]	2	0 (0)	2 (100)	
Histologic grade				0.0072
1	17	7 (41)	10 (59)	
2	53	9 (17)	44 (83)	
3	49	4 (8)	45 (92)	
Ki-67 LI[d] (median)		29.3	51.0	<0.0001
p53				0.150
Positive	66	8 (12)	57 (88)	
Negative	54	12 (22)	42 (78)	
Bcl-2				0.249
Positive	40	4 (10)	36 (90)	
Negative	79	16 (20)	63 (80)	
5-year survival (%)		73.6	52.0	0.387[e]

[a] International Federation of Gynecology and Obstetrics
[b] *CR* Complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease
[c] Not evaluated
[d] Labeling Index
[e] Calculated by log-rank test

Univariate and multivariate analyses of disease-specific survival

The results of univariate analysis for disease-specific survival according to various clinicopathologic factors, including the intensity of WT1 immunoreactivity, are summarized in Table 4, a. Among the clinicopathologic factors, residual tumor (2 cm in size or more; P=0.007) was

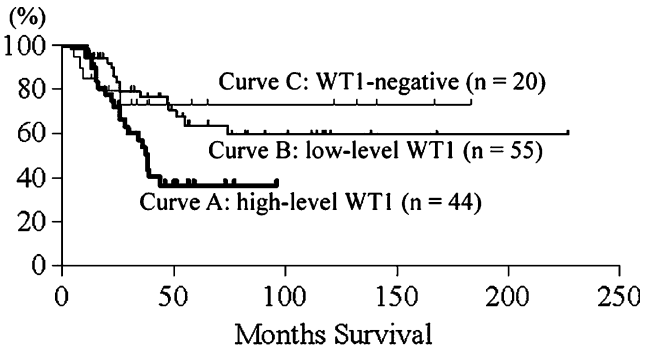

Fig. 3 Overall survival curves for 119 patients with ovarian serous adenocarcinoma (OSA), stratified by WT1 immunoreactivity status. *Curve A* for 44 OSA patients with high-level WT1 immunoreactivity, *Curve B* for 55 OSA patients with low-level WT1 immunoreactivity, and *Curve C* for 20 OSA patients with negative WT1 immunoreactivity. Significant differences were evident between the high-level WT1 immunoreactivity group (*Curve A*) and the low-level immunoreactivity group (*Curve B*; P=0.0082, log-rank test)

Table 3 Comparison of clinicopathologic factors and intensity of WT1 immunoreactivity in WT1-positive ovarian serous adenocarcinomas

Factor	Total	Number of cases (%)		*P* value
		WT1 low-level (*n*=55)	WT1 high-level (*n*=44)	
Age (year, median)		55.9	57.8	0.397
FIGO[a] stage				0.595
I	8	5 (63)	3 (37)	
II	5	4 (80)	1 (20)	
III	64	33 (52)	31 (48)	
IV	22	13 (59)	9 (41)	
Residual tumor				0.464
0 cm	15	9 (60)	6 (40)	
2 cm	25	16 (64)	9 (36)	
≥2 cm	56	28 (50)	28 (50)	
Details unknown	3	2 (67)	1 (33)	
Response to chemotherapies				0.563
CR/PR[b]	36	19 (53)	17 (47)	
SD/PD[b]	18	8 (44)	10 (56)	
NE[c]	2	1 (50)	1 (50)	
Histologic grade				0.003
1	10	10 (100)	0 (0)	
2	44	26 (59)	18 (41)	
3	45	19 (42)	26 (58)	
Ki-67 LI[d] (median)		46.0	57.3	0.012
p53				0.133
Positive	57	28 (49)	29 (51)	
Negative	42	27 (64)	15 (36)	
Bcl-2				0.003
Positive	36	13 (36)	23 (64)	
Negative	63	42 (67)	21 (33)	
5-year survival (%)		63.8	36.5	0.008[e]

[a] International Federation of Gynecology and Obstetrics
[b] *CR* Complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease
[c] Not evaluated
[d] Labeling Index
[e] Calculated by log-rank test

significantly correlated with worse patient outcome. Higher histological grade (grade 3/2/1) and the intensity of WT1 immunoreactivity (high-level immunoreactivity or others) showed only marginal significance (P=0.052 and 0.083, respectively). Patient age greater than or equal to 57 years (median; P=0.801), advanced FIGO clinical stage (stage III or IV; P=0.113), Ki-67 LI greater than or equal to 47.4 (median; P=0.563), p53 positivity (P=0.244), and Bcl-2 positivity (P=0.649) were not correlated with disease-specific survival in the present analysis.

We also performed a multivariate analysis using the Cox proportional hazards model including residual tumor size (≥2 vs <2 cm), histologic grade (3/2/1), and the intensity of WT1 immunoreactivity (high-level immunoreactivity vs others) as parameters (Table 4, b). The residual tumor size was identified as an independent prognostic value for overall survival (P=0.0052). The high-level WT1 immunoreactivity also showed nearly significant prognostic value (P=0.054).

Discussion

In the present, real-time PCR analysis clearly demonstrated that the intensity of WT1 immunoreactivity in OSAs was associated with the mRNA expression level, indicating that the intensity of WT1 immunoreactivity is a good indicator of the level of true WT1 protein expression. Taken together with clinicopathologic features, it appears that WT1 could assist the progression of OSAs in proportion to the level of expression of both its mRNA and protein.

The frequency of immunohistochemical detection of WT1 protein expression in OSA was 83.2% (99 of 119

Table 4 Cox model estimates of the significance of prognostic factors

Variables	*P* value	RR (95% CI)
a. Univariate Cox regression model		
Age (≥57[a] vs <57 years)	0.801	0.92 (0.49–1.72)
FIGO stage (IV or III vs II or I)	0.113	2.13 (0.84–5.45)
Residual tumor (≥2 vs <2 cm)	0.007	2.40 (1.26–4.55)
Histologic grade (3/2/1)	0.052	1.58 (1.00–2.50)
Ki-67 (LI≥47.4[a] vs <47.4)	0.563	1.20 (0.65–2.20)
p53 (positive vs negative)	0.244	0.68 (0.36–1.30)
Bcl-2 (positive vs negative)	0.649	0.86 (0.44–1.67)
WT1 immunoreaction (high level vs other)	0.083	1.73 (0.93–3.20)
b. Multivariate Cox regression model		
Residual tumor (≥2 vs <2 cm)	0.005	2.64 (1.34–5.21)
Histologic grade (3/2/1)	0.874	1.04 (0.63–1.73)
WT1 immunoreaction (high level vs no)	0.054	1.92 (0.99–3.73)

Other abbreviations as in Tables 2 to 3
RR Relative risk, *CI* confidence interval
[a] Mean values

cases), which was compatible with the range reported in previous studies (50.5–97%) [1, 13]. The introduction of TMA technology allowed a large number of specimens to be processed and was especially useful for comparative scoring of WT1 protein expression with specimens subjected to IHC under identical conditions.

Principally, the clinicopathologic significance of the *WT1* gene and its gene product in tumor tissues has been discussed from the viewpoints of (1) diagnostic utility, (2) functional roles in tumor progression, and (3) potential as a target for antigen-specific immunotherapy. From the viewpoint of diagnostic utility, a high level of WT1 protein expression could be a marker of the clinical aggressiveness of OSA. In relation to clinicopathologic parameters, the WT1-positive group showed a more advanced stage and higher histological grade than the WT1-negative group. Moreover, compared with the low-level WT1 immunoreactivity group, the group with a high immunoreactivity level showed a higher histological grade and a poorer patient outcome. Hylander et al. [13] demonstrated that WT1-positive OSAs tended to have a higher grade and more advanced stage than WT1-negative tumors. Netinatsunthorn et al. [21] divided WT1-positive OSAs into subgroups by the intensity of their immunostaining, as we did in the present study, and demonstrated that a high intensity of WT1 immunoreactivity had an independent prognostic value. In our multivariate analysis, the prognostic value of the high-level WT1 immunoreactivity was also nearly significant (P=0.054).

Some reports have advocated the practical utility of IHC for detecting the WT1 protein in differentiating OSA from other histological subtypes of ovarian carcinoma or from adenocarcinomas of other primary sites such as the endometrium or the pancreatobiliary system [8, 9]. However, its application to differential diagnosis in surgical pathology is currently limited and controversial, as a recent study by Nakatsuka et al. [20] demonstrated WT1 immunoreactivity in carcinomas of various primary sites such as the stomach, prostate, biliary tract, and urinary tract, and even in malignant melanomas.

The functional role of the *WT1* gene and its product in ovarian carcinomas is largely unknown, and few studies have investigated the issue. Shimizu et al. [30] hypothesized that the *WT1* gene is related to cell differentiation in OSAs, based on the detection of WT1 expression in normal female genital tissues such as the ovarian surface epithelium, the lining of inclusion cysts, and the tubal epithelium, but not in the cervical or endometrial epithelium.

The *WT1* gene encodes a zinc-finger transcription factor that represses the transcription of growth factor genes such as the platelet-derived growth factor A chain [7], colony-stimulating factor 1 [11], insulin-like growth factor (IGF)-II [5], and growth factor receptor genes such as the IGF-I receptor (IGF-IR) [38] and epidermal growth factor receptor [6]. Because the transcription of these growth factors and growth factor receptors can be regulated by WT1 via its interaction with wild-type p53, it has been recognized that WT1 functions as a transactivator in the absence of wild-type p53 [14, 17]. In OSAs, especially those with a histologically high grade, p53 mutation, which commonly contributes to nuclear p53 immunoreactivity, is associated with tumor progression [3, 29, 36]. Genetic or molecular interaction between WT1 and mutant p53 may be related to tumor progression and aggressive biological activity of OSA cells.

The different subcellular distribution of the WT1 protein among tumor types is of interest. In the reports by Nakatsuka et al. [20], a majority of WT1-positive tumors showed diffuse or granular immunoreactivity in the cytoplasm, whereas ovarian tumors and desmoplastic small round cell tumors frequently showed nuclear immunoreactivity. To date, the pathological significance of such contrasting distribution (nucleus vs cytoplasm) is unclear. Although the WT1 protein is principally a DNA-binding transcription factor distributed in the nucleus, recent reports have demonstrated that phospholylation in the DNA-binding domain alters the affinity for DNA and subcellular distribution of the WT1 [40]. Moreover, nucleus-cytoplasmic shuttling of WT1 and the association between WT1 and actively translating polysomes were reported [22]. Therefore, distinct subcellular localization of WT1 might alter the properties of tumor cells through the regulation of variable genes and/or proteins.

The WT1-positive group showed a higher Ki-67 LI than the WT1-negative group. Moreover, compared with the low-level WT1 immunoreactivity group, the group showing high immunoreactivity had a higher Ki-67 LI. Interestingly, expression of Bcl-2, an apoptosis regulator, was more frequent in the high-level than in the low-level WT1 immunoreaction group. WT1 can also directly activate the transcription of several apoptosis-related genes including Bcl-2 [18]. The present study demonstrated that a high level of WT1 immunoreactivity was significantly associated with both Ki-67 LI and Bcl-2 expression. These findings suggest transcriptional interaction of WT1 with cell cycle accelerators and apoptosis inhibitors.

It is intriguing that WT1-negative OSAs showed lower mRNA expression than non-neoplastic ovarian tissues. Moreover, the mRNA expression level of OSAs with low WT1 immunoreactivity was similar with that of non-neoplastic ovarian tissues. We additionally studied the WT1 immunoreactivity in the ovarian surface epithelium and/or the lining of inclusion cysts in seven unremarkable ovaries and the tumor cells of seven cases of serous tumor of borderline malignancy, which is thought to be a precursor of OSAs. Consequently, all the ovarian surface

epithelia, linings of inclusion cysts, and tumor cells of serous borderline tumors studied showed a weak WT1 immunoreactivity (data not shown), corresponding to the low-level immunoreactivity demonstrated in the present study. These findings suggest that low-level expression of WT1 may be associated with cell differentiation rather than an accelerator of tumor progression, and moreover, the low-level WT1 positivity might be a native characteristic of ovarian serous neoplasms. However, at present, it is unknown what kind of molecular mechanisms give rise to the different WT1 expression pattern, i.e., high-level immunoreactivity and the negativity, in OSAs.

Comprehension of WT1 expression in ovarian carcinomas should aid its utility for diagnosis or prognostication and might also help to clarify whether the *WT1* gene product could be an attractive tumor antigen for immunotherapy. Recent evidence that the presence of intratumoral T cells in advanced ovarian carcinomas is correlated with better clinical outcome has prompted a search for a well-characterized tumor antigen [42]. Generally, the ideal tumor antigen for immunotherapy should demonstrate a high level of expression in tumors, limited expression in normal tissues, and a functional role in tumor progression. Therefore, WT1-positive OSAs, especially those with a high level of WT1 immunoreactivity, could be a prime target for antigen-specific immunotherapy.

In conclusion, the results of the present study suggest that WT1 can be an accelerator of tumor progression in OSAs, possibly with a dose-dependent manner. A high level of WT1 immunoreactivity in high-grade and advanced-stage OSA may have not only a diagnostic and prognostic value but also a potential for application of antigen-specific immunotherapy against this highly lethal malignancy.

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ORIGINAL ARTICLE

Human pheochromocytomas show reduced p27Kip1 expression that is not associated with somatic gene mutations and rarely with deletions

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Abstract Pheochromocytomas are neuroendocrine tumors arising in the neural crest-derived chromaffin cells of the adrenal gland or in extra-adrenal sympathetic ganglia (paragangliomas). In a rat model of multiple endocrine neoplasia (MEN), absence of functional p27Kip1 protein predisposes to pheochromocytoma and paraganglioma development. As no data is available regarding the involvement of p27Kip1 in human pheochromocytoma and/or paraganglioma, we set out to determine the expression pattern of p27Kip1 in those tumor types. A panel of 25 pheochromocytomas and 23 paragangliomas was collected. Two pheochromocytomas were from MEN2 patients. The paragangliomas included 15 tumors that developed at the carotid bifurcation, three in the jugulo–tympanic area, and five at other sites. Except for the MEN2 cases, all others were apparently sporadic. Immunohistochemistry for p27Kip1 and the proliferation marker Ki67 was performed. We found that p27Kip1 expression is reduced/lost in 56% of pheochromocytomas, but only in 18.1% of paragangliomas. Downregulation of p27Kip1 was not associated with increased proliferation. Cases showing reduced/lost p27Kip1 expression were screened for the presence of somatic mutations in *CDKN1B* (p27Kip1) and for allelic imbalance at the p27Kip1 locus. Three cases had allelic imbalance but none had mutations. In conclusion, pheochromocytomas display extreme reduction/loss of p27Kip1 expression at high frequency.

Keywords Pheochromocytoma · Paraganglioma · p27Kip1 · Ki67 · Immunohistochemistry · Allelic imbalance

Abbreviations

CDK	cyclin-dependent kinase
MEN	multiple endocrine neoplasia
VHL	von Hippel–Lindau
SDH	succinate dehydrogenase
AI	allelic imbalance

Introduction

Pheochromocytomas are neuroendocrine tumors that arise from neural crest-derived chromaffin cells of the adrenal gland and extra-adrenal sympathetic ganglia (referred to as paragangliomas). About 25% of patients with pheochromocytoma and/or paraganglioma present with a family history of von Hippel–Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2), one of the three familial paraganglioma syndromes (PGL1, PGL3, PGL4), or, more rarely, neurofibromatosis type 1 (NF1). The causative germline mutations in these syndromic cancers have been identified in *VHL*, rearranged during transfection (*RET*), succinate dehydrogenase (*SDH*) subunits B, C, D, and *NF1*, respectively [14]. Approximately 10–15% of pheochromocytomas/paragangliomas are malignant and present very challenging problems for therapy. Currently, the malignant

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status of pheochromocytomas/paragangliomas is established primarily by the presence of metastases at non-chromaffin sites [18], and although several markers have been proposed to predict malignancy in those tumors, there is no consensus as to the existence of histological, clinical, or molecular indicators of worse prognosis.

We have identified a MEN-like syndrome in the rat (named MENX) that shows phenotypic overlap with both MEN1 and MEN2 human syndromes [21]. Affected rats develop bilateral pheochromocytoma and parathyroid adenoma, multifocal anterior pituitary adenoma, and thyroid C-cell hyperplasia, as well as paraganglioma [21]. The histopathology of the rat lesions resembles that of their human counterpart [21]. Noteworthy, pheochromocytoma has complete penetrance in MENX rats: Adrenal medullary hyperplasia is already evident at 2 months of age and progresses to pheochromocytoma with age. We recently mapped the *MenX* locus [35] and identified the genetic mutation that causes MENX. Affected rats harbor a germline loss-of-function mutation of *Cdkn1b*, a gene in which germline mutations predisposing to cancer had not been previously reported [34]. Noteworthy, we also identified a mutation-negative, suspected MEN1 patient who carries a germline heterozygous nonsense mutation in the human *CDKN1B* gene [34]. *Cdkn1b* encodes the cyclin-dependent kinase (Cdk) inhibitor p27Kip1, an important cell-cycle regulatory protein that controls the progression from G1 to the S phase by interacting with cyclinE/Cdk2 and cyclinD1/Cdk4 complexes [36, 39]. Emerging evidence suggests that p27Kip1 can regulate cellular functions other than cell-cycle progression, such as cell differentiation and migration [6, 8]. Due to its central role in many important cellular processes, p27Kip1 has been analyzed in several human malignancies. The *CDKN1B* gene, encoding p27Kip1, usually is not somatically mutated in human cancers and is inactivated through epigenetic mechanisms only in a small subset of tumors including lymphoid malignancies and hepatocellular carcinoma [27, 31]. In contrast, p27Kip1 expression level is frequently reduced or absent in human tumors, and the downregulation of p27Kip1 is a statistically significant predictor of survival and tumor behavior in a variety of cancers, including prostate, breast, hepatocellular, and gastrointestinal cancers (reviewed in [28]). In the tissues of MENX-affected rats, the level of p27Kip1 protein is extremely reduced or absent compared to wild-type animals [34], and likely, this inactivation triggers tumor development in neuroendocrine tissues. Due to the tumor spectrum of MENX rats with inactivation of p27Kip1, we decided to study the expression pattern of p27Kip1 in human pheochromocytoma and paraganglioma. In this paper, we report that p27Kip1 expression is frequently reduced/lost in pheochromocytoma (56%) but less so in paraganglioma (18.1%; P=0.0151, phaeochromocytoma versus paraganglioma). This suggests that, despite their histopathological similarities, those two tumor types follow distinct molecular pathways. The downregulation of p27Kip1 represents the single most-frequent molecular alteration so far reported in pheochromocytoma, implicating p27Kip1 in the pathogenesis of this tumor type.

Materials and methods

Samples

Formalin-fixed, paraffin-embedded tissues from 25 pheochromocytomas and 22 paragangliomas were retrieved from the archives of the Institute of Pathology, Technical University of Munich. The histological classification of the cases was reviewed and is reported in Tables 1 and 2. Of the pheochromocytoma cases, two belonged to MEN2A patients (PC21 and PC22) and were both bilateral and associated with medullary thyroid carcinoma (MTC); one case (PC20) was associated with a renal cell carcinoma (RCC; Table 1), but the patient was negative for *VHL* gene mutations. The paragangliomas included 13 tumors that developed at the carotid bifurcation, three in the jugulo–tympanic area, and 6 in other sites (Table 2). All cases, except PC21 and PC22, were apparently sporadic.

Immunohistochemistry

Conventional sections of the tissue samples mentioned above were used for immunohistochemistry. Immunohistochemistry was performed using monoclonal antibodies against p27Kip1 (clone 57, 1:1,000, BD Transduction Laboratories, San Jose, CA, USA) and the proliferation marker antigen Ki-67 (MIB-1, 1:500, DAKO, Carpinteria, CA, USA). Sections were cut at 3 μM and stained immunohistochemically using an automated immunostainer (Ventana Medical System, Tucson, AZ, USA) according to previously published procedures [37]. Positive controls (tonsils) were included in each staining run to confirm the adequacy of the staining. The degree of staining was evaluated using a semi-quantitative scoring system at ×200 magnification. To score the p27Kip1 staining, both the intensity of the staining and the percentage of positive tumor cells were taken into consideration. Three positive signs indicate >80% of tumor cells showing strong nuclear staining for p27Kip1, two positive signs indicate >80% of tumor cells showing very weak nuclear positivity, one positive sign indicate very weak to moderate positivity in <20% of tumor cells, and the negative sign indicate that tumor cells have negative staining. Only distinct nuclear staining of tumor cells was used for scoring the Ki67 immunoreactivity, which was determined semiquantitatively and is indicated as the percent of positive cells on the entire

Table 1 Clinicopathological, immunohistological, and molecular characteristics of pheochromocytomas

Case ID	Age/sex	p27Kip1 staining	Ki-67 (%)	p27Kip1 sequence	AI
PC1	64/M	++	<5		
PC2	49/F	+	<5		
PC3[a]	32/M	+	<5	Ex1+387 c/t	–
PC4[a]	50/M	+++	<5		
PC5	68/F	+[b]	<5		D12S391
PC6	40/F	+++[b]	<5		
PC7	58/M	+	<5	Ex1+387 c/c; G109G	D12S1580
PC8	70/F	+	<5	Ex1+387 c/c	
PC9	74/M	–	<5	Ex1+387 c/t	–
PC10	48/M	+++	<5		
PC11	41/F	–	<5		–
PC12	54/F	+	<5		–
PC13	54/M	+++	<5		
PC14	68/F	+++	<5		
PC15	70/M	+++	<5		
PC16[a]	49/F	–	<5	Ex1+387 c/c	–
PC17	76/M	+++	<5		
PC18	44/M	+++	<5		
PC19	73/F	+++	<5		
PC20[c]	64/F	+	<5		–
PC21[d]	45/F	+++	<5		
PC22[d]	31/F	+[b]	<5		–
PC23	66/F	+++	<5		
PC24	73/F	+[b]	<5	Ex1+387 c/c; V109G	
PC25	64/F	++	<5		–

Cases in bold were sequenced for the 5′ region and coding exons of the *CDKN1B* (p27Kip1) gene. p27Kip1 polymorphisms were named according to the SNP500Cancer database: Ex1+387 c/t corresponds to SNP rs34330 and V109G to SNP rs2066827. Except when indicated, cases were apparently sporadic.

AI Allelic imbalance, *M* male, *F* female, *negative sign* no AI

[a] Vascular invasion

[b] Some cytoplasmic staining

[c] Tumor associated to RCC

[d] MEN2A

tumor represented in the section. The immunohistochemistry of the clinical data in all cases was reviewed blindly by two pathologists (LQ-M and FF). Images were recorded using a Hitachi camera HW/C20 installed in a Zeiss Axioplan microscope with Intellicam software (Zeiss MicroImaging, Thornwood, NY, USA). Adobe PhotoShop (San Jose, CA, USA) was used for image processing.

Molecular analyses

Normal and tumor tissue areas were grossly macrodissected from five 5-μm sections of formalin-fixed, paraffin-embedded tissue blocks to obtain homogeneous cell populations. The tumor tissue samples contained >80% tumor cells. DNA was extracted from both cell populations as follows. Briefly, tissues were deparaffinized using xylene and ethanol, centrifuged, and dried. Pellets were resuspended in 200 μl of lysis buffer (0.05 M/l Tris–HCl at pH 7.5, 1 mM/l ethylenediaminetetraacetic acid (EDTA), 0.5% Tween-20) with 1 mg/ml proteinase K. After overnight digestion at 55°C, samples were boiled for 10 min to inactivate the proteinase K and used for polymerase chain reaction (PCR) amplification. Sequencing of the complete coding region and 140 bp of the 5′ untranslated region of the *CDKN1B* gene was performed with the Big-Dye terminator kit (Applied Biosystems), and sequences were run on an ABI377 sequencer. Primers for sequencing the 5′ region and exon 1 were: p27Kip1_1a FW GTCGGGGTCT GTGTCTTTTG, p27Kip1_1a Rev CCATGTCTCTG CAGTGCTTC; p27Kip1_1b FW TGTCTAACGG GAGCCCTAGC, p27Kip1_1b Rev AGTAGAACTCGGG CAAGCTG; p27Kip1_1c Fw AGTTAACCCGGGACTTG GAG, p27Kip1_1c Rev GTCCGACGGATCAGTCTTTG; p27Kip1_1d Fw AGGAGAGCCAGGATGTCAGC, p27Kip1_1d Rev GCCAGGTAGCACTGAACACC. Primers for exon 2 were: p27Kip1_2 Fw CTGACTATGGGGCCAA CTTC, p27Kip1_2 REV GCCAGCAACCAGTAAGATCAG. Sequencing of the *VHL* gene was performed using previously

Table 2 Clinicopathological, immunohistological, and molecular characteristics of paragangliomas

Case ID	Location	Age/sex	p27Kip1 staining	Ki-67 (%)	p27Kip1 sequence	AI
PG1[a]	Nasal sinus	67/F	+	>20	Ex1+387 c/t	D12S391,D12S358D12S1580
PG2	Cbt	70/F	+++	<5		
PG3	Rtp	63/F	++[b]	<5		–
PG4_1[a]	Cbt	40/F	+++	<5		
PG4_2	Recurrence of PG4_1	45	+++	<5		
PG4_3	Recurrence of PG4_1	48	+++	<5		
PG5	Cbt	61/F	+++	<5		
PG6	Cbt	40/F	+++	<5		
PG7[a]	Cbt	38/F	+++	<5		
PG8	Cbt	69/F	+++	<5		
PG9	Cbt	59/M	+++	<5		
PG10	Jtt	59/F	+++	<5		
PG11	Cbt	48/F	–	<5	Ex1+387 c/t	–
PG12	Base of skull	62/M	+++	<5		
PG13	Cbt	62/M	+++	<5		
PG14	Jtt	62/M	+++	<5		
PG15	Mediastinal	54/F	+++	<5		
PG16	Cbt	34/M	+++	<5		
PG17	Cbt	40/M	+++[b]	<5		
PG18	Retrosternal	54/M	+++	<5		
PG19	Rtp	61/M	+++	<5		
PG20	Cbt	71/F	+++	<5		
PG21	Jtt	52/F	–	<5	Ex1+387 c/c	
PG22	Cbt	38/F	+++	<5		

Cases in bold were sequenced for the 5′ region and coding exons of the *CDKN1B* (p27Kip1) gene. p27Kip1 polymorphisms were named according to the SNP500Cancer database: Ex1+387 c/t corresponds to SNP rs34330 and V109G to SNP rs2066827. All cases were apparently sporadic.
AI Allelic imbalance, *Cbt* carotid body tumor, *Rtp* retroperitoneal tumor, *Jtt* jugulotympanic tumor, *M* male, *F* female, *negative sign* no AI
[a] Local infiltration
[b] Some cytoplasmic staining

reported primers and conditions [40]. Allelic imbalance (AI) analysis at the *CDKN1B* locus was performed using the chromosome 12 p markers D12S391, D12S358, and D12S1580 in cases showing abnormal p27Kip1 staining. PCR products were loaded onto a 377XL sequencer (PE Biosystems). Allele size and fluorescent intensity were determined by Genescan and Genotyper software (PE Biosystems). AI was established by determining the fluorescent intensity of each allele and calculating their ratio in both normal (*N*) and tumor (*T*) DNA using the formula (allele1−*N*/allele2−*N*)/(allele1−*T*/allele2−*T*). Samples were scored as showing AI if an allelic ratio of <0.67 or >1.5 was obtained [10].

Statistical analyses

The number of patients with a reduced expression level of p27Kip1 and that of patients showing the typical high expression was compared between the two tumor types (pheochromocytoma versus paraganglioma cases) using the Fisher's exact test. Two sided P value<0.05 was considered statistically significant.

Results

Expression patterns of p27Kip1 and Ki-67 in pheochromocytoma

Normal adrenal medulla typically shows strong nuclear positivity for p27Kip1 in virtually all cells and low percentage of Ki67-positive cells (below 5%), indicating low proliferative activity (data not shown). From the 25 analyzed pheochromocytomas, 11 cases (44%) showed strong nuclear p27Kip1 staining in >80% of the tumor cells (classified as '+++,' Fig. 1a, b; Table 1), while 14 cases (56%) showed a very weak (Fig. 1c–f) to negative nuclear staining for p27Kip1 (Fig. 1h). The case illustrated in Fig. 1g, showed complete loss of p27Kip1 staining except for one small nodular area of the tumor, which had very strong nuclear expression of the protein. In the negative cases, infiltrating lymphocytes and residual normal adrenal gland were strongly stained with p27Kip1 and, thus, served as internal positive controls for the adequacy of staining. The immunohistochemical staining was mainly nuclear also in the cases with reduced p27Kip1 protein

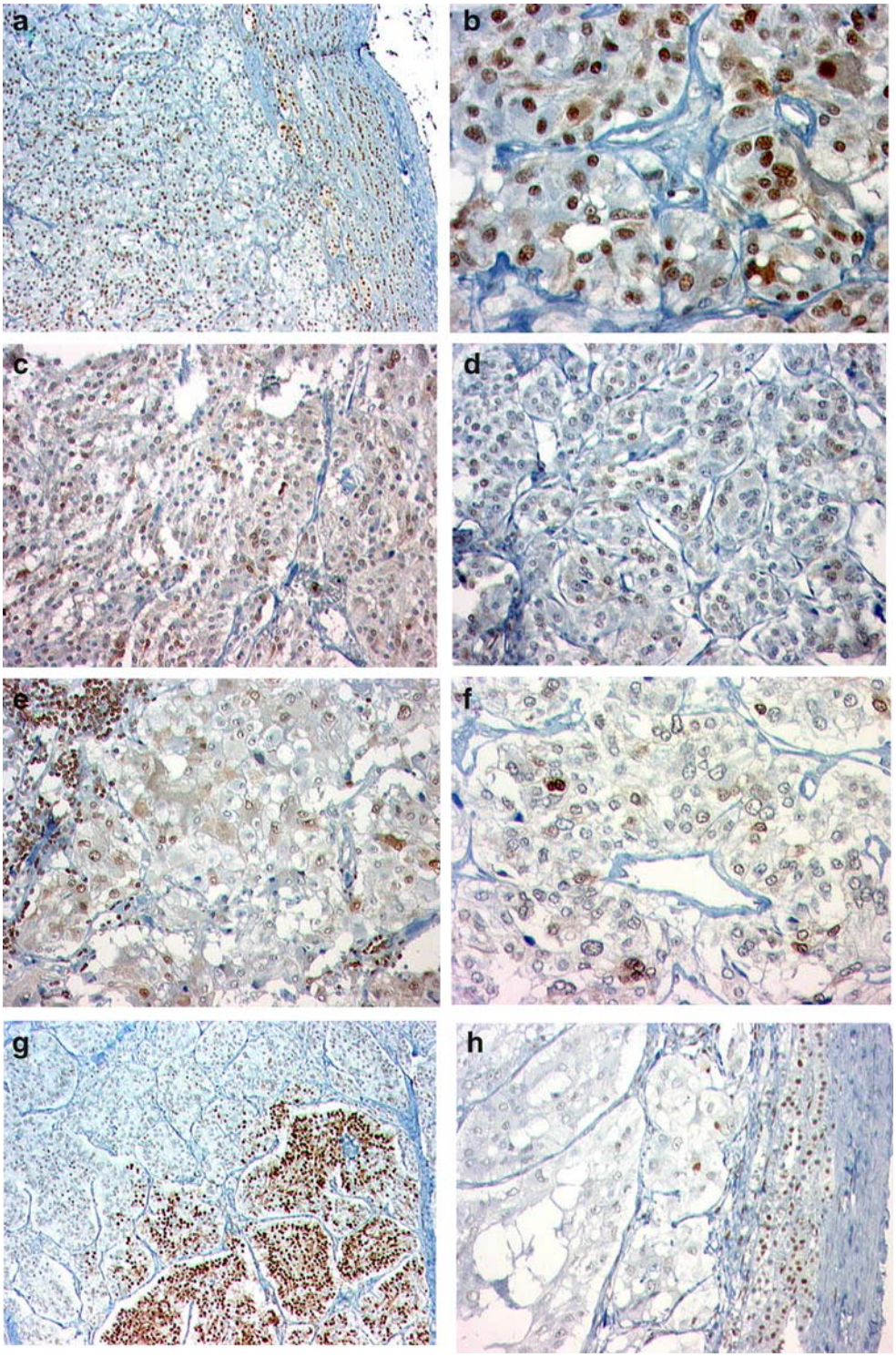

Fig. 1 Immunohistochemical analysis of pheochromocytoma. Sections were scored for the p27Kip1 staining intensity, and the percentage of stained cells in the tumor (see "Materials and methods"). **a**, **b** Strong p27Kip1 staining (+++). **a** Note on the right side that the residual normal adrenal cells are strongly positive for p27Kip1 (100×). **b** Higher magnification demonstrates that the vast majority of the tumor cells have a strong, nuclear positivity for p27Kip1 (400×). **c**–**f** Cases with reduced expression of p27Kip1. **c**, **d** Weak diffused expression of p27Kip1 in the majority of the cells (++; 200×). **e**, **f** Very weak or moderate expression of p27Kip1 in <20% of the cells (+; **e** 200×, **f** 320×). Note that in case **e**, in addition to the reduced expression of p27Kip1, there is also cytoplasmic staining. On the upper-left side of **e**, there are numerous lymphocytes with strong nuclear positivity used as internal control. **g**–**h** Cases of pheochromocytoma with lost p27Kip1 expression or negative for p27Kip1 (−). **g** Only one nodule within the tumor showed nuclear positivity for p27Kip1; the rest of the tumor was negative (100×). **h** Pheochromocytoma negative for p27Kip1. On the right side, there are residual normal adrenal cells strongly positive for p27Kip1 used as internal control (200×)

expression. Four cases (PC5, PC6, PC22, PC24) showed in addition some cytoplasmic reactivity (as an example, see Fig. 1e). The extent and distribution of the cytoplasmic p27Kip1 staining mirrored the nuclear staining in each sample; i.e., when the nuclear staining was very faint and present in only a few cells, the same also held true for the cytoplasmic staining. All pheochromocytomas showed immunoreactivity for Ki-67 in <5% of the cells (Table 1) irrespective of p27Kip1 status.

Expression pattern of p27Kip1 and Ki-67 in paraganglioma

Of the 22 paraganglioma cases, 18 (77.3%) showed strong nuclear immunoreactivity in >80% of the tumor cells

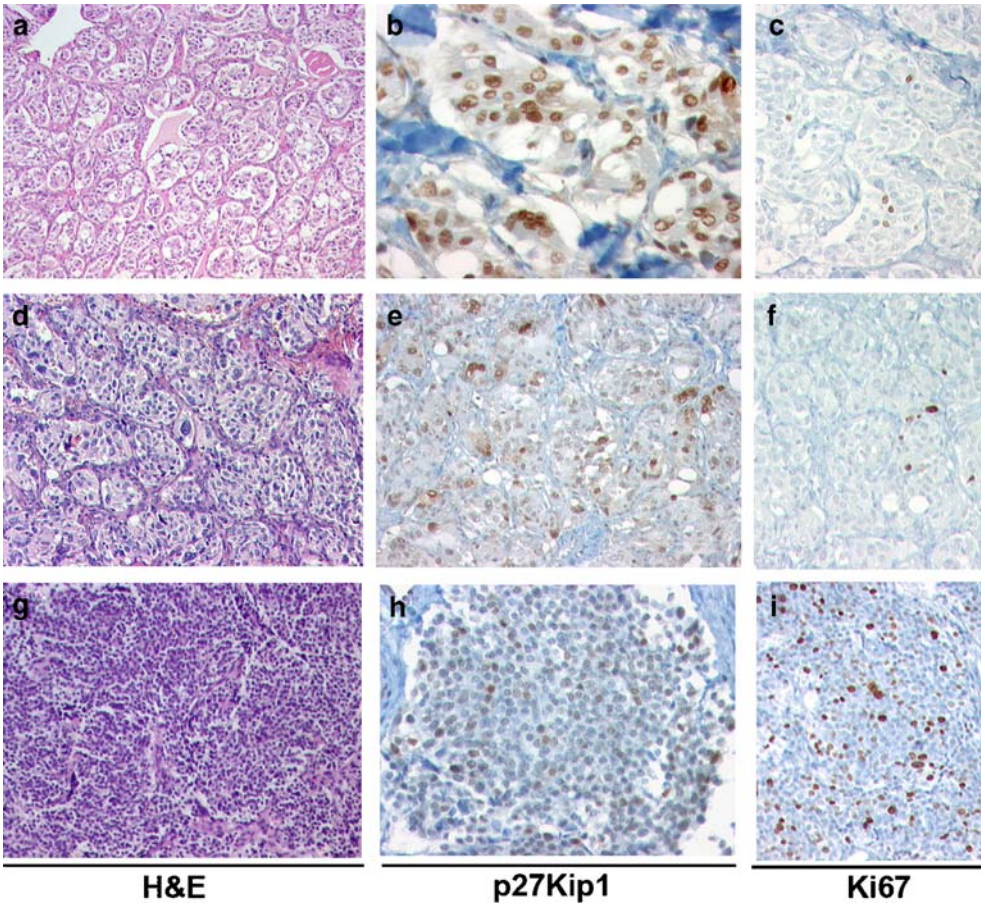

Fig. 2 Histological and immunohistochemical analysis of paraganglioma. **a**–**c** H&E stain of a paraganglioma (**a** 100×) with strong p27Kip1 nuclear positivity (+++) in virtually all tumor cells (**b** 400×) and very low proliferation rate as demonstrated by the Ki67 immunoreactivity (**c** 200×). **d**–**f** H&E stain of a paraganglioma (**d** 160×) with weak to moderate p27Kip1 staining in >80% (++) of the tumor cells (**e** 100×) and very low proliferation rate (**f** 200×). **g**–**i** H&E stain of the aggressive paraganglioma case PG1 (**g** 100×) with p27Kip1 staining in less than 20% (+) of the tumor cells (**h** 400×) and high proliferation rate (**i** 200×)

(Fig. 2b), while 4 cases (18.1%) had weak (Fig. 2e and h) or negative p27Kip1 staining (Table 2). The staining was mainly nuclear also in the cases with reduced p27Kip1 positivity. Only two cases (PG3 and PG17) showed some cytoplasmic reactivity for p27Kip1, and the extent and distribution of the cytoplasmic p27Kip1 staining mirrored the nuclear staining in each sample (as described above for the PC cases). Paraganglioma cases with abnormal p27Kip1 staining included tumors at different locations throughout the body (Table 2). All paraganglioma cases showed immunoreactivity for Ki-67 in <5% of the cells, except one (PG1) that showed positivity in more than 20% of the cells (Fig. 2i). PG1 was classified as an aggressive paraganglioma, as it was a tumor in the nasal cavity associated with infiltration into the base of the skull, both frontal sinuses, and both orbitae.

Genetic analyses of the samples showing abnormal p27Kip1 immunoreactivity

The reduction in the level of expression of p27Kip1 in these tumors (both phaeochromocytoma and paraganglioma cases) could be due to loss of the chromosomal region harboring the gene. Therefore, we searched for allelic imbalance (AI) at the p27Kip1 locus using three highly polymorphic microsatellite markers (D12S391, D12S358, D12S1580) flanking the p27Kip1 locus on chromosome 12p13 (Fig. 3). These markers present a centromere-to-telomere orientation, covering 890 Kb of chromosome 12 that include the *CDKN1B* gene (encoding p27Kip1). The 5′ end of the *CDKN1B* gene is approximately 230 kb downstream of D12S358 and the 3′ end 472 kb upstream of D12S1580. For ten (out of 14) pheochromocytomas and 3 paragangliomas with reduced/lost p27Kip1 expression (see Tables 1 and 2), normal tissue was available for AI analyses. Two samples were non-informative for D12S391, four were non-informative for D12S358, and two for D12S1580. The remaining samples were informative for at least one marker in the region (Fig. 3a). One pheochromocytoma showed AI for D12S391 (PC5; Fig. 3b), another showed AI for D12S1580 (PC7), and the aggressive paraganglioma (PG1) showed AI for all three markers (not shown). Overall, the AI frequency calculated as the ratio of samples showing AI versus the total number of informative cases for each marker is quite low, ranging from 11% (D12S358) to 18% (D12S391, D12S1580).

Nine cases showing a decreased expression of p27Kip1 were analyzed for the presence of somatic mutations in the coding region of the *CDKN1B* gene (Tables 1 and 2). Control DNA from the adjacent normal tissues was also

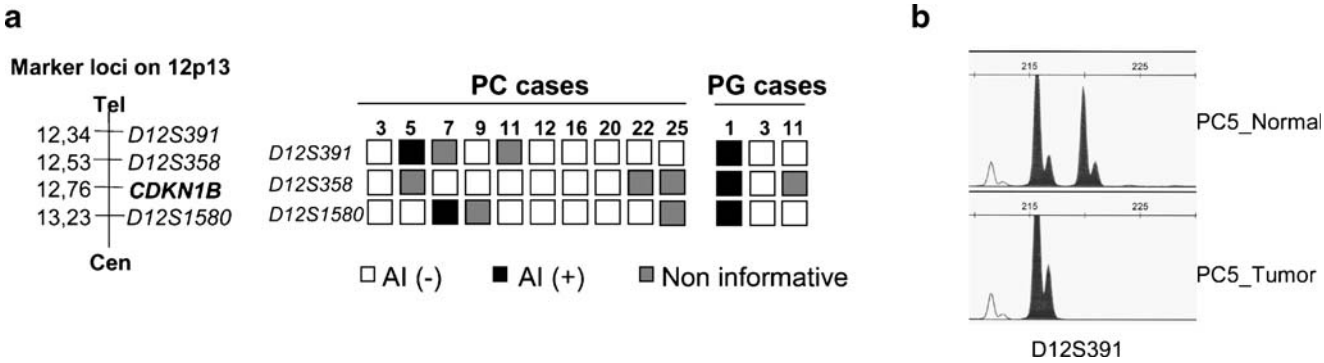

Fig. 3 Allelic imbalance analysis for markers at 12p13. **a** The panel summarizes the status of thirteen cases having abnormal p27Kip1 immunohistochemical staining. Cases described as retaining heterozygosity; having AI and non-informative cases are indicated by *open*, *close*, and *gray* squares, respectively. On the left is reported the position of the markers along chromosome 12 in megabases [according to the National Center for Biotechnology Information (NCBI) genome browser], as well as the position of the *CDKN1B* gene encoding p27Kip1. PG1 was the only sample showing AI for all the markers tested. PC5 and PC7 showed AI for one marker each. **b** Example of AI for marker D12S391 in sample PC5. The normal DNA shows two alleles, while the tumor DNA retains the smaller allele but has lost the larger one

sequenced. PCR amplification of the 5′ region and the two coding exons was performed followed by direct automated sequencing of the PCR products. Although potential pathogenic mutations in *CDKN1B* were not found, known polymorphisms in the 5′ untranslated region of exon1 (SNP rs34330) and at codon 109 (Val109Gly, SNP rs2066827) were identified (Tables 1 and 2).

Patient PC20 could be affected by VHL disease, as the tumor spectrum of this syndrome includes RCC and pheochromocytoma. Therefore, we sequenced the *VHL* gene in the DNA from the normal tissue of case PC20 but we found no mutations. Furthermore, no mutations were identified in *MEN1* and *RET* in this patient (data not shown).

Statistical analysis

The number of patients with a reduced expression level of p27Kip1 (scored −, +, or ++) or showing the high p27Kip1 expression typical of normal tissues (scored +++) was compared between the two tumor types (pheochromocytoma versus paraganglioma) using the Fisher's exact test. The results showed that the number of pheochromocytoma samples having reduced p27Kip1 expression is significantly higher than that of paraganglioma cases showing reduced p27Kip1 positivity (P=0.0151). Age at presentation, malignant potential, and tumor location (for the paragangliomas) were not found to be significantly associated with downregulation of p27Kip1 in both tumor types.

Discussion

In this study, we examined the expression of the cyclin-dependent kinase inhibitor p27Kip1 in a panel of apparently sporadic pheochromocytoma and paraganglioma cases. Two syndromic pheochromocytomas (MEN2) were among the cases analyzed. A striking decrease/loss of p27Kip1 expression was observed at a high frequency in pheochromocytoma (56% of cases), as demonstrated by the low immunostaining and decreased number of cells/field stained with the anti-p27Kip1 antibody. In the remaining cases, the typical strong nuclear expression was observed. In contrast to the high frequency of decreased p27Kip1 expression observed in pheochromocytoma, only 18.1% of the paragangliomas showed reduction of p27Kip1 immunoreactivity. These two tumor types share many similarities: They both arise from chromaffin cells derived from the embryonal neural crest, they are histologically very similar, and they both can be caused by germline mutations in the same susceptibility genes (*VHL*, *SDHD*, *SDHB*). However, our data points to a different genetic etiology of those tumor types with a significantly higher percentage of downregulation of p27Kip1 in pheochromocytomas compared to the paragangliomas (P=0.0151). This finding strongly implicates reduction of p27Kip1 in adrenal but less so in extra-adrenal pheochromocytoma development. These observations are in agreement with our studies on the MENX rat model system. Indeed, affected rats (homozygous for a loss-of-function mutation in p27Kip1 [34]) develop pheochromocytoma with complete penetrance at young age (<6 months), while paragangliomas occur later in life (>10 months), and their incidence is only about 10% (Pellegata, unpublished observation).

In contrast to what is observed by immunohistochemistry for many antigens, we did not find a continuum of p27Kip1 immunoreactivity in our samples, but instead a discontinuous expression pattern. Indeed, we observed samples with virtually all tumor cells highly positive for p27Kip1 (as seen in normal adrenal chromaffin cells), while the others had a dramatic reduction of positivity in either the number of stained tumor cells and/or the intensity of staining.

The molecular mechanism underlying the reduction/loss of p27Kip1 expression is as yet uncertain. We have examined allelic status of chromosome 12p, where the *CDKN1B* gene is located, but changes occur at a low incidence in these tumors: One aggressive paraganglioma showed loss of p27Kip1 through allelic imbalance (AI), and two pheochromocytomas showed AI at markers in the vicinity of p27Kip1. The overall incidence of AI at the analyzed loci (D12S391, D12S358, D12S1580) was 11–18% (calculated as the ratio of AI/informative cases for each marker). Although microdeletions are a formal possibility, it is likely that AI is not the most important mechanism for loss of protein expression. In addition, we did not find mutations in the coding sequence of the *CDKN1B* gene. Both these results are in agreement with the reported low incidence of AI at the p27Kip1 locus and the scarcity of somatic mutations in *CDKN1B* in other solid tumors [24, 25, 30, 38]. It has been shown that the intracellular level of the p27Kip1 protein is mainly regulated at the post-translational level via proteasome-mediated degradation [33]. Indeed, in most cases of decreased p27Kip1 concentrations, increased proteolytic activity toward the protein has been observed [12, 19, 29]. In contrast, no changes in the level of *CDKN1B* messenger RNA (mRNA) have been found in tumors that show downregulation of the p27Kip1 protein [11, 23, 29]. Our studies on the p27Kip1-negative tissues of MENX rats [34] have also failed to detect changes in the level of p27Kip1 mRNA. Thus, the mechanism causing loss of p27Kip1 expression in human pheochromocytomas and paragangliomas might be post-translational.

Cytoplasmic relocation due to phosphorylation or sequestration by partner proteins plays an important role in regulating p27Kip1 functions. It has been proposed that, while p27Kip1 in the nucleus acts primarily as a tumor suppressor by modulating cyclin-cdk complexes, cytoplasmic p27Kip1 has oncogenic properties. Indeed, expression of a p27Kip1 protein that can only localize in the cytoplasm was recently found to increase motility, survival, and Akt levels in MCF7 breast cancer cells [42]. Cytoplasmic p27Kip1 staining has been observed in several tumor types and is in general associated with more aggressive behavior and poorer prognosis [3]. Among the samples analyzed, six showed cytoplasmic staining for p27Kip1 in addition to nuclear staining (see tables). Four of these cases (PC5, PC6, PC24, PG3) had an overall downregulation of p27Kip1 expression (three classified as "+" and one as "++"), indicating that, in those samples, cytoplasmic relocation could contribute to the loss of p27Kip1 tumor suppressor function.

PC21 and PC22 are MEN2A-associated tumors arising in patients who carry a germline mutation in the *RET* protooncogene. It has been reported that younger age of onset, multifocality of the tumors, and the presence of extra-adrenal tumors is parameters usually associated with a genetic predisposition to pheochromocytoma [32]. Thus, cases PC3 (32 years of age) and PC11 (41 years) could also be syndromic pheochromocytomas. Interestingly, three of the above cases (PC3, PC11, PC22) showed reduction/loss of p27Kip1. This observation warrants further studies in a larger cohort of syndromic pheochromocytomas to determine whether p27Kip1 inactivation may occur at a higher incidence in hereditary cases.

The biological consequences of the downregulation of p27Kip1 in pheochromocytoma are not yet known. Except one, all the tumors we analyzed have relatively low proliferative activity (Ki67 immunoreactivity, <5%) irrespective of the p27Kip1 expression level. The fact that downregulation of p27 does not cause massive increase in cell proliferation is in agreement with the phenotype of MENX-affected rats. These animals display increase in overall body size and weight compared to their normal littermates and show a remarkable increase in the size of some organs [34], but the proliferative activity of most tissues (including neuroendocrine tissues) is similar to that observed in unaffected animals (Pellegata, unpublished). It is, therefore, likely that impaired p27Kip1 function may affect not only proliferation but additional cellular processes required to maintain tissue homeostasis (such as differentiation and senescence).

Our observation that reduction of p27Kip1 expression in pheochromocytoma does not increase tumor cell proliferation is in contrast to what has been reported for other tumor types (including medulloblastomas, endocrine tumors of the pancreas, and gastrointestinal tract and most lymphoma subtypes) in which immunoreactivities for p27Kip1 and Ki67 are inversely correlated [1, 9, 13]. When an inverse relationship exists, it is not easy to discern whether the low p27Kip1 expression is the pathological cause or the physiological consequence of increased proliferative activity. In our cases, the decreased p27Kip1 concentration is not the result of an increased proliferation of the tumor cells, but it is due to an altered regulation of the protein's expression. Studies performed in mice have shown that neuroendocrine cells are especially sensitive to defects in cell-cycle regulatory proteins affecting the Rb pathway. In fact, mice heterozygous for Rb [22, 26], chimeric for Rb −/− [41], and deficient for the Cdk-inhibitors p18Ink4c and p27Kip1 develop tumors with high penetrance in the pituitary gland, pancreatic islets, and thyroid [20]. Hyperplasia of the adrenal medulla also develops in mice chimeric for Rb −/− [41]. Our results on the frequent reduction of p27Kip1 in human adrenal medullary tumors further support the hypothesis that this protein plays a role in regulating endocrine tissue homeostasis, not necessarily via controlling cell proliferation.

While the molecular mechanisms underlying the development of hereditary pheochromocytoma and paraganglioma

have been largely elucidated, advances in understanding the molecular pathogenesis of their sporadic counterparts has lagged behind. Somatic mutations in the six known pheochromocytoma/paraganglioma susceptibility genes occur at relatively low incidence (10–25%) in sporadic tumors [5, 7]. Candidate gene approaches have identified tumor suppressor genes inactivated in pheochromocytomas, such as the RAS-association domain family 1, isoform A (*RASSF1A*), and the p16 tumor suppressor genes. *RASSF1A* [2, 4] and p16 [17] were found to be down regulated through promoter region hypermethylation in 22–48% and in 24% of pheochromocytomas, respectively. Interestingly, the incidence of p16 and of *RASSF1A* inactivation was higher in hereditary pheochromocytomas compared to sporadic cases [2, 4, 17]. Recently, genome-wide approaches have been used to identify molecular pathways involved in sporadic and hereditary pheochromocytomas and paragangliomas development [15, 16, 18]. These studies have not yet provided us with individual candidate genes to further analyze. The limited knowledge of the molecular pathogenesis of sporadic pheochromocytomas and paragangliomas has hampered the identification of predictors of malignancy and the development of novel therapeutic approaches to target specific dysregulated pathways. In view of the newly established role of p27Kip1 downregulation in pheochromocytomas, cell cycle regulatory pathways can now be further explored in this tumor type, as well as new therapeutic modalities able to restore p27Kip1 expression (function) in adrenal medullary tumor cells.

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ORIGINAL ARTICLE

Submucosal connective tissue-type mast cells contribute to the production of lysophosphatidic acid (LPA) in the gastrointestinal tract through the secretion of autotaxin (ATX)/lysophospholipase D (lysoPLD)

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Abstract Lysophosphatidic acid (LPA) is involved in a broad spectrum of biological activities, including wound healing and cancer metastasis. Autotaxin (ATX), originally isolated from a melanoma supernatant as a tumor cell motility-stimulating factor, has been shown to be molecularly identical to lysophospholipase D (lysoPLD), which is the main enzyme in the production of LPA. Although ATX/lysoPLD is known to be widely expressed in normal human tissues, the exact distribution of ATX-producing cells has not been fully investigated. In this study, we evaluated ATX/lysoPLD expression by immunohistochemical staining using a rat anti-ATX mAb in the human gastrointestinal tract and found that submucosal mast cells (MC) highly expressed this enzyme. This was confirmed by immunofluo rescent double staining using mAbs to tryptase and chymase. Then, we isolated MC from human gastric tissue by an immunomagnetic method using CD117-microbeads and showed that a subpopulation of CD203c-positive MC showed positive staining for intracellular ATX/lysoPLD on flowcytometry. This was confirmed by Western blotting of the isolated cells. Moreover, a significant level of ATX/lysoPLD release could be detected in the culture supernatants of human MC by Western blot analysis. Our data suggest that submucosal MC play significant roles in various aspects of pathophysiology in the gastrointestinal tract by locally providing bioactive LPA through the production of ATX/lysoPLD.

Keywords Autotaxin · Lysophpsphatidic acid · Mast cell · Gastrointestinal tract

Abbreviations

ATX	autotaxin
lysoPLD	lysophospholipase D
LPA	lysophosphatidic acid
MC	mast cell
NPP	ecto-nucleotide pyrophosphatase / phosphodiesterase

Introduction

Autotaxin (ATX) was originally isolated from a melanoma cell supernatant as a 125-kD glycoprotein that stimulates tumor cell motility in a pertussis toxin-sensitive manner [55, 57]. Thereafter, more evidence for a positive role of ATX in tumor metastasis has been demonstrated by both in vivo and in vitro studies [36–38, 56, 70]. ATX is strongly detected in the culture media of various cancer cells, and its mRNA is overexpressed in various human malignancies, such as lung, breast, and renal cell carcinoma [53, 70, 71]. These findings support that ATX functions as an autocrine motility factor in the step of tumor progression. ATX was initially identified as a member of the ecto-nucleotide

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pyrophosphatase/phosphodiesterase (NPP) family and was termed NPP2 [56]. The NPP family is composed of three ecto-enzymes that hydrolyze phosphodiester and pyrophosphate bonds in nucleotides [9, 18]; however, its molecular structure relating to stimulation of cell motility is an enigma. On the other hand, lysophospholipase D (lysoPLD), which converts lysophosphatidylcoline (LPC) to lysophosphatidic acid (LPA, 1- or 2-acyl-*sn*-glycero-3-phosphate), was found to be abundant in plasma and serum [3, 62]. LPA is a lipid mediator of a broad range of cellular responses, including smooth muscle contraction, platelet aggregation, neurite retraction/cell rounding, regulation of proliferation, protection from apoptosis, and modulation of chemotaxis and transcellular migration [19, 35]. LPA elicits most of its cellular responses via transduction cascades downstream of its specific G protein-coupled receptors, LPA1/Edg-2, LPA2/Edg-4, and LPA3/Ede-7, which belong to the endothelial cell differentiation gene (Edg) family [2, 4, 11, 24]. Some of these cellular responses implicate LPA as a mediator of tumor progression [31, 34, 50–52]. Recently, molecular cloning of lysoPLD revealed that this enzyme was identical to ATX [63, 66]. As LPC is abundantly present in plasma and tissues (exceeding 100 μM), ATX/LysoPLD is considered to be a key enzyme in the production of LPA in vivo [3, 12, 61]. Therefore, it is generally accepted that the effects of ATX/LysoPLD in cancer progression (including proliferation, migration, and angiogenesis) are mostly attributable to the production of LPA from LPC or more complex lysophospholipids [66, 67]. Although the mechanism of catalysis of ATX/lysoPLD is well characterized, its post-translational processing, regulation of expression, and mechanism of release from cells are not well understood. ATX is widely expressed, with highest mRNA levels detected in brain, placenta, intestine, and ovary [36]. However, the cellular expression of ATX/lysoPLD in each tissue remains unclear. Herein, by immunohistochemical study using a specific monoclonal antibody, we assessed the cellular distribution of ATX/LysoPLD in the human gastrointestinal tract, and found that submucosal mast cells (MC) constitutively express ATX/lysoPLD.

Materials and methods

Reagents and human samples

Rat anti-ATX/lysoPLD monoclonal antibodies (2A12, 4F1) were generated by the immunization of rat with a polypeptide (amino acids 58–182 of human autotaxin) at the Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan. The specificity [60] and immunoreactivity for tissue section of these mAbs were evaluated previously [5, 46]. Antihuman tryptase mAb (AA1) and antihuman chymase mAb (CC1) were purchased from Dako (Carpinteria, CA) and Serotic (Oxford, UK), respectively, and both of their subclasses were IgG1.

Surgical specimens were obtained from seven patients with primary gastric carcinoma treated by total gastrectomy and seven patients with primary colon cancer treated by right hemicolectomy in the University of Tokyo, with written informed consent. Normal parts of the stomach or colon in those specimens, more than 5 cm from the tumor edge, were used for staining experiments. The ileum of the oral margin of right hemicolectomy specimens and the jejunal margin used for reconstruction after gastrectomy was used for evaluation of the small intestine.

Immunohistochemical study

Paraffin-embedded sections, 5 μm thick, were deparaffinized in xylene, hydrated through a graded series of ethanol, then immersed in 3% hydrogen peroxide in 100% methanol for 30 min to inhibit endogenous peroxidase activity. To activate the antigens, the sections were boiled in 10 mM citrate buffer pH 6.0 for 15 min. After being rinsed in phosphate-buffered saline (PBS), the sections were incubated with normal rabbit serum for 30 min and incubated overnight at 4°C in humid chambers with primary antibodies to tryptase (AA1, dilution 1:100), chymase (CC1, dilution 1:100), or ATX/lysoPLD (2A12, dilution 1:50). After washing with PBS, the sections were incubated with biotinylated rabbit anti-mouse immunoglobulin (Nichirei, Tokyo, Japan) for 20 min. After washing again with PBS, the slides were treated with peroxidase-conjugated streptavidin for 10 min and developed by immersion in 0.01% H_2O_2 and 0.05% diaminobenzidine tetrahydrochloride. Light counterstaining with Mayer's hematoxylin was performed. All cases had a negative control that was run simultaneously with the test slide, in which control rat IgG was used as the primary antibody. Tryptase-, chymase-, and ATX/LysoPLD-positive cells were counted in five different fields selected at random, using a microscope (and expressed as number per ×200 field).

Immunofluorescent double staining

To identify the two molecules in the same specimen, immunofluorescent double staining was performed on frozen sections. The surgically removed specimen was immediately fixed with acetone for 10 min and immersed in 3% hydrogen peroxide in 100% methanol for 30 min. Then, sections were incubated with normal rabbit serum for 30 min and incubated overnight at 4°C in shaded humid chambers with primary antibodies to tryptase or chymase. After washing with PBS, sections were incubated for 2 h with rhodamine-conjugated rabbit anti-mouse IgG (Chemicon International, Temecula, CA). Then, sections were incubated overnight with rat anti-

ATX antibody chemically conjugated with fluorescein isothiocyanate (FITC) (FITC-conjugated 4F1, dilution 1:10). The sections were observed with a confocal laser microscope (Fluoview, Olympus, Tokyo, Japan).

Buffer for cell preparation

Tris–ethylenediaminetetraacetic acid (EDTA) (TE) buffer is Eagle's minimum essential medium (MEM, Gibco, Berlin, Germany) containing 2 mM EDTA. TGMD buffer is TE buffer supplemented with 1 mg/ml gelatin, 1.23 mM $MgCl_2$, and 15 μg/ml DNAse. Hepes buffer contains 20 mM Hepes, 125 mM NaCl, 5 mM KCl, and 0.5 mM glucose. HA buffer is Hepes buffer plus 0.25 mg/ml bovine serum albumin (BSA).

Cell preparation

The normal parts of the gastric wall were resected, and tissues containing the mucosal and submucosal layers were separated from the muscularis propria layer using scissors and used for cell separation. MC were isolated by a four-step enzymatic tissue dispersion method as described previously with some modifications [7, 8, 49]. The tissues were cut into fragments with scissors and incubated in TE buffer containing 1 mg/ml acetylcysteine for 10 min at room temperature to remove mucus and then in TE buffer containing 5 mM EDTA for 15 min at 37°C to detach epithelial cells. After washing in TE buffer, the tissue was incubated in TE buffer containing 3 mg/ml pronase and 0.75 mg/ml chymopapain at room temperature. Pronase and chymopapain were purchased from Roche (Mannheim, Germany). During this first digestion step, the tissue was cut into smaller pieces using scalpels. After 30 min, the free cells (fraction 1) were separated from tissue fragments by filtration through a polyamide Nybolt filter (pore size 300 μm). The remaining tissue fragments were washed in TE buffer, and the first digestion step was repeated at 37°C (fraction 2). The tissue fragments were then washed in TGMD buffer and incubated twice for 30 min at 37°C in TGMD buffer containing 1.5 mg/ml collagenase D (Roche, Mannheim, Germany) and 0.15 mg/ml elastase (Roche, Mannheim, Germany). The freed cells were separated from the digested tissue by filtration (fraction 3 and 4). Fraction 3 and 4 were filtered through a Nyblot filter (pore size 100 μm) again and resuspended in HA buffer. Cell viability was measured using trypan blue staining, and MC counts were performed using Alcian blue at each step.

Mast cell purification

Dispersed cells containing approximately 1% MC were purified as described previously with some modifications [7, 39, 49]. In brief, the dispersed cells were incubated with anti-CD117 mAb-conjugated microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) for 15 min at 4°C. Then, CD117-positive cells were enriched by passing through a magnetic column twice at 1 ml/min using the "POSSEL DS" program of the AutoMACS system which enables the fast and efficient positive selection of rare population with low immunogenic epitope (Miltenyi Biotec, Bergisch Gladbach, Germany). "POSSEL DS" means "double sensitive positive selection".

The purified MC were dispersed on slides by centrifugation for 10 min (×500 rpm) and fixed with acetone. Then, the slide samples were incubated with biotinylated anti-tryptase (AA1) or anti-chymase (CC1) mAbs followed by incubation with peroxidase-conjugated streptavidin and development by diaminobenzidine tetrahydrochloride as described above. Under the microscope, 100 cells were randomly selected in five to seven different fields, and cells positive for tryptase or chymase in those cell populations were calculated (per ×200 field).

Detection of ATX/lysoPLD by flow cytometry

Dispersed cells obtained after the four enzymatic steps were washed twice with PBS and fixed with acetone for 10 min at 4°C. Then they were permeabilized with 4% paraformaldehyde (PFA) for 10 min at 4°C. For two-color staining, cells were incubated with FITC-conjugated anti-ATX mAb and PE-conjugated anti-CD203c antibody (Immunotech, Marseille, France). Data were collected in a fluorescent activated cell sorting (FACS) Calibur (Becton Dickinson, Mountain View, CA) and analyzed using CellQuest software (Becton Dickinson).

Detection of ATX/lysoPLD by Western blot analysis

Immunoselected MC (2×10^6), which contained more than 70% Alcian blue-stained cells, were cultured in 8 ml Roswell Park Memorial Institute (RPMI) supplemented with 0.1% BSA, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc., Grand Island, NY). As a control, the same numbers of negatively selected cells were also cultured in the same medium. After culture for 48 h, the proteins of the culture supernatants were extracted as described previously [66]. Then, 8 ml culture supernatant was concentrated to 100 μl by a Centricon Plus-20 (Millipore, Bedford, MA), and electrophoresed in sodium dodecyl sulfate (sodium dodecyl sulfate (SDS)) 7.5% polyacrylamide gel for 45 min at 200 V. Then, the protein was transferred onto an Immobilon transfer membrane (Millipore, Bedford, MA) for sequential incubation with 5% reconstituted nonfat milk powder to block nonspecific sites, dilutions of anti-ATX antibody, and horseradish peroxidase-labeled rabbit polyclonal anti-rat IgG, before development with a standard enhanced chemiluminenscence kit (Amersham, Inc., Buckinghamshire, United Kingdom).

Results

Immunostaining of MC in the gastrointestinal tract

MC were clearly identified in the stomach, small intestine, and colon by immunostaining with anti-tryptase antibody (AA1), as reported previously [27, 69]. Granules in the MC cytoplasm were stained brown. In the stomach, tryptase-positive MC were diffusely present from the mucosal to the muscular layer, but the number of MC in the submucosal layer was significantly greater than that in the mucosal or muscular layer (Fig. 1b). MC were also diffusely present in the small intestinal and colonic tissues (Fig. 1d).

Immunodetection of ATX/lysoPLD in the gastrointestinal tract

As shown in Fig. 1, there were many large oval cells whose cytoplasm was clearly stained by anti-ATX/lysoPLD antibody (2A12). The positive cells were detected in the mesenchymal area in the submucosal layer of the gastrointestinal tract, while they were rarely observed in the mesenchymal area of the mucosal or muscular layers (Table 1). Interestingly, in serial sections, most of the ATX-positive cells were morphologically similar to MC detected by anti-tryptase antibody (Fig. 1e,f).

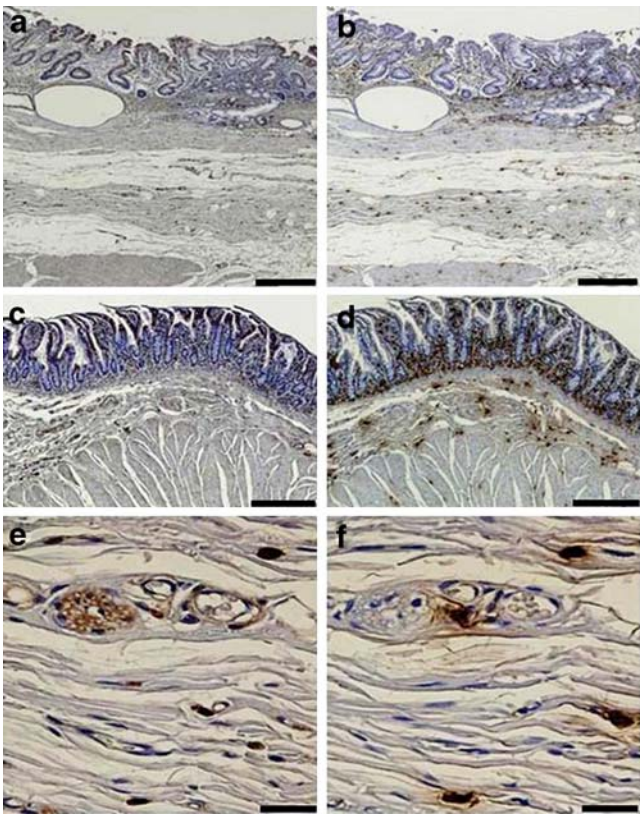

Fig. 1 Immunohistochemical staining of the stomach (**a**, **b**, **e**, **f**) or terminal ileum (**c**, **d**) with anti-ATX/lysoPLD mAb (2A12) (**a**, **c**, **e**) or anti-tryptase mAb (AA1) (**b**, **d**, **f**). Serial sections of submucosal area of the stomach with high magnification (**e**, **f**). *Bar* indicates 1,000 μm (**a**, **b**), 500 μm (**c**, **d**), and 50 μm (**e**, **f**). Immunoreactivity for ATX/lysoPLD was clearly detected in a subpopulation of tryptase positive cells

ATX/lysoPLD-positive cells were identified as a subtype of MC

To confirm that ATX/lysoPLD-positive cells are identical to MC, we performed immunofluorescent double staining in surgically removed gastric tissue. As shown in Fig. 2, when tryptase-positive MC were stained red and ATX/LysoPLD-positive MC green, ATX/LysoPLD-positive cells were detected with yellow fluorescence in merged views (Fig. 2c). On the other hand, some MC were detected as red in merged views, suggesting that some MC did not express ATX/LysoPLD (Fig. 2f). No cells were stained green in these frozen sections. This clearly indicates that the cells expressing ATX/LysoPLD in merged views are confined to a subpopulation of MC in the gastrointestinal tract.

Then, we performed double immunofluorescent staining with anti-chymase antibody (CC1). Most of the ATX/lysoPLD-positive MC were stained yellow in the merged view (Fig. 2i). When the number of ATX/lysoPLD-positive MC in tryptase- or chymase-positive MC was counted in 20 fields selected at random, 92% (82–96%) of chymase-positive cells expressed ATX/lysoPLD, whereas 68% (61–86%) of tryptase-positive cells expressed ATX/LysoPLD (Table 2). A previous study on human intestine showed that tryptase-positive MC exist in both the mucosal and submucosal layers, while most chymase-positive MC exist in the submucosal layer [27]. This appears to be consistent with our result that most of the MC in submucosal, but not mucosal layers, highly expressed ATX/lysoPLD.

Proportion of ATX/lysoPLD-positive MC in the gastrointestinal tracts

The proportion of ATX/lysoPLD-positive cells in tryptase-positive MC was comparatively evaluated in the stomach, intestine, and colon. As shown in Table 1, more than 50% of MC in the submucosal layer expressed ATX/lysoPLD in all organs examined (stomach: 52%, small intestine: 63%, colon: 56%). In contrast, only a small proportion of MC in the mucosal or muscular layer expressed ATX/lysoPLD (stomach: 7.1%, 8%, small intestine: 6.7%, 8%, colon: 14%, 4.1%).

Detection of intracellular ATX/lysoPLD in MC isolated from gut tissue

Enzymatic isolation provided at least 2×10^8 single cells containing about 1% MC, and their viability was at least 90%. After positive selection using magnetic affinity purification with anti-CD117, the majority of the cells were

Table 1 Distribution of tryptase and autotaxin (ATX/lysoPLD)-positive cells in each layer of gastrointestinal tract

Tryptase- and ATX/LysoPLD-positive cells were counted in five different fields using a microscope (×200 field), and the mean (min–max) of seven different samples were expressed.

Parts of the gastrointestinal tract	Layer	Tryptase(+)	ATX(+)	ATX(+)/Tryptase(+)
Stomach	m	16 (13–27)	1 (0–5)	7.1% (0–27%)
	sm	40 (17–77)	20 (3–35)	52% (18–69%)
	mp	56 (6–94)	4 (0–6)	8% (0–17%)
Small intestine	m	33 (10–54)	2 (1–4)	6.7% (2.7–10%)
	sm	41 (16–48)	26 (10–30)	63% (32–95%)
	mp	18 (3–35)	2 (0–6)	8% (0–50%)
Colon	m	20 (18–45)	3 (1–5)	14% (4–18%)
	sm	27 (18–29)	16 (14–36)	56% (47–90%)
	mp	15 (2–28)	1 (0–4)	4.1% (0–27%)

stained blue by Alcian blue (Fig. 3a) and anti-tryptase mAb (Fig. 3b). In contrast, cells stained with anti-chymase mAb were fewer than tryptase-positive cells (Fig. 3b). Then, to detect intracellular expression of ATX/lysoPLD, we performed double staining of the dispersed cells with anti-ATX/lysoPLD and anti-CD203c, which was determined as a specific cell surface antigen of human MC and basophils [17]. FACS analysis showed that more than 70% of the cell population positively expressed CD203c (Fig. 3c). When the CD203c-positive population was gated, 22% (13–27%) of CD-203c positive cells were clearly positive for intracellular ATX/lysoPLD, whereas the CD203c-negative population lacked expression of ATX/lysoPLD (Fig. 3d).

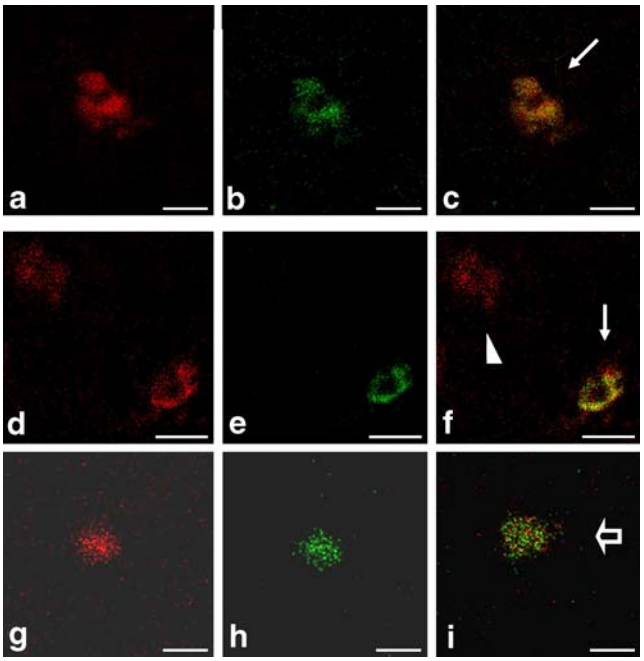

Fig. 2 Immunofluorescent double staining with anti-tryptase (AA1), anti-chymase (CC1), and anti-ATX/lysoPLD (4F1) mAbs. A frozen section of normal gastric tissue was stained as described in the Materials and Methods section. The section was observed with a confocal laser microscope (Fluoview, Olympus, Tokyo, Japan). The *bar* indicates 10 μm **(a–f)**. Double staining for tryptase in *red* (**a**, **d**) and ATX/lysoPLD (**b**, **e**) in *green*. In the merged view, a double-positive cell is stained *yellow*, indicating that the cell expresses ATX/lysoPLD and tryptase (*white arrows* in **c**, **f**). In contrast, a cell stained *red* in the merged view expresses tryptase but not ATX/lysoPLD (*white arrow head* in **f**). No cells are stained green in the merged view, indicating that ATX/lysoPLD-producing cells are restricted to a subtype of MC. **(g–i)** Double staining for chymase in *red* (**g**) and ATX/lysoPLD (**h**) in *green*. In the merged view, most of the cells are stained *yellow* [*open arrow* in (**i**)], indicating that most chymase-positive MC coexpress ATX/lysoPLD

Expression of tryptase or chymase in cell population isolated from gut tissue

As shown in Fig. 3b, 82% of the cells were positive for tryptase, while only 17% MC were positive for chymase (Table 3). This result is consistent with Gebhardt's report, showing that about 20% of purified MC expressed both tryptase and chymase [16]. From these results, it is supposed that the cell isolates from gut tissue by this method contain relatively more mucosal MC than submucosal MC.

Detection of the release of ATX/lysoPLD from MC by Western blot analysis

Finally, we examined the protein expression of ATX/lysoPLD and tryptase and chymase in MC-rich population.

Table 2 Proportion of ATX/lysoPLD-positive cells in tryptase- or chymase-positive cells

Cells	ATX/lysoPLD-positive cells	ATX/lysoPLD-negative cell	Percentage of ATX/lysoPLD-positive cells
Tryptase-positive cells	30 (27–44)	16 (5–19)	68% (61–86%)
Chymase-positive cells	26 (23–38)	2 (1–5)	92% (82–96%)

Frozen section of normal gastric or intestinal tissues were immunostained red by anti-tryptase or anti-chymase mAbs and green, by anti-ATX/lysoPLD mAbs. Positive cells were counted in 20 different fields, and the mean (min–max) in five different samples were expressed.

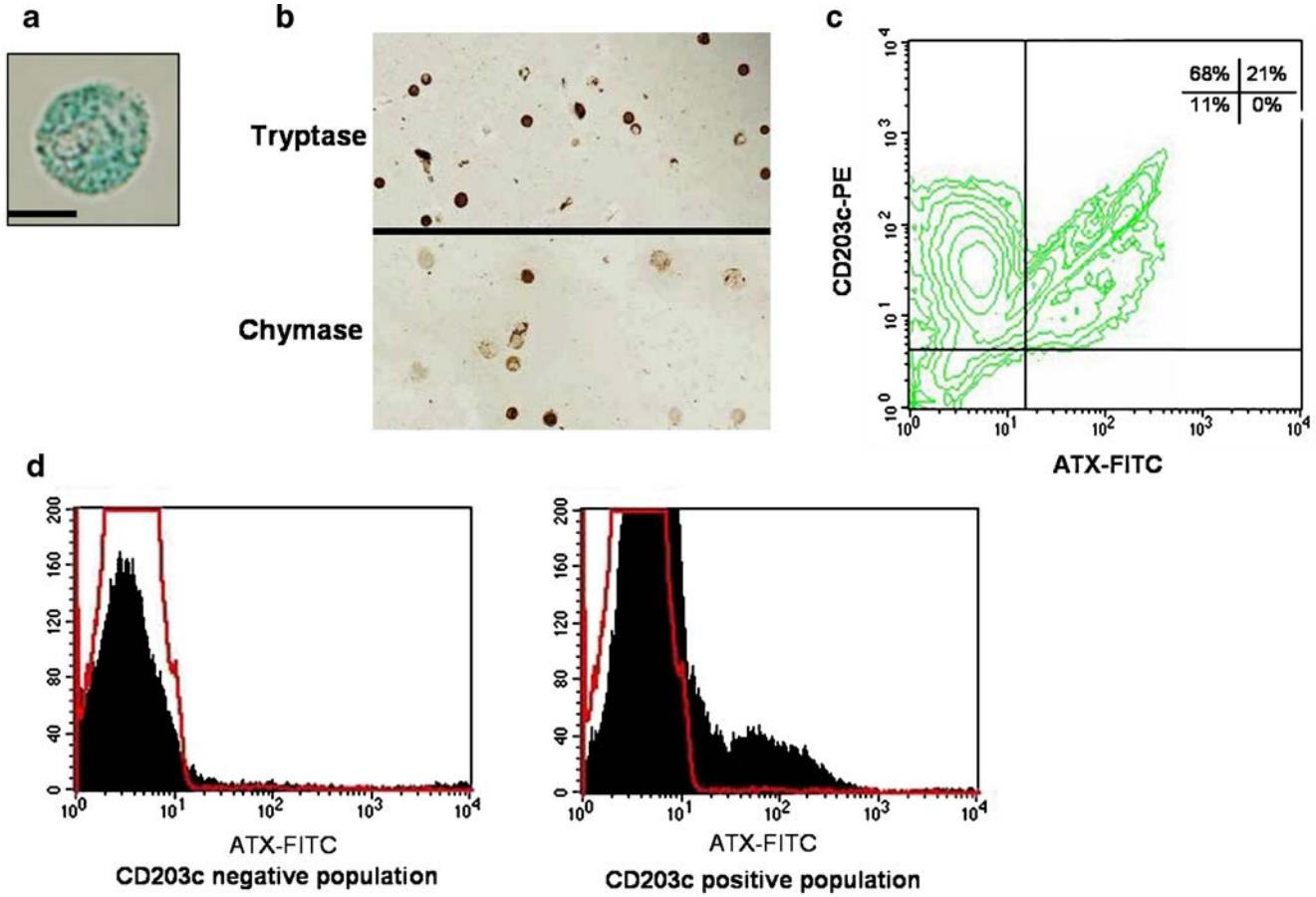

Fig. 3 Characterization of MC isolated from gastrointestinal tissue. The cells recovered after enzymatic digestion of gastrointestinal mucosal tissue were positively selected using anti-CD117 mAb. **a** A representative MC stained by Alcian blue (**b**); Immunochemical staining with anti-tryptase (*upper*) and anti-chymase (*lower*). The majority of cells are positive for tryptase, but not for chymase. **b** Flowcytometric profile of double staining by CD203c and ATX/lysoPLD. The MC-enriched fraction was fixed, permeabilized, and double stained with PE-conjugated anti-CD203c mAb and FITC-conjugated anti-ATX/lysoPLD mAb (**c**). The CD203c-negative (*left*) and -positive (*right*) areas were gated, and the FITC level was examined in each gated fraction. The *red line* represents negative control. The figure shows the representative data from four different experiments (Table 4)

Positively selected cells expressed ATX/lysoPLD, whereas negatively selected cells did not (Fig. 4a). As shown in Fig. 4a, tryptase and chymase were also detectable in cell lysates of MC-rich population.

Then, we evaluated whether MC extracellularly secrete ATX/lysoPLD. As shown in Fig. 4b, ATX/lysoPLD protein was detected in the concentrated culture supernatant of MC-enriched population cells isolated from gastric tissue, but not in the supernatant of negatively selected cell populations. This finding indicates that a subpopulation of the human MC in gastric tissue release soluble ATX/lysoPLD into the culture medium.

Discussion

LPA is involved in the etiology of a variety of diseases such as atherosclerosis, obesity, and cancer and in physiological responses such as wound healing, vascular tone, vascular integrity, and reproduction [34, 48]. However, the molecular mechanism of the production of this bioactive phospholipid is poorly understood, although a large amount of LPA is known to be produced by activated platelets or stimulated fibroblasts in pathological conditions. Recently, it has been postulated that at least two pathways exist for LPA production, intracellular synthesis from phosphatidic acid and

Table 3 Distribution of tryptase- and chymase-positive MC in the immunomagnetically purified population

Tryptase (+)	Chymase (+)	Chymase (+)/Tryptase(+)
82 (79–90)	17 (12–25)	21% (13–29%)

The purified MC were stained with avidin–biotin peroxidase method using biotinylated anti-tryptase or anti-chymase mAbs. Under the microscope, a hundred cells were randomly selected in five to seven different fields and positive cells for tryptase or chymase in those cell populations were calculated. Mean (min–max) in five different samples were expressed (Fig. 3b).

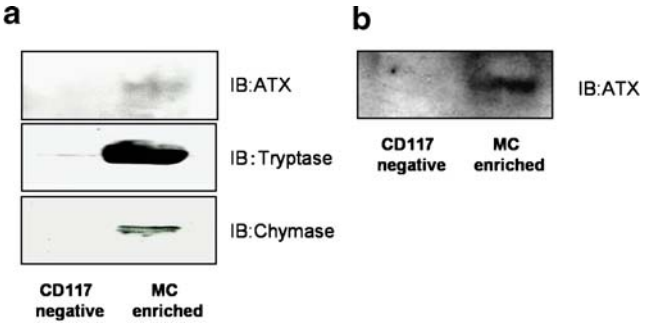

Fig. 4 **a** Western blotting of lysates of MC-enriched cell population from gastrointestinal tract for ATX/lysoPLD, tryptase, and chymase. **b** Detection of release of ATX/lysoPLD from MC-enriched population. MC-enriched cells (2×10^6) were cultured. Then, the supernatant was concentrated and the protein expression of ATX/lysoPLD was evaluated by Western blotting. For negative control, negatively selected cells with anti-CD117 mAb were cultured in the same conditions, and the culture supernatant was assessed for the presence of ATX/lysoPLD. The figure shows representative data from three different experiments

extracellular conversion from other lysophospholipids. In the latter pathway, ATX/lysoPLD is shown to be a key enzyme in the production of LPA by the catalysis of LPC, which is known to be abundantly present in plasma and tissue fluids [3, 64, 66]. In this study, we found a subtype of MC in the human gastrointestinal tract that showed strong staining for ATX/lysoPLD. This reports characterized the cellular producer of ATX/lysoPLD in normal tissue, while a recent study has demonstrated that ATX/LysoPLD is overexpressed in Epstein–Barr virus-infected Hodgkin's lymphoma cells [5]. That study used the same antibody as us and showed similar cytosolic staining of ATX/LysoPLD in lymphoma cells, suggesting that ATX/lysoPLD is stored in the cytosol in contrast to the other NPP family proteins, which are predominantly distributed in the plasma membrane [54]. By Western blotting of culture supernatants, we confirmed the secretion of ATX/lysoPLD from the MC-enriched fraction. These data clearly indicate that those MC undergo membrane-proximal cleavage to yield soluble ATX/lysoPLD, and thus, have important physiological roles through the local supply of a high concentration of LPA in gastrointestinal mucosal tissue.

Tissue MC are known to derive from specific bone marrow progenitor cells and migrate into tissues, where they mature depending on the microenvironment and acquire the capability to produce a wide range of mediators including histamine, heparin, prostaglandins, leukotrienes, and many inflammatory cytokines such as interleukin-8, vascular endothelial cell growth factor (VEGF), and platelet-derived growth factor (PDGF), and exert their biological effects by releasing such mediators [6, 15, 21, 26, 30]. In rodents, MC are phenotypically divided into two subtypes, mucosal-type MC (MMC) and connective tissue-type MC (CTMC), that produce distinct types of mediators. MC in the human gastrointestinal tract are also subdivided by immunohistochemical detection of two proteases; CTMC that contain tryptase and chymase, and MMC containing tryptase but not chymase [1, 27–29]. Those studies have shown that MMC are preferentially located in the mucosal surface, while CTMC are detected mainly in submucosal tissue in the gut. Our immunostaining results revealed that MC in the submucosal layer highly expressed ATX/lysoPLD, but MC located in the mucosal and muscular layers did not. Moreover, double staining experiments showed that most of the chymase-positive MC also expressed ATX/lysoPLD, while about half of the tryptase-positive cells lacked the expression of ATX/lysoPLD. This is consistent with previous findings and indicates that ATX/lysoPLD is constitutively produced mainly by submucosal CTMC in the gastrointestinal tract.

Using flowcytometry, we confirmed that intracellular staining of ATX/lysoPLD was detected by CD203c-positive MC isolated from gut tissue. In FACS analysis, however, only 10–20% CD203c-positive MC are positive for ATX / lysoPLD, which does not agree with the immunostaining data indicating that most of the submucosal MC are positive for ATX /lysoPLD (Table 4). In fact, cells separated by this immunomagnetic method contained many tryptase-positive MC but fewer chymase-positive MC. This suggests that submucosal MC are collected less efficiently than mucosal MC by this isolation process, which may contribute to the discrepancy between FACS analysis and immunohistochemistry.

Table 4 Percentages of each quadrant for different four FACS plots

Quadrant	Ex.1 (%)	Ex.2 (%)	Ex.3 (%)	Ex.4 (%)	Mean (%)	(Max–min) (%)
Left upper	68	66	60	55	62	(55–68)
Right upper	21	10	22	17	18	(10–22)
Left lower	11	24	18	28	20	(11–28)
Right lower	0	0	0	0	0	0

The MC-enriched fraction was fixed, permeabilized and double stained with PE-conjugated anti-CD203c mAb and FITC-conjugated anti-ATX/lysoPLD mAb. Percentages of each quadrant for different four FACS plots were shown.

The expression of ATX/lysoPLD in submucosal MC is considered to have physiological relevance. MC are essential for allergic and late phase reactions and T-cell-mediated immunity [14, 33, 42]. LPA has been reported to lead to overexpression of adhesion molecules and chemokines in endothelial cells, and thus, induces the recruitment of mononuclear phagocytic cells [40, 45]. For helper T cells, LPA has been shown to induce chemotactic migration of naïve CD4(+) T cells expressing the specific receptor Edg4 (LPA2), while enhancing proliferation and IL-2 production in activated CD4(+) T cells expressing Edg2 (LPA1) predominantly, but rather, inhibit the migration of these activated CD4 (+) T cells [20, 73]. This strongly suggests that the production of ATX/lysoPLD in submucosal MC has an important role in preparation of CD4(+) helper T cells' repertoire to respond to a variety of antigens in gastrointestinal mucosal tissue. Once T cells are stimulated with certain antigens, ATX/lysoPLD can augment T cell-mediated responses in mucosal tissue through the production of LPA.

On the other hand, LPA is considered to have suppressive effects on neutrophil-induced inflammation in vivo. LPA reduces IL-8-induced migration of human neutrophils in patients with pneumonia and inhibits the production of oxygen species in human neutrophils in response to stimulation with PMA [10, 43]. In the gastrointestinal tract, LPA reduces the degree of colonic inflammation induced by ethanol and trinitrobenzene sulfonic acid in a rat colitis model in vivo [59]. These findings suggest the possibility that ATX/lysoPLD released from submucosal MC plays a protective role against acute phase inflammatory bowel disease (IBD) [23]. Because the number of MC is markedly increased in IBD, it would be intriguing to examine the production of ATX/lysoPLD in MC in the intestinal mucosa in patients with IBD. In addition, recent studies have demonstrated that LPA also activates enteric glia cells, which are considered to play critical immunoregulatory roles in gut tissue [47, 68]. Taken together, these findings suggest that MC-derived ATX/lysoPLD has pivotal effects on mucosal immunity in the gastrointestinal tract.

LPA is also essential in wound healing. When superficial mucosal injury occurs, the mucosal defect is rapidly closed through a process termed epithelial restitution in physiological conditions [25, 58]. LPA is considered to play major roles in this step, as it strongly promotes epithelial cell migration and proliferation in vitro [41]. In fact, rectally applied LPA stimulates wound healing of the intestinal epithelium and reduces the size of ulcers in the rat [58]. Therefore, the production of ATX/lysoPLD in gastrointestinal CTMC is considered to be necessary for the maintenance of mucosal barrier function, as they can constitutively provide a considerable amount of LPA in the submucosal area, and thus, epithelial cells can rapidly respond to repair the mucosal injury.

Finally, ATX/lysoPLD has an essential role in the progression of malignant diseases in the gastrointestinal tract. Many studies have provided evidence for positive roles of LPA in the initiation or progression of malignancy, including melanoma, ovary, prostate, breast, head and neck, colon, and stomach cancer [13, 22, 72]. This is reasonable, because LPA can function as a growth factor for cancer cells and also stimulates angiogenesis [34]. More than 40 years ago, it has been reported that a large number of MC accumulate around solid tumors and a large number of tumor-associated MC were significantly correlated with high vascular density and poor outcome [32, 44, 65]. In those studies, MC-derived secretalogues, such as histamine, heparin, or VEGF are considered to be responsible for the new vessel formation. However, our results strongly support additional mechanisms of MC to promote the malignant potential of cancer through the production of ATX/lysoPLD.

In summary, we discovered that a subpopulation of submucosal CTMC constitutively secrete ATX/lysoPLD. MC are considered to play important roles in the pathophysiology of gastrointestinal diseases, such as allergic enteritis, inflammatory bowel disease and malignancy. The effects of MC may be partly attributable to ATX/lysoPLD by providing LPA in gastrointestinal mucosal tissue, although circulating ATX/lysoPLD synthesized in different locales may also modulate LPA levels. As ATX/lysoPLD is a relatively stable protein compared to the bioactive phospholipid product, further research on this enzyme may be useful for the evaluation of functional role.

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ORIGINAL ARTICLE

Unexpected diagnosis of cystic fibrosis at liver biopsy: a report of four pediatric cases

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Abstract We report here four cases of pediatric patients in whom the diagnosis of cystic fibrosis was made only after the histological examination of a liver specimen obtained by biopsy (three cases) or at autopsy (one case). There were two boys and two girls, aged 13 months to 7.5 years. None had a personal or familial history suggestive of cystic fibrosis. One patient, presenting with myocardial lesion and hepatomegaly, died of heart failure; at autopsy, the liver showed a typical aspect of focal biliary cirrhosis. In the three other cases, liver disease was the only manifestation of cystic fibrosis at the time of diagnosis. Liver biopsy examination showed focal biliary cirrhosis in one case and massive steatosis in two. In all four cases, the diagnosis was confirmed by the existence of known pathogenic mutations in the *CFTR* gene. The evolution was variable; one patient had progressive liver disease with severe portal hypertension after 7 years; another one had lung complications after 1 year. In conclusion, our experience recalls that the diagnosis of cystic fibrosis must be considered in children presenting with unexplained liver disease; its confirmation by molecular techniques makes it possible to set up an appropriate follow-up.

Keywords Cystic fibrosis · Liver · Focal biliary cirrhosis · Steatosis

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Introduction

Cystic fibrosis (CF), with a prevalence of 1/2,500 newborns, is the most frequent lethal genetic disease among whites [9, 26]. The disease is caused by mutations in the *CTFR* gene, which maps on chromosome 7 (7q31) [39]. About 1,000 disease-causing mutations have been identified [43], among which, the most common is ΔF508 [9]. Pathogenic mutations result in the synthesis of an abnormal CFTR protein. CFTR is a glycoprotein located at the apical pole of most secretory epithelial cells including those of airways, intestine, pancreatic ducts, and bile ducts. It forms a cAMP-mediated low-conductance channel by which chloride ions pass from the cell into the lumen [9].

The large tissue distribution of CFTR protein explains why cystic fibrosis may affect multiple organs. The most common targets are the respiratory tract, the pancreas, and the digestive tract [9]; their involvement may be responsible for life-threatening complications which are the main determinants of the natural history of the disease and of its prognosis. Among the various other manifestations

which may be encountered in cystic fibrosis, hepatobiliary complications are frequent but are often overlooked because of their insidious development, usually mild course and slow progression [3, 6, 20, 22, 27, 30, 42, 49]. It is usually assumed that only 20% of cystic fibrosis patients would present clinical liver disease during their evolution; however, because of the increased duration of survival in patients with cystic fibrosis, the apparent incidence and the severity of hepatobiliary disease have recently increased [12]. In two recent large prospective studies including respectively, 177 [5] and 241 [27] patients with previously diagnosed cystic fibrosis, the prevalence of liver disease was 27 and 35%, and the median age at diagnosis was 7 and 3 years, respectively.

Despite their high incidence during the course of cystic fibrosis, it is highly unusual for hepatobiliary complications to reveal the disease. Only a few cases of patients presenting with end-stage liver disease as the only manifestation of cystic fibrosis have been described in children or even in adults. Even more rarely, severe liver disease may reveal some unusual forms of cystic fibrosis, such as the rare cases of cystic fibrosis masquerading as neonatal hepatitis or neonatal cholestasis mimicking extrahepatic biliary atresia [31, 46] or the cases of adult [15] or pediatric [40] patients presenting as sclerosing cholangitis.

It is, therefore, very much unusual to raise the diagnosis of cystic fibrosis at the histological examination of a liver biopsy taken during the evaluation of a pediatric patient without any prior history or any other symptom suggestive of the disease and presenting only with alterations in liver blood tests and/or hepatomegaly. We report four such cases to discuss the difficulties encountered in the diagnosis and management of these unusual patients, to underline the criteria necessary for a definitive diagnosis, and to point out the limits of routine screening for the early diagnosis of cystic fibrosis in asymptomatic patients.

Materials and methods

Study group

The files of our departments have been searched for identifying all the patients fulfilling the following criteria: (a) patients referred between 1990 and 2005 to the gastroenterology unit of the Department of Pediatrics, Hôpital Edouard Herriot, Lyon, (b) for the evaluation of abnormal liver tests and/or hepatomegaly, and (c) in whom the diagnosis of liver disease associated with cystic fibrosis was made after the histological examination of a liver biopsy (d) in the absence of known personal or familial history of the disease. Four patients fulfilled these criteria and are the subjects of the present report.

Clinical data

In each case, complete clinical charts were available. The following clinical data were recorded: mode of presentation, clinical course, and outcome. Biochemical tests of liver function included serum measurements of free and conjugated bilirubin, transaminases (aspartate transferase and alanine transferase), and gamma-glutamyl transpeptidase. Sweat test was performed in all patients; it was considered positive when chloride value was >60 mmol/l in at least two examinations as recommended [10]. Molecular genetic screening for the most common mutations of *CFTR* gene was performed in all cases by PCR (Elucigene CF30, Tepnel, Abingdon, UK). After diagnosis, standard protocols for the follow-up of cystic fibrosis patients were applied; in particular, complete chest and abdominal explorations were done at least once a year.

Histological data

Liver tissue samples were obtained by percutaneous transthoracic biopsy (in three cases) or at autopsy (in one case). They were fixed in Bouin's fluid or formalin and embedded in paraffin. Three micrometer thick sections were stained with hematoxylin–eosin–saffron, Perls reaction, periodic acid Schiff (PAS) reaction with diastase digestion and chromotrope. All the available tissue material was reviewed. The following histological variables were assessed: overall architecture, presence and extent of fibrosis, presence and extent of ductular proliferation, presence of cholangitis, presence, pattern, extent and distribution of steatosis, occurrence of giant cell transformation, cholestasis, iron deposition, and hemopoiesis.

Results

Clinical data are summarized in Table 1 and histological features in Table 2.

Clinical features

There were two boys and two girls, all born at term. Pregnancies were uneventful in all cases. There was no previous family history of cystic fibrosis. The median age at presentation was 47 months (range, 13 to 90 months).

The clinical presentation was variable. One case presented with growth retardation since birth and progressive heart failure of unknown cause associated with hepatomegaly and a fourfold increase in serum transaminase activities. A sweat test was performed at 7 months of age, but was in the normal range. The child died of heart failure at the age of 13 months. The histological examination of autopsy

Table 1 Clinical features

Patients	1	2	3	4
Age at clinical presentation	43 months	13 months	42 months	7 1/2 years
Sex	Male	Female	Male	Female
Mode of presentation	Abdominal pain Chronic diarrhea	Growth retardation Heart failure causing death	No symptom	Abdominal pain Chronic constipation
Clinical examination	Hepatomegaly	Hepatomegaly	Hepatomegaly	Hepatomegaly
Liver blood tests	↑ Transaminases	↑ Transaminases	↑ Transaminases	No abnormality
Ultrasound findings	Heterogeneous pseudotumoral hepatomegaly	Hepatomegaly	Hepatomegaly	Heterogeneous pseudotumoral hepatomegaly
Sweat test highest chloride value (mmol/l) and age of realization	102 (at 43 months, after liver biopsy)	27 (at 7 months)	135 (at 42 months, after liver biopsy)	102 (at 7 1/2 years, after liver biopsy)
CFTR mutations	ΔF508 N1303K	ΔF508 ΔF508	1717-1 (G-A) 1303 (N-K)	585 (E-X) 1066 (R-C)
Clinical outcome	Alive No sign of pancreatic insufficiency or bronchitis	Dead of heart failure due to myocardial necrosis and fibrosis	Alive First bronchitis at 5 years	Alive Portal hypertension (splenomegaly and esophagus varicoses) at 15 years
Duration of follow-up	6 months	–	11 years	14 years

samples showed the existence of scattered myocardial necrosis and fibrosis and of severe liver alterations; hepatic lesions were not in keeping with a diagnosis of chronic cardiac liver disease, but suggested a diagnosis of cystic fibrosis.

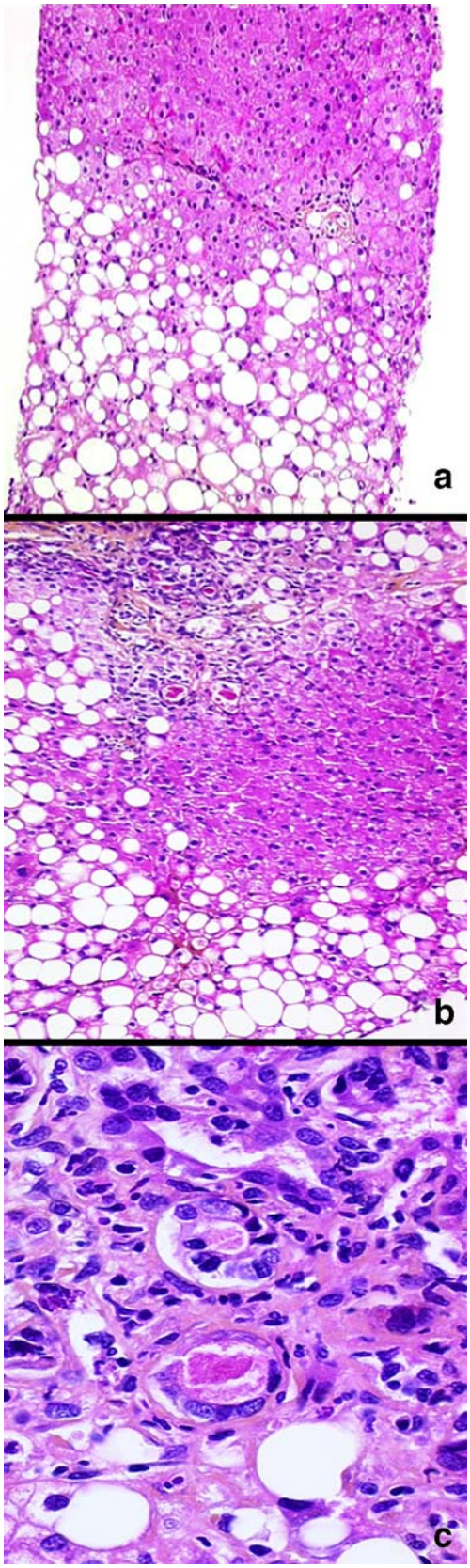
In the three other patients, clinical symptoms were mild. Chronic abdominal pain with intestinal transit dysfunction was the only symptom in two cases. One child was asymptomatic. Hepatomegaly was present in all three cases; in two cases, ultrasound examination showed an enlarged liver with steatosis and a heterogeneous pseudotumoral appearance. Liver blood tests showed increased transaminase serum levels in two cases; they were normal in one case. There was no evidence of viral infection or autoimmune disease; metabolic diseases (including alpha1-antitrypsin deficiency and Wilson's disease) were ruled out by biochemical and molecular tests. In all cases, the decision of liver biopsy was taken because of persistent abnormal liver blood tests and/or dysmorphic liver at imaging studies.

Histological findings

Typical histological features of focal biliary cirrhosis were observed in two cases (cases no. 1 and no. 2; Fig. 1). Focal periportal fibrosis was associated with ductular proliferation

Table 2 Histological findings

	1	2	3	4
Tissue sample	Liver biopsy	Autopsy	Liver biopsy	Liver biopsy
Number of portal spaces	24		12	25
Steatosis and % hepatocytes	80	80	90	80
Giant cell transformation	−	−	−	−
Expansion of portal tracts	+	+	−	+
Fibrosis	Extensive bridging Focal cirrhosis	Extensive bridging Focal cirrhosis	−	Focal, periportal
Cholangiolitis	+	+	−	−
Ductular proliferation	+	+	−	−
Cholestasis	−	−	−	−
Mucous plugs PAS +	+	+	−	−
Iron deposition	−	−	−	−
Extramedullary hemopoiesis	−	−	−	−



◀ **Fig. 1** Typical focal biliary cirrhosis (case no. 1). In this case, the histological picture is typical of focal biliary cirrhosis. At low magnification (**a**), periportal fibrosis and steatosis are readily visible. At higher magnifications (**b** and **c**), the periportal neoductular proliferation is obvious; numerous bile ducts contain large plugs made of amorphous proteinaceous material. Hematoxylin–eosin–saffron; original magnifications: **a** ×120; **b** ×240; **c** ×380

and pericholangitis, defined by the presence of a rim of inflammatory cells, including polymorphonuclear leukocytes and lymphocytes around the bile ducts. Amorphous pink deposits were present in dilated ductules; this material stained with PAS and resisted diastase digestion. There was no evidence of cholestasis. In both cases, macrovesicular steatosis occurred in 80% of hepatocytes without preferential distribution in the acini. The combination of portal fibrosis, ductular proliferation, PAS+ diastase resistant bile plugs and steatosis was highly suggestive of cystic-fibrosis-associated focal biliary cirrhosis. This hypothesis was therefore raised, and it was suggested to confirm the diagnosis by biochemical and molecular techniques.

In the two other cases, the only lesion was massive macrovesicular steatosis present in 80% of hepatocytes (cases no. 3 and no. 4; Fig. 2). It was panacinar in distribution. There was no evidence of steatohepatitis; portal spaces were not enlarged; there was no ductular proliferation or cholestasis; no bile plug was visible; there was no iron deposition. There was no clinical evidence for the more common causes of steatosis or steatohepatitis in children, including obesity, chronic hepatitis C, and drug-induced injury. Metabolic diseases associated with steatosis, such as Wilson's disease and mitochondrial diseases, have been ruled out. The hypothesis of massive steatosis associated with cystic fibrosis was therefore considered, and it was suggested to confirm the diagnosis by biochemical and molecular techniques.

Final diagnosis

After the results of the liver biopsy, a sweat test was performed in the three children in whom it has not been performed before. In all cases, the result was positive. Molecular screening revealed the presence of known pathogenic *CFTR* mutations in all four cases. The ΔF 508 mutation was present in two cases (cases no. 1 and no. 2); it was homozygous in one case, heterozygous and associated with a N1303K mutation in the other. In the two other cases, different combinations of heterozygous mutations were observed (Table 1).

Evolution and follow-up

Three patients were alive after the diagnosis. They presented no clinical, radiological, and/or biological sign

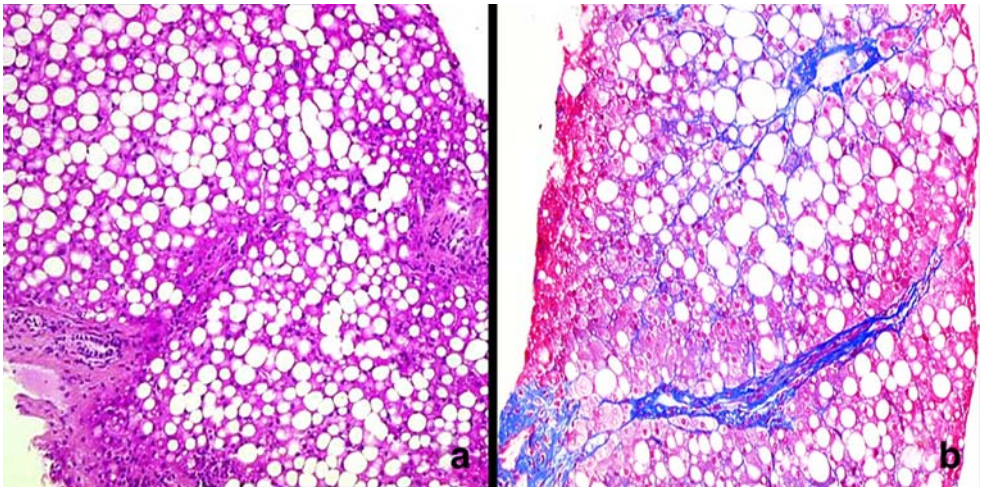


Fig. 2 Massive steatosis (case no. 3). In this case, macrovesicular steatosis is present in most hepatocytes, with a pancinar distribution (**a**); chromotrope staining confirms the presence of an extensive periportal fibrosis. **a** hematoxylin–eosin–saffron, **b** chromotrope; original magnifications ×260

suggestive of pancreatic insufficiency or lung involvement. Treatment consisted in fat soluble vitamin supplements, ursodeoxycholic acid, and pancreatic enzymes.

During further evolution, liver disease was progressive in only one case: Portal hypertension, with splenomegaly and esophageal varices, appeared after 7 years of follow-up. Imaging studies confirmed the existence of a dysmorphic liver; there was no biological sign of hepatocellular insufficiency, and no additional liver biopsy was taken during the course of the disease. In the other two cases, liver disease was stable; no complication was apparent. In one of these cases, lung disease appeared after 1 year of follow-up. At the time of submission of the manuscript, all three children were alive after, respectively, 6 months, 11 years, and 14 years of follow-up.

Discussion

The four patients included in this report are characterized by a much unusual mode of presentation. In all four cases, there was no known prior personal or familial history of cystic fibrosis. In three cases, liver disease was the only manifestation of cystic fibrosis at the time of diagnosis; in the remaining case, liver disease was present early in the course of the disease, but its correct interpretation was delayed because of its association with progressive heart failure due to myocardial lesion. In all four cases, the diagnosis of cystic fibrosis was first suggested only after the histological examination of liver tissue obtained either at biopsy or at autopsy. In two cases, the histological pattern, with a typical aspect of so-called focal biliary cirrhosis, was highly suggestive of cystic fibrosis; in the other two patients, presenting only with massive steatosis, the diagnosis of cystic fibrosis was considered in the absence of clinical, biochemical, or histological argument for another etiology. The presentation of the four cases included in our study is therefore in sharp contrast with the usual clinical pattern of the hepatobiliary manifestations of cystic fibrosis, which preferentially occur in patients with a known history of the disease.

Apart from their mode of presentation, the hepatobiliary manifestations observed in our patients were typical in their clinical, biological, and histological features. Two cases corresponded to typical examples of focal biliary cirrhosis. It is usually assumed that focal biliary cirrhosis results from bile duct injury secondary to the accumulation of an abnormal bile secretion in the lumen of bile ducts [19]; this results in the obstruction of bile ducts and in a reactive ductular proliferation usually associated with pericholangitis, which, in turn, promotes the development of periportal fibrosis in injured areas. It is likely that the abnormal bile secretion which triggers this sequence of events is related to the expression of an abnormal CFTR protein by biliary epithelial cells [13, 44]. However, the absence of genotype–phenotype correlations explaining the development of hepatobiliary manifestations in cystic fibrosis [18] suggests the existence of "modifier" genes [7, 8, 16], which remain, however, to be identified with certainty, even if several gene candidates have been proposed, including alpha1-antitrypsin, defensins, glutathione S transferase P1, mannose-binding lectin, and TGFbeta1 [2, 14, 21, 23, 43].

Focal biliary cirrhosis occurs in 10 to 70% of patients according to the series [1]: Such important differences may be explained by differences in the composition of the series reported so far as well as in the diagnostic procedures employed and in the criteria retained for the diagnosis [12]. In most cases, as in the two patients reported here, the clinical and biological abnormalities associated with focal biliary cirrhosis are mild [38]. Hepatomegaly, as observed in the two cases reported here, is present in only 30 to 43% of patients at diagnosis [1]. There is usually no sign of portal hypertension or hepatocellular insufficiency until late

in the course of the disease. Liver blood tests are normal in 70 to 80% of patients [1]. In contrast, the two patients reported here presented with alterations in liver blood tests: Both had a persistent increase in serum transaminase levels. In contrast to its clinical expression, the histological picture of focal biliary cirrhosis is highly suggestive [24]; it results from the variable combination of the following lesions: focal portal and periportal fibrosis, reactive ductular proliferation, presence of amorphous, proteinaceous PAS+ diastase-resistant material in dilated bile ducts, periductular inflammation, and steatosis. In the two cases reported here, all the histological features were present, and the resulting picture was therefore a strong argument supporting the unexpected diagnosis of cystic fibrosis.

The main risk of focal biliary cirrhosis is the evolution toward multilobular cirrhosis, which occurs in 5 to 15% of patients with cystic fibrosis [5, 27, 30]; the prevalence increases with age and duration of follow-up [27]. In some cases, multilobular cirrhosis results in end-stage liver disease, dominated by portal hypertension and its complications [11, 17], which may require liver transplantation [34]. We could not evaluate the pattern of evolution in our two patients: One died at diagnosis of heart failure due to scattered myocardial necrosis and fibrosis, a rare but known complication of cystic fibrosis [35, 48], and the other one has a follow-up of only 6 months.

In the two other cases included in this report, the clinical and histological picture was that of a massive steatosis of the liver in an asymptomatic child. Hepatomegaly and/or mild alterations of liver blood tests were present. The only histological lesion observed at liver biopsy was massive macrovesicular steatosis without preferential zonal distribution. Cystic fibrosis is known to be one of the possible etiologies of fatty liver disease in children [41]. Indeed, steatosis is a frequent manifestation of cystic fibrosis: It occurs in 20 to 60% of patients (23% in an autopsy series [33]), but is massive in less than 5% of cases [12]. Its pathogenesis is not fully understood. As hepatocytes do not express CTFR [25, 44], steatosis has been usually assumed to be of secondary, toxic, or nutritional origin [3]. In particular, it has been associated with dietary factors such as essential fatty acid deficiency [30]. However, this pathogenetic mechanism is unlikely in asymptomatic patients like those included in our study. In addition, our data show that steatosis associated with cystic fibrosis may precede the onset of severe hepatobiliary complications. In one of our cases, steatosis was the first sign of progressive liver disease, culminating with the development of severe portal hypertension after 7 years of follow-up. This lends support to the concept that in cystic fibrosis as in other liver diseases, steatosis may contribute to the promotion of liver fibrosis. Taken as a whole, our data therefore show that the pathogenesis of steatosis in cystic fibrosis needs to be reevaluated and that its role in the progression of liver disease must not be underestimated.

The very unusual presentation of the patients included in our study made it necessary to enforce and confirm the diagnosis of cystic fibrosis by specific arguments. The classical diagnostic and screening test for the identification of cystic fibrosis remains the sweat test, even if other screening tests have been recently proposed, such as the trypsin test which is gaining large acceptance [47]. The principle of the sweat test is simple: In the presence of an abnormal CFTR protein, the chloride concentration of the sweat is increased; a value >60 mmol/l is considered positive [28]. However, the results of a negative sweat test must be interpreted with caution, especially when the clinical context is suggestive. False negative results are possible because of unreliable methods, technical errors, and errors in interpretation [28, 29]. Moreover, sweat tests may be normal in atypical cases of cystic fibrosis, as shown by our own experience and by previous reports [4, 36, 45]; indeed, some recent series have shown that 1 to 2% of patients with cystic fibrosis may have sweat chloride concentrations of less than 60 mmol/l, likely as a result of heterozygous mutations preserving some biological activity for the residual CFTR protein [32, 36, 37]. This was observed in one of our patients (case no. 2) in whom the sweat test performed at 7 months was negative. In our experience, it is important to control such negative results after some months of evolution, as delayed positivity of the sweat test may be observed in a few instances.

Currently, molecular techniques are necessary to confirm the diagnosis of cystic fibrosis. They have to show the presence of pathogenic mutations in the *CFTR* gene. In our series, such mutations were detected in all four cases. One patient was homozygous for ΔF508. The other patients were heterozygous. The variety of mutations found in this small group of patients confirms the absence of genotype–phenotype correlation underlying the development of hepatobiliary manifestations in cystic fibrosis. We have no information regarding the expression of possible modifier genes involved in the pathogenesis of liver disease [2, 14, 43] in our patients, except for α1-antitrypsin, which had a normal phenotype in all four cases.

In conclusion, we report here four unusual pediatric cases to underline the variety of clinical presentations which may be associated with cystic fibrosis. They recall that this diagnosis has to be considered in children presenting with unexplained liver disease. The confirmation of the diagnosis by molecular techniques makes it possible to set up an appropriate follow-up to detect the possible complications of liver disease and the late involvement of other target organs.

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ORIGINAL ARTICLE

Angiogenic switch during tumor progression of carcinoma ex-pleomorphic adenoma

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Abstract We analyzed the tumor vascularization in carcinomas ex-pleomorphic adenoma (CXPA) to investigate the angiogenic switch during the malignant transformation of pleomorphic adenoma (PA) to carcinoma and during tumor progression. In eight cases of early CXPA (intracapsular and minimally invasive tumors), eight of advanced CXPA (widely invasive tumors), and ten of PA without malignant transformation, tumor vascularization was assessed in histological samples by measuring total microvascular area (TVA) and microvessel density (MVD) using CD34 and CD105 antibodies. MVD for CD105 increased significantly during tumor progression, whereas this was not the case for CD34 MVD. Comparing widely invasive CXPA with and without myoepithelial differentiation, CXPA with myoepithelial differentiation showed a significantly lower number of CD105 positive vessels but revealed higher TVA values. In these tumors, the neoplastic cells usually formed larger hypovascularized aggregates that were often surrounded by large-sized vessels. In conclusion, the antibody CD105 reveals an angiogenic switch during the progression from adenoma to carcinoma in salivary glands. The degree of angiogenesis and the total vascular area have distinctive patterns in CXPA with and without myoepithelial differentiation. Low angiogenesis associated with high TVA value is more characteristic of CXPA with myoepithelial differentiation.

Keywords Carcinoma · Pleomorphic adenoma · Angiogenesis · Malignant transformation

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Introduction

Angiogenesis, the formation of new blood vessels from preexisting ones, has been considered a critical process for tumor growth, invasion, and metastasis [20, 24]. The ability to induce and sustain angiogenesis occurs during tumor development via an angiogenic switch from vascular quiescence [12]. Besides that, blood can be supplied by a non-angiogenic process, the so-called co-option mechanism, well-documented in some human cancers, where the neoplastic cells use the existing blood vessels without eliciting an angiogenic response [9, 22, 28].

Tumor vascularization has rarely been studied in salivary gland tumors [5, 30].

In humans, the most common salivary gland tumor, the pleomorphic adenoma (PA), usually shows a poorly vascularized myxoid and chondroid stroma [26]. Carcinoma ex-pleomorphic adenoma (CXPA) is the principal form of malignancy arising in PA and accounts for 4.5–15% of all cancers of the salivary glands [7, 10, 18]. They are usually high-grade carcinomas, with frequent metastases and a poor clinical outcome [7, 10, 11, 17, 18].

In salivary carcinoma spontaneously arising in HER-2/neu transgenic mice, it has recently been suggested that the

richly vascularized preexisting salivary tissue is able to support tumor onset and progression with no need for an angiogenic switch [4].

Taking these results into consideration, we investigated the question whether an angiogenic switch would take place during the malignant transformation of PA into carcinoma and during tumor progression.

In a series of CXPAs, which represent the different phases of the adenoma–carcinoma sequence, i.e., in situ (intracapsular), minimally invasive and invasive carcinoma, tumor vascularization was assessed in histological samples by measuring the total microvascular area (TVA) and microvessel density (MVD) using CD34 and CD105 antibodies. PA without malignant transformation was used as control. CD34 has been reported as an optimal pan-endothelial marker [9] that stains not only "newly forming" vessels but also normal ones trapped within tumor tissues. In contrast, CD105 (endoglin), a proliferation-associated and hypoxia-inducible protein expressed in angiogenic endothelial cells, has been considered as a marker of neoangiogenesis [9, 24, 27, 29].

Materials and methods

The study was performed in 16 cases of CXPA which were retrieved from the files of the Department of Pathology of the University of Campinas. All diagnoses were reviewed by two pathologists (AA and VCA) using 50-μm sections obtained from formalin-fixed paraffin-embedded samples and routinely stained with hematoxylin–eosin. CXPA was defined as a malignant epithelial neoplasm arising in association with a primary or recurrent PA. The tumors were classified according to extension of invasion beyond the capsule of the previous PA as intracapsular (without invasion)—4 cases, minimally invasive (≤1.5 mm of invasion)—4 cases, and widely invasive—8 cases. Demographic and clinical information was obtained from the patients' medical records.

Immunohistochemistry

One paraffin block from each case was chosen for the immunohistochemical study [23], and the following antibodies were used (Table 1): CD34 and CD105 for detection of blood vessels and α-smooth-muscle actin (α-SMA), vimentin; cytokeratins 7 (CK7) and 14 (CK14) for the detection of epithelial and myoepithelial cells. The 5-μm sections were deparaffinized and hydrated, and endogenous peroxidase activity was quenched by immersion of the slides in 3% hydrogen peroxide. For all antibodies, except for α-SMA and anti-CD105, antigen retrieval (AR) was achieved by boiling in a steamer immersed in citrate buffer (pH 6.0, for 30 min). For CD105, AR was performed using 0.4% pepsin for 30 min (Table 1). Only the sections for CD105 were incubated at 37°C with protein block serum-free (code x0909, Dako, SA, Denmark) for 30 min. Subsequently, for all antibodies, the sections were incubated overnight at 4°C with the primary antibody and afterwards with the EnVision polymer HRP and Envision+ (code K1491, DAKO, SA, Denmark) for 1 h at 37°C. Sections were stained for 5 min at 37°C with 3.3′-diaminobenzidine tetrahydrochloride (DAB) and counter-stained with hematoxylin. Negative controls were run by omitting primary antibodies.

Microvessel density and microvessel area assessments

Immunohistochemical reactions for CD34 and CD105 were interpreted by two authors (AA and AS) using a double-headed microscope, and the most vascularized areas at low power magnification (hotspots) within each tumor were chosen for further analysis. For quantitative study, ten digital images per case were obtained using a charge-coupled device camera adapted to an Olympus CX30 microscope (X40 objective, 0.44 mm field diameter) and analyzed with Imagelab analysis software (version 2.4), which allows manual segmentation of target areas (vessels). Microvascular density for CD34 as well as for CD105 was considered the mean number of microvessel count, and

Table 1 Details of the antibodies used for immunohistochemistry

Specificity	Clone	Isotype	Dilution	Source	Buffer (AR)
CD34	QBEnd 10	IgG_1	1:50	Dako[a]	Citrate
CK7	OV-TL 12/30	IgG_1	1:100	Dako[a]	Citrate
CK14	LL002	IgG_3	1:1000	Neomarkers[b]	Citrate
Vimentin	V9	IgG_1	1:100	Dako[a]	Citrate
α-SMA	1A4	IgG_{2a}	1:200	Dako[a]	None
CD105	SNG	IgG_1	1:10	Dako[a]	Pepsin

[a] Dako Corporation, Glostrup, Denmark
[b] LabVision Neomarkers, Fremont, CA, USA

total vascular area the mean area occupied by microvessels per unit area of the tumor, i.e., that correspondent to one tumor field using the ×40 objective—0.152 mm^2. Vessels with muscular walls were excluded.

Statistical analysis

For comparison of the variables between the different tumor types, we used the Kruskal–Wallis test. To assess changes during the tumor progression, we codified adenomas as 0, minimally invasive carcinomas as 1, and widely invasive carcinomas as 2 and followed the changes of the variables with a Sperman rank order correlation. Results with $p<0.05$ were considered significant

Results

The CXPA group included 11 women and 5 men, and the tumors were located in the parotid glands in nine cases, in the submandibular glands in five, and in the minor salivary glands in two. The average age of the intracapsular CXPA group was 47, in the minimally invasive 67, and in frankly invasive 65. The CXPAs were classified into two main groups according to the extent of invasion beyond the capsule of the previous PA: early tumors—eight cases (four intracapsular and four minimally invasive carcinomas) and advanced tumors—eight cases (eight widely invasive carcinomas).

The PA group without malignant transformation included five women and five men, the average age was 41, and the tumors were located in the parotid glands in eight cases and in the submandibular glands in two.

Microvascular density (MVD) for CD34 and CD105

Figures 1, 2, 3, and 4 illustrate positive vessels and MVD for CD105 and CD34 in all groups. The mean vessel density of CD105 stained vessels was significantly different between the groups ($p<0.0001$; Kruskal–Wallis test). During the adenoma carcinoma sequence, the MVD of CD105 gradually increased (Fig. 3) with a highly significant strong positive correlation ($R=0.793$; $p<0.0001$). In PA without malignant transformation (Figs. 1 and 3), a low number of small CD105 positive vessels were detected (mean 3.33). However, in the early CXPA (intracapsular and minimally invasive carcinomas), these vessels were more numerous (mean 9.0; Figs. 1 and 3). The greatest increment in CD105 MVD was seen in the widely invasive CXPA (mean 33.6), with carcinomas without myoepithelial differentiation revealing higher values (mean 46.5) than those with such cellular differentiation (mean 16.33; Figs. 2 and 3).

In relation to MVD for CD34, no significant differences ($p=0.15$) were observed in the subgroups that represent the adenoma–carcinoma sequence (Fig. 4; adenoma mean 10.49, early CXPA 7.66, advanced CXPA 8.71, CXPA without myoepithelial differentiation 9.35, and CXPA with myoepithelial differentiation 7.87).

Total vascular area (TVA)

Total vascular area was significantly different among the tumors ($p=0.005$); Kruskal–Wallis test.

Figure 5 illustrates TVA in all groups of adenoma–carcinoma sequence. In widely invasive CXPAs, the TVA value was higher (mean 179.4) than in intracapsular and minimally invasive CXPAs (mean 63.8). In widely invasive CXPA, comparing tumors with and without myoepithelial differentiation, TVA value was higher in the former (mean 260.2 and 118.9, respectively). In widely invasive tumors with myoepithelial differentiation, the carcinoma cells often formed large hypovascularized cellular aggregates that were surrounded by large vessels (Fig. 2c), whereas in those without such cellular differentiation, the carcinomatous aggregates usually contained a smaller quantity of cells, and the vessels around them were thinner (Fig. 2d).

Discussion

Studies over the last decade have assumed that quantification of microvessel density may reveal the degree of angiogenic activity in a tumor [13]. However, a crucial question in angiogenesis is what proportion of the tumor vascular network is due to preexisting normal host vessels or newly formed tumor vessels that truly represent tumor angiogenesis [24]. Therefore, it has been suggested that markers that are preferentially expressed in angiogenic vessels, such as CD105, would be more appropriate to quantify angiogenesis [6, 8]. In this context, in recent works a distinctive pattern of expression of pan-endothelial markers (CD34 and CD31) and CD105 was found in benign and malignant lesions, where MVD for CD105 was more indicative of tumor angiogenesis [3, 19].

In the current study, a series of CXPAs that represent the lesion in the different carcinogenesis phases was analyzed in relation to MVD for CD34 and CD105 as well as total vascular area, thus allowing the characterization of tumor vascularization and the assessment of angiogenic activity during tumor progression.

In our series, when the different subgroups that represent the adenoma–carcinoma sequence (adenoma–intracapsular/minimally invasive CXPA–widely invasive CXPA) were compared, MVD for CD105 showed a strongly significant

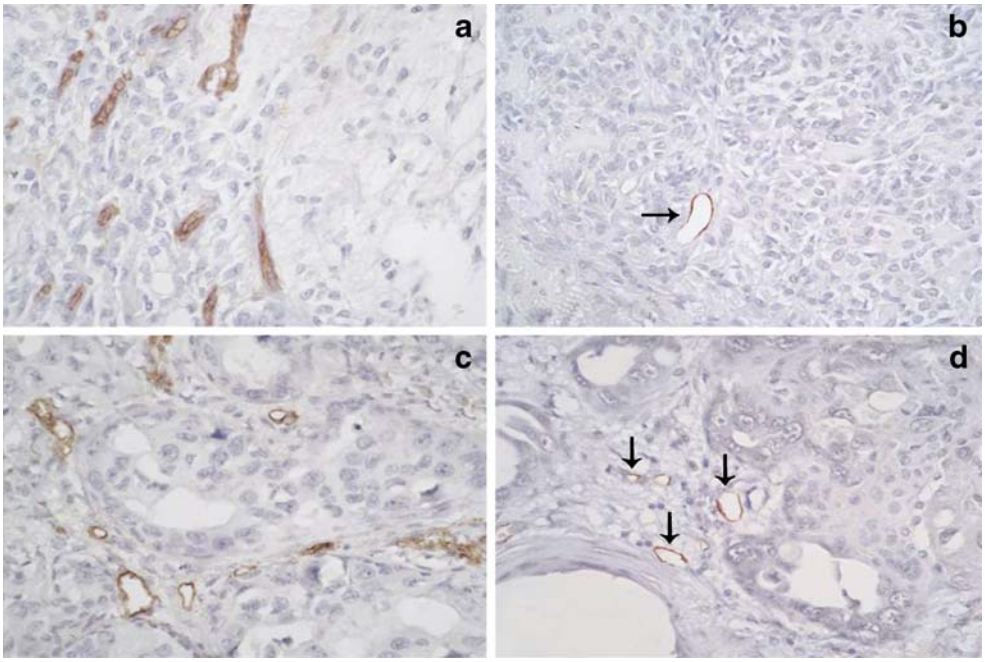

Fig. 1 CD34 (*at left*) and CD105 (*at right*) positive vessels: in pleomorphic adenoma without malignant transformation (**a**, **b**) and in intracapsular CXPA (**c**, **d**)

positive correlation with tumor progression, whereas MVD for CD34 revealed no difference. These results suggest that an angiogenic switch was required during the process of malignant transformation of PA into carcinoma and also reinforce the idea that MVD for CD105 may give a more accurate measure of the tumor angiogenic activity than a pan-endothelial marker [1]. Furthermore, our findings in salivary adenoma–carcinoma sequence were similar to those reported in the process of human colorectal cancer development where CD105, but not CD34, gradually increased in adenoma–carcinoma sequence [1]. Nevertheless, CXPA differs markedly from salivary carcinoma sponta-

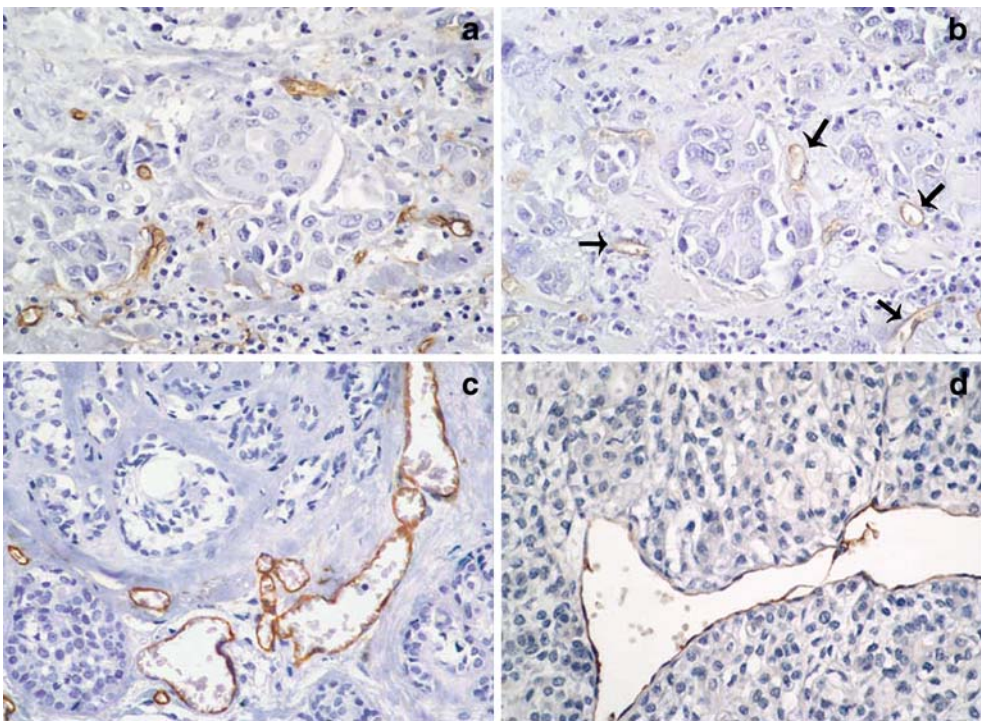

Fig. 2 CD34 (*at left*) and CD105 (*at right*) positive vessels: in widely invasive CXPA with myoepithelial component (**c**, **d**) large hypovascularized carcinomatous aggregates are surrounded by larger vessels than seen in widely invasive CXPA without myoepithelial differentiation (**a**, **b**); carcinomatous aggregates contain fewer cells, and the vessels around them are thinner

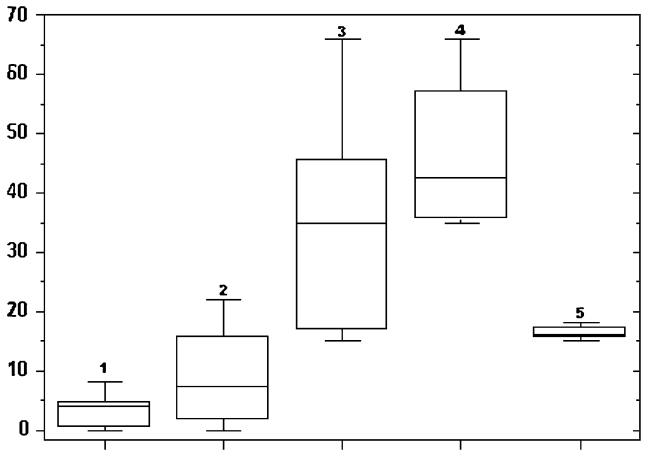

Fig. 3 Box and whisker plot of the microvascular density (MVD) for CD105: *1* pleomorphic adenoma without malignant transformation, *2* intracapsular and minimally invasive CXPA, *3* widely invasive CXPA, *4* CXPA without myoepithelial differentiation, *5* CXPA with myoepithelial differentiation. The *limits of the box* represent the 25 and 75% percentiles and the *center bar* the median. The *whiskers* are equivalent to the 5 and 95% percentiles

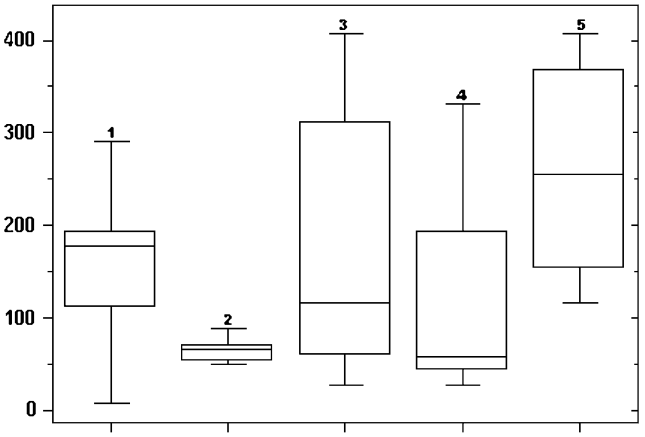

Fig. 5 Box and whisker plot of the total microvascular area (TVA) for CD34: *1* pleomorphic adenoma without malignant transformation, *2* intracapsular and minimally invasive CXPA, *3* widely invasive CXPA, *4* CXPA without myoepithelial differentiation, *5* CXPA with myoepithelial differentiation. The *limits of the box* represent the 25 and 75% percentiles and the *center bar* the median. The *whiskers* are equivalent to the 5 and 95% percentiles

neously arising in HER-2/neu transgenic mice, as in this animal model an angiogenic switch was not detected in any phase of carcinogenesis. However, it should be noted that CXPAs are usually high-grade carcinomas with frequent metastases, whereas the salivary carcinoma in the murine model is a slow-growing tumor with low metastatic capability, and therefore, there are probably differences in terms of oxygen and nutrient consumption rates of the tumor cells.

The onset of angiogenesis has been considered a hallmark of neoplastic transformation [13]. In benign lesions, the expression of CD105 in the microvessels has been described as barely visible, contrasting with that in carcinoma where it is marked [3, 19]. In our series, in PA without malignant transformation, CD105-positive vessels were rare and usually small. Nevertheless, in PA harboring intracapsular or minimally invasive carcinoma, they were more numerous, suggesting that angiogenesis was activated in these early malignant lesions. These findings are in agreement with those reported in multistep tumorigenesis models. In these models, angiogenesis was found to be activated in midstage lesions before the appearance of full-blown tumors suggesting that neovascularization is a prerequisite for the rapid clonal expansion associated with the formation of macroscopic tumors [12]. However, in macroscopic tumors, the major factor contributing to vessel density is metabolic demand, which frequently increases during tumor progression to accommodate an increased metabolic need of cancer cells [13, 24]. In this sense, in our salivary adenoma–carcinoma sequence, the most significant increment regarding MVD for CD105 was observed in the widely invasive CXPA group, suggesting that a marked modification in metabolic burden occurred when the carcinoma cells infiltrate the tissues outside the confines of the original PA.

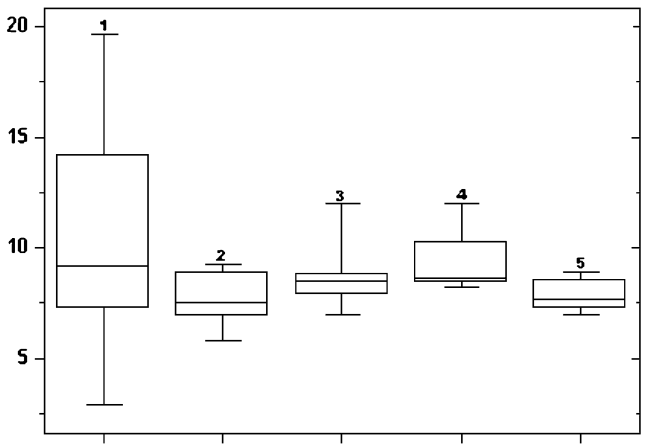

Fig. 4 Box and whisker plot of the microvascular density (MVD) for CD34: *1* pleomorphic adenoma without malignant transformation, *2* intracapsular and minimally invasive CXPA, *3* widely invasive CXPA, *4* CXPA without myoepithelial differentiation, *5* CXPA with myoepithelial differentiation. The *limits of the box* represent the 25 and 75% percentiles and the *center bar* the median. The *whiskers* are equivalent to the 5 and 95% percentiles

However, it is known that MVD varies widely with tumor type. Carcinomas arising in PA encompass a wide spectrum of histological tumor types [2]. In our series, comparing widely invasive CXPA with and without myoepithelial differentiation, a marked difference in terms of MVD for CD105 (neoangiogenesis) was detected. CXPA with myoepithelial differentiation showed a lower number of CD105-positive vessels. Angiogenesis is influenced by the net balance between angiogenic factors that stimulate

and those that inhibit vessel growth [12], and it has been suggested that myoepithelial cells may inhibit angiogenesis [25]. Therefore, this non-angiogenic property of the myoepithelial cell could be an explanation for the lower MVD for CD105 found in CXPA with such cellular differentiation. In our laboratory, studies are being carried out to investigate if other types of salivary carcinomas (non-ex-pleomorphic adenoma) with and without myoepithelial differentiation also show difference regarding degree of angiogenesis.

Total vascular area (TVA) has been less studied than MVD, but recent studies have emphasized its prognostic value [14–16, 24]. Our series showed significant findings in relation to TVA. Although widely invasive CXPA with or without myoepithelial component presented a MVD for CD34 without significant difference, the total vascular area assessed by this antibody revealed a marked difference among them. Widely invasive CXPA with myoepithelial component showed the highest TVA mean value. In these tumors, the neoplastic cells usually formed larger hypovascularized aggregates that were often surrounded by large-sized vessels. In contrast, in CXPA without myoepithelial differentiation, the carcinomatous aggregates usually contained a smaller quantity of cells with several small-sized vessels among them. These differences in vessel shape, size, and distribution between the two types of carcinomas could also be explained by some properties of myoepithelial cells. These may produce an abundant extracellular matrix devoid of vessels and containing bound angiogenic inhibitors [21]. It is likely that large-sized blood vessels are needed for cellular nutrition in carcinomas with myoepithelial component to compensate for the low angiogenesis inside the large cellular aggregates. Regarding benefit from antiangiogenic therapies and measuring the response to such a therapy, it has been suggested that localization of CD105 to angiogenic tissue could have potential when selecting patients [6]. Whether these differences appointed in our study could be relevant in terms of antiangiogenic treatment is only a topic of speculation at the present moment.

In conclusion, the antibody CD105 reflects more clearly the angiogenic changes during the progression from adenoma to carcinoma in salivary glands than CD34, revealing an angiogenic switch during this process. The degree of angiogenesis and the total vascular area have distinctive patterns in CXPA with and without myoepithelial differentiation. Low angiogenesis associated with high TVA value is more characteristic of CXPA with myoepithelial differentiation.

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ORIGINAL ARTICLE

CD30 and Epstein–Barr virus RNA expression in sclerosing angiomatoid nodular transformation of spleen

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Abstract Sclerosing angiomatoid nodular transformation (SANT) is a splenic lesion composed of angiomatoid/vascular nodules surrounded by hyalinized/sclerotic stroma, fibroblasts, myofibroblasts, and inflammatory cells. The endothelium within the nodules has a phenotype resembling splenic sinusoids, capillaries, and small veins. Martel et al. (Am J Surg Pathol 28:1268–1279, 2004) suggested that SANT may represent the final pathway of a variety of splenic lesions including inflammatory pseudotumors (IPTs). Epstein–Barr virus (EBV) has a role in the genesis of some splenic IPTs, but its presence in SANT has not been investigated. Six cases of SANT are reported. All were stained for CD31, CD34, CD8, CD68, smooth muscle actin, muscle-specific actin, and CD30 and were tested for EBV by in situ hybridization (EBER). All cases showed angiomatoid nodules with complex expression of CD31, CD34, and CD8, with focal CD68. Expression of CD30 by endothelial cells was also seen. One case had small diffuse areas lacking nodules resembling an IPT and was positive for EBV. The inflammatory cells and the normal spleen were negative for CD30 and EBER. In conclusion, SANT shows upregulation of CD30 with respect to normal spleen. The presence of EBV in the stromal cells of a case supports the notion that a subset of SANT may be related to IPT.

Keywords Spleen · Sclerosing angiomatoid nodular transformation · Inflammatory pseudotumor · Epstein–Barr virus · CD30

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Introduction

Sclerosing angiomatoid nodular transformation (SANT) is a recently recognized splenic vascular lesion characterized by multiple well-circumscribed individual and confluent vascular/angiomatoid nodules with interstitial hemorrhage set in a heterogeneous fibrocollagenous stroma admixed with inflammatory cells [5, 14, 16]. The nodules contain a complex mixture of endothelial phenotypes resembling splenic sinusoids (CD34−/CD31+/CD8+), capillaries (CD34+/CD31+/CD8−), and small veins (CD34−/CD31+/CD8−). Focal expression of CD68 can also be seen [5, 14]. The internodular stroma of SANT is composed of variable numbers of fibroblasts, myofibroblasts, plasma cells, lymphocytes, macrophages, and hemosiderin-laden macrophages. SANT appears to be a benign process, and splenectomy has been curative in all cases reported to date. Martel et al. [16] hypothesized that SANT may represent a peculiar sclerotic process with transformation of splenic red pulp in response to an exaggerated stromal proliferation. These authors also suggested that some SANTs might have started off as inflammatory pseudotumors (IPTs) due to the

appearance of the internodular stroma and the fact that some patients had fever or elevated erythrocyte sedimentation rate (ESR) at presentation, features often seen in splenic IPTs.

Herewith, we describe six examples of SANT. One case was prospectively identified as SANT on core biopsy, a diagnosis confirmed in a subsequent splenectomy. A second patient had an original diagnosis of IPT on core biopsy and was managed conservatively without splenectomy. The role of core biopsy in the diagnosis and management of SANT has not been previously addressed. One case had small diffuse non-nodular areas closely resembling an IPT. Epstein–Barr virus (EBV) was detected in this lesion. This is the first reported example of SANT associated with EBV supporting the notion that a subset of SANTs may be related to IPTs. CD30 is an activation marker expressed in the endothelial cells of approximately one third of non-splenic vascular lesions [7] but has not been studied in SANT. All six cases described herein expressed CD30 in endothelial cells.

Materials and methods

The material studied consists of eight specimens originating from six patients and includes five splenectomies and three core biopsies of splenic masses. One patient had two core biopsies followed by a splenectomy. Hematoxylin and eosin, Gomori methenamine silver (GMS), and Ziehl–Nielsen (ZN) stains were performed on 3- to 4-μm thick sections of formalin-fixed paraffin-embedded tissue on all cases. Immunohistochemical stains were performed in sections from one or more representative paraffin blocks from each case using the ultrastreptavidin–HRP detection system (ID Labs Biotechnology, London, ON, Canada). Color development was performed using the NovaRed® substrate kit (Vector Labs, Burlingame, CA). The antibodies employed are listed in Table 1. All the immunohistochemical studies were performed in an automated Ventana BenchMark® instrument (Ventana Systems, Tucson, AZ). In situ hybridization for the presence of EBV-encoded small RNAs (EBER) was performed on all cases using a pre-diluted probe (Ventana Systems) following standard protocols [3]. Polymerase chain reaction (PCR) analysis for EBV was performed on a single case in the mass and adjacent uninvolved spleen from formalin-fixed paraffin-embedded tissue using standard methods with appropriate positive and negative controls [18].

Table 1 Immunohistochemical antibodies employed

Antibody	Source	Dilution
CD31	Dako	1/100
CD34	Ventana	Pre-diluted
CD8	Novacastra	1/50
CD68	Dako	1/50
CD21	Dako	1/50
CD23	Novocastra	1/100
CD30	Dako	1/50
SMA	Ventana	Pre-diluted
MSA	Dako	1/100
ALK-1	Ventana	Pre-diluted
EBER	Ventana	Pre-diluted

SMA Smooth muscle actin; *MSA* muscle-specific actin; *EBER* Epstein–Barr virus encoded RNA.

Results

Clinical features

Clinical features are summarized in Table 2. There were six patients, two men and four women with an average age of 57.8 years and range of 41–73 years. The masses ranged in size from 1.9 to 12.0 cm with an average of 5.8 cm. Clinical and follow-up information was available in five patients. One patient was lost to follow-up. In three patients, SANT was an incidental imaging finding: one in a lung cancer patient undergoing staging CT scan (case 1), one during investigation for chronic cough (case 3), and another undergoing yearly serial ultrasounds for persistent hydronephrosis due to a neurogenic bladder secondary to myelomeningocele (case 5). The latter patient underwent two core biopsies with a tentative diagnosis of SANT but due to rapid change in the mass size, underwent splenectomy for definitive diagnosis and treatment. Case 4 presented with a 4-year history of persistent anemia of unknown cause and an elevated ESR and C-reactive protein. A full hematological workup including bone marrow biopsy revealed no additional abnormalities. Case 6 had a remote history of periampullary carcinoma treated with a Whipple's procedure 20 years earlier. This patient presented with epigastric pain and anemia and was found on CT scan to have an anastomotic bowel "mass" and multiple small splenic nodules. The splenic lesions had hyper-dense central zones suggestive of vascular structures. The bowel lesion was biopsied numerous times and revealed only an inflammatory fibroblastic lesion, which has remained clinically stable. A core biopsy of the largest splenic mass was undertaken 4 years after initial presentation due to growth of the lesion. This was the only material available for review in this patient. A year after biopsy, the splenic lesions have remained stable, and the patient has been discharged from our hospital. No splenectomy was performed.

Follow-up information was available on four of the five splenectomized patients and the patient with a core biopsy only. All five patients are alive and well with either stable disease or no recurrence. Case 4 had normalization of all blood counts after splenectomy.

Table 2 Clinicopathological features

Case	Age	Sex	Spleen weight (gm)	Mass size (cm)	Presentation	Original Dx	Follow-up
1	58	F	110	1.9	Lung ca staging	SANT	12 months, NER
2	65	M	320	6.5	Not available	Hamartoma	Lost to follow-up
3	73	F	324	6.0	Hypertension, hyperlipidemia, remote lung abscess, chronic cough	hemangioendothelioma	16 months, NER
4	51	M	2,720	12.0	Anemia NYD	IPT	5 months, NER
5	41	F	256	6.5	Growing mass on two serial ultrasounds	SANT	1 month, NER
6	59	F	Unknown	2.1[a]	Multiple splenic lesions 20 years post Whipple's procedure for periampullary carcinoma	IPT	12 months, NEP

Dx Diagnosis; *IPT* inflammatory pseudotumor; *SANT* sclerosing angiomatoid nodular transformation; *NER* no evidence of recurrence; *NEP* no evidence of progression of unresected splenic mass.
[a] Size for the splenic mass in case # 6 is based on radiological estimates as only a core biopsy has been performed to date in this case.

Macroscopic findings

Of the five splenectomies, three cases were multinodular and had well-demarcated red-brown nodules ranging individually from 0.1 to 1.0 cm with intervening white fibrotic areas (Fig. 1a). One case was mostly red-brown, and one case was tan-white, with both showing only vague nodularity giving the masses a variegated appearance (Fig. 1b). The surrounding splenic tissue appeared normal.

Microscopic findings

All cases exhibited a multinodular vascular proliferation admixed with a variable inflammatory infiltrate and fibrosis (Figs. 2a,b and 3a,b). A partial pseudocapsule was seen in five cases. The coalescing "angiomatoid" nodules were made up of thin walled veins, small capillary-like or slit-like vessels, and plump spindle cells with extravasated red blood cells (Figs. 2a,b and 3b). The nodules also contained inflammatory cells comprised of lymphocytes, plasma cells, and smaller numbers of neutrophils. The nodules were well circumscribed, of varying sizes and shapes, and many had serpiginous borders with a surrounding rim of either sclerotic tissue or fibrinous material (Figs. 2b and 3a,b). Two cases had the entire internodular stroma replaced by dense hypocellular fibrocollagenous tissue (Fig. 3b), while four others had a dense chronic lymphoplasmacytic infiltrate (Figs. 2b, 3a) and a fibroblastic proliferation in between nodules, with only focal collagen or myxoid matrix deposition. There were varying numbers of hemosiderin-laden macrophages in the internodular stroma (Fig. 3b).

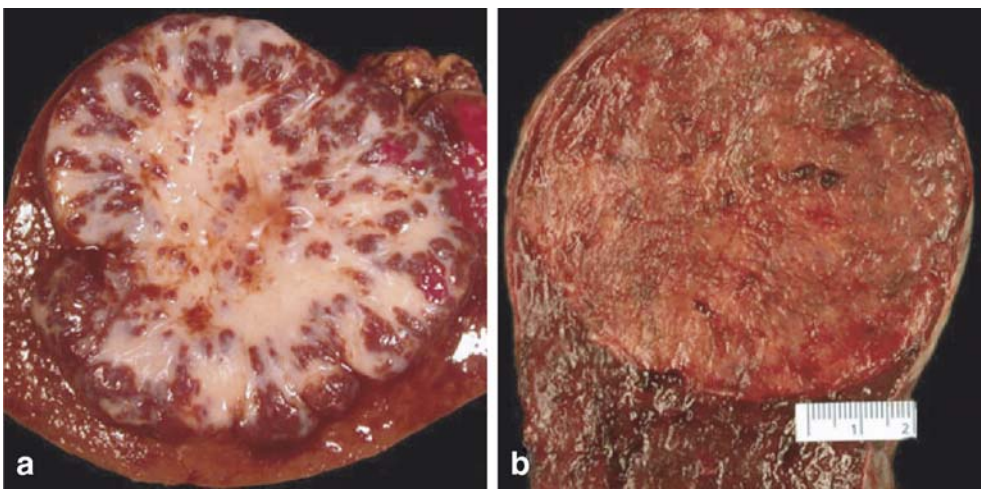

Fig. 1 Case 5 showing red-brown nodules of varying size surrounded by stellate white fibrous tissue (**a**). Case 4 showed a well-defined tan mass with poorly defined nodules (**b**)

Occasional entrapped lymphoid follicles with germinal centers were also seen.

In case 4, in addition to the multinodular vascular proliferation, there were focal areas completely devoid of angiomatoid nodules. These regions represented approximately 5% or less of the total surface area of the mass and contained dense chronic inflammation, consisting predominantly of plasma cells with smaller numbers of lymphocytes, and neutrophils (Fig. 3c). Merging imperceptibly with these cells were larger spindled and ovoid cells, some with irregular nuclear contours, which had vesicular chromatin and eosinophilic nucleoli. Some of these cells were seen in small clusters.

There was no mitotic activity, and no necrosis was identified in any of the cases. No evidence of carcinoma or lymphoma was seen, and ZN and GMS stains revealed no fungal or mycobacterial organisms. The normal surrounding spleen contained lipogranulomas in the white pulp in one case and another had abundant extra-medullary hematopoiesis (case 4). In the remaining cases, the surrounding splenic parenchyma was unremarkable.

Immunohistochemical findings

All cases demonstrated similar immunohistochemical findings. The angiomatoid nodules showed diffuse CD31 (Fig. 2c) staining of all the vascular spaces. CD34 demonstrated a smaller degree of staining with intense expression in capillaries only (Fig. 2d). CD8 was seen lining a smaller number of channels and showed the weakest staining of all

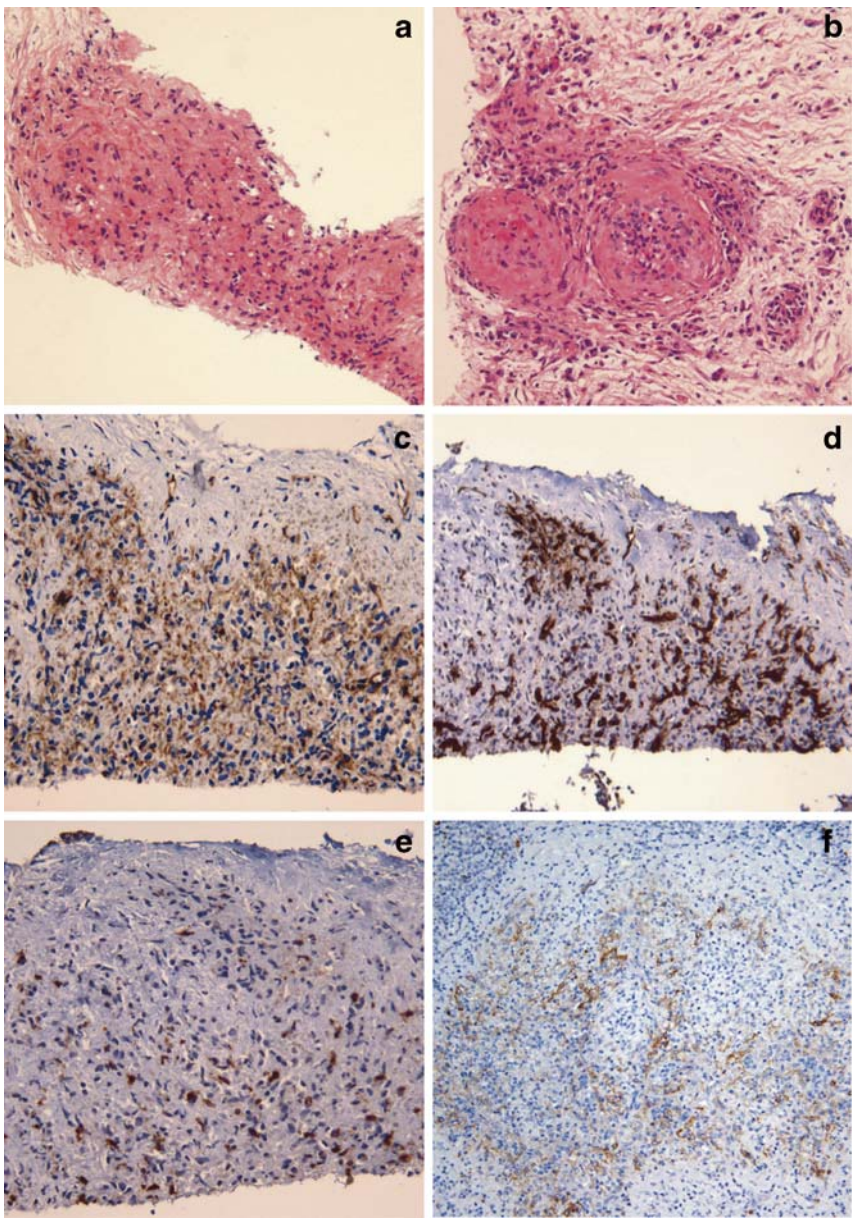

Fig. 2 Core biopsy of SANT demonstrating well-defined angiomatoid nodule composed of endothelial cells, inflammatory cells, and extravasated red cells (**a**); nodule with a fibrinoid ring with interstitial hemorrhage surrounded by lymphocytes, plasma cells, and eosinophils (**b**); and variable expression for CD31 (**c**), CD34 (**d**), CD8 (**e**), and CD30 (**f**)

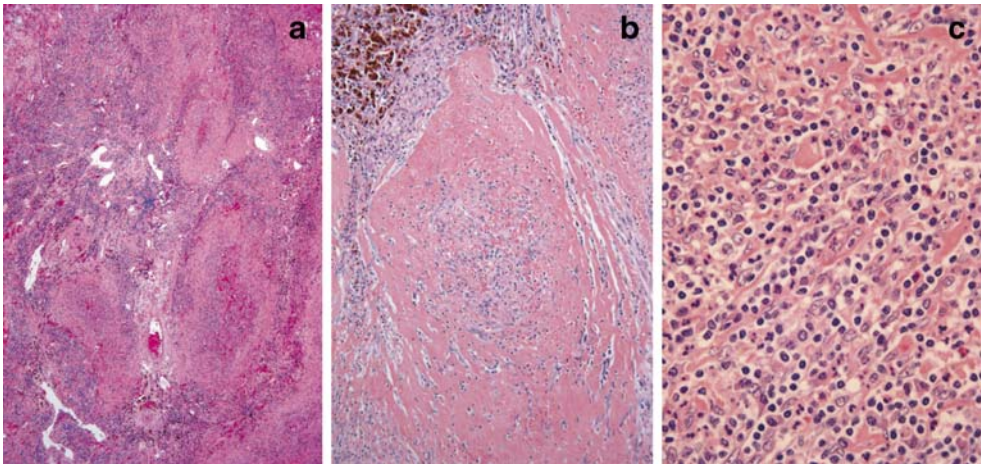

Fig. 3 Low-power magnification of Case 4 showing multiple nodules with interstitial hemorrhage surrounded by sclerotic stroma and inflammatory cells (**a**). Case 4 showing angiomatoid nodule with prominent interstitial hemorrhage surrounded by a sclerotic rim and inflammatory cells with hemosiderin-laden macrophages (**b**). High-power magnification of Case 4 showing internodular IPT-like areas with spindle cells admixed with numerous inflammatory cells (**c**)

vascular markers (Fig. 2e), with some nodules lacking reactivity altogether. CD68 stained isolated cells within the nodules in all cases, whereas CD21 and CD23 were negative. Three cases tested for ALK-1 exhibited no staining. CD30 was strongly and diffusely expressed by the endothelial cells of the angiomatoid nodules in all cases (Fig. 2f). The expression was stronger than endothelial markers in most cases. The distribution of CD30 positive cells was somewhat similar to the pattern seen with CD31. No CD30 staining was seen in between the nodules or in normal splenic parenchyma. Smooth muscle actin (SMA) and muscle-specific actin (MSA) were strongly expressed by spindle cells within and between the nodules. In case 4, the areas devoid of angiomatoid nodules demonstrated MSA and SMA staining only (Fig. 4a).

In situ hybridization

Strong nuclear expression for EBER was noted in case 4. The positivity was present in the spindle cells within and between angiomatoid nodules (Fig. 4c), with no staining in inflammatory cells and complete absence in normal spleen.

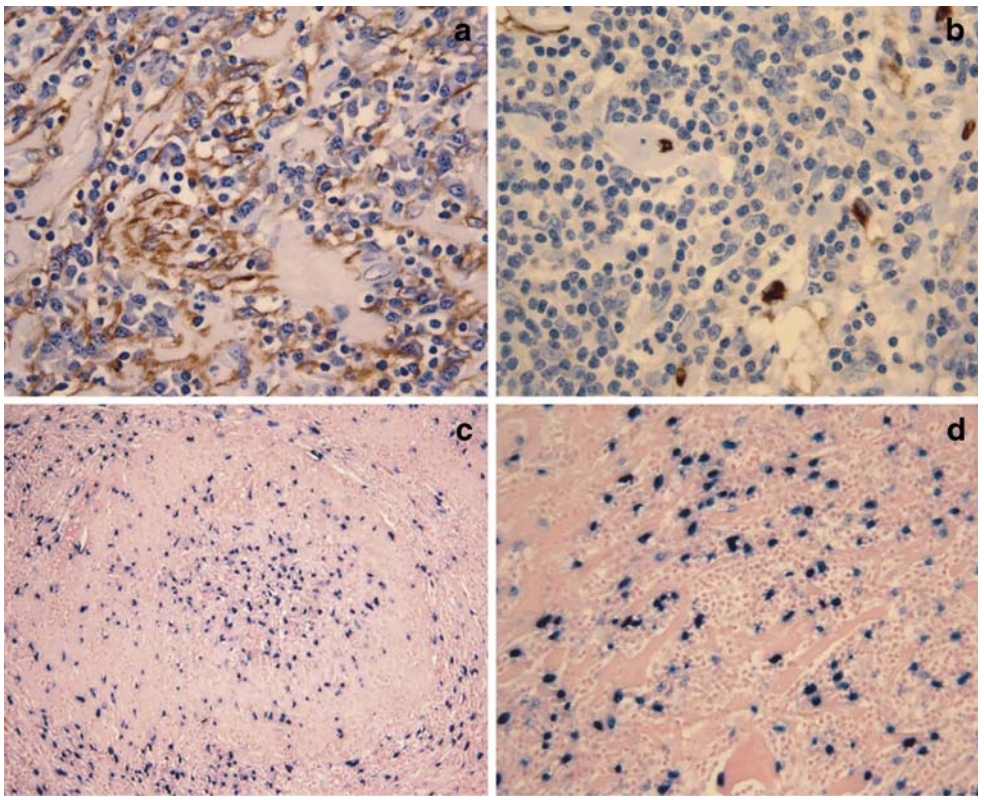

Fig. 4 Case 4 demonstrating MSA staining in IPT-like area staining spindle cells (**a**). Same area stained with CD34 demonstrating only a few isolated endothelial cells (**b**). The spindle cells are negative. Strong nuclear staining with EBER in angiomatoid nodules (**c**) and IPT-like area (**d**)

EBER was also noted in the areas devoid of angiomatoid nodules (Fig. 4d) suggesting that the expression was limited to fibroblasts and myofibroblasts. Double staining for EBER and CD31 was attempted, but the results were technically unsatisfactory; therefore, the presence of EBV in endothelial cells could not be completely excluded. EBER was negative in the remaining five cases.

Molecular analysis

A strong PCR band was present in the splenic mass in case 4 consistent with the presence of EBV DNA. There was no evidence of EBV in the normal spleen.

Discussion

Since its first description by Martel et al. [16] in 2004 and including the cases described herein, there have been approximately 33 reported cases of SANT [5, 14, 16]. Additional cases have been reported as splenic hamartoma and hemangioendothelioma [9, 10, 21]. The usual clinical presentation is of a middle-aged adult with abdominal discomfort, splenomegaly, raised ESR, or an incidental finding on routine cancer staging/medical imaging. Women are affected twice as often as men. SANT can be separated from other vascular lesions by its unique nodular angiomatoid proliferation with distinctive immunohistochemical profile, namely, a mixture of CD31+/CD8+/CD34− red pulp sinusoid-type endothelial cells, CD31+/CD34+/CD8− capillary-like endothelial cells, and CD34−/CD31+/CD8− small vein-type endothelium [16]. Focal CD68 staining has also been described in the nodules [14]. The internodular stroma and nodules themselves contain SMA and MSA positive fibroblasts/myofibroblasts and mixed inflammatory infiltrate [16]. SANT is considered a benign reactive process, and there have been no recurrences after splenectomy [5, 14, 16].

Martel et al. [16] noted that most SANTs often have an internodular stroma indistinguishable from IPT and suggested that SANT could represent the end stage or a final common pathway of a variety of splenic lesions, including IPT [16]. Like IPT, SANT has been found in association with extrasplenic malignancies [8, 17], is more common in women, and some patients have fever or elevated ESR at presentation. All the cases in our series had internodular areas resembling IPT, and one patient who presented with persistent anemia of unknown cause and elevated serum ESR and C-reactive protein also had small diffuse IPT-like areas. The presence of EBV was demonstrated in the spindle cells of the angiomatoid nodules, internodular stroma, and diffuse IPT-like areas of this case. This is the first description of EBV associated with SANT. None of the previously described cases was tested for EBV [5, 14, 16]. IPT of spleen is associated with the presence of EBV in tumor cells in a significant number of cases [1, 11, 17]. The presence of EBV in this case supports the suggestion that at least a subset of SANTs may represent transformation of an EBV-associated IPT. EBV has been shown to be associated with two types of mesenchymal tumors in the spleen, IPT, and "IPT-like" follicular dendritic cell tumor (FDCT) [1, 2, 4, 13, 17, 19, 22]. The relationship between IPT and "IPT-like" FDCT is not entirely understood, but recently, Lewis et al. [13] postulated that EBV positive mesenchymal tumors of the spleen may represent a group of related lesions arising from an EBV infected mesenchymal stem cell. This cell of origin could proceed to differentiate down a myofibroblastic pathway with actin positivity, a follicular dendritic cell pathway with CD21 or CD35 positivity, or an IPT with no specific differentiation. Some IPTs contain clonal EBV genomes strongly suggesting that they are true neoplasms [4, 13]. Additional studies investigating the morphologic spectrum and the presence and clonal status of EBV in SANT are necessary to gain further insight into the nature and pathogenesis of SANT.

In the six cases described herein—in addition to the usual immunoprofile of SANT—there was strong CD30 expression in the angiomatoid nodules. CD30 is an activation marker inconsistently expressed in vitro by endothelial cells [7]. It has been hypothesized that CD30 acts as a receptor for a number of as yet unidentified growth factors thereby regulating cytokine-mediated cell growth [6, 20]. The expression of CD30 suggests that the angiomatoid transformation of splenic red pulp in SANT is a cytokine-mediated process. A single study looking at CD30 staining in other non-splenic vascular lesions demonstrated moderate CD30 expression in 17 of 52 benign vascular tumors, 1 of 6 hemangioendotheliomas, 3 of 8 angiosarcomas, 0 of 8 Kaposi sarcomas, and 0 of 8 telangiectatic granulomas [20]. CD30 may prove to be a useful marker in supporting a diagnosis of SANT; however, additional studies of CD30 expression in littoral cell angioma, hemangioma, and hemangioendothelioma of spleen are needed.

Core biopsy of spleen appears to be a useful and noninvasive procedure in the recognition of SANT. In our series, two cases of SANT were diagnosed by core biopsies. One patient was initially called an IPT and chose to be followed with no additional treatment. The diagnosis in this patient was made before the description of SANT as a pathologic entity. The second patient had a presumptive diagnosis of SANT and was initially managed conservatively with repeat imaging but proceeded to surgery as the size of the lesion tripled over the course of a few months. Given the excellent prognosis of SANT, it is possible that some patients may be managed conservatively by "watchful" waiting and follow-up imaging studies. Splenic core biopsies are typically employed in the diagnosis of splenic

masses with a view to triage patients in need of splenectomy. Core biopsy of spleen is considered sensitive and specific for both hematological and non-hematological diagnoses—including vascular lesions—and they correlate well with final splenectomy diagnoses [12, 15]. Littoral cell angioma, hemangioma, hemangioendothelioma, and angiosarcoma are on the differential diagnosis of SANT on core biopsy; however, the nodular pattern, lack of atypia, and distinct immunohistochemical profile of SANT make this distinction possible. Potential indications for splenectomy in patients with a core biopsy diagnosis of SANT may include increase in mass size, marked splenomegaly, blood count abnormalities, or suspicion of malignancy.

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ORIGINAL ARTICLE

Human brain tumors: multidrug-resistance *P*-glycoprotein expression in tumor cells and intratumoral capillary endothelial cells

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Abstract Malignant brain tumor is a lethal disease with currently available treatment options having a limited impact on outcome. Nevertheless, novel therapeutic approaches combined with genetic prediction of chemosensitivity have, in the last decade, significantly improved clinical benefit for the treated patients. The fine characterization of the MDR1 gene encoding for *P*-glycoprotein (MDR1–Pgp) in brain tumors may be a crucial determinant for evaluating the long-term efficiency of specific anticancer compounds. By using a very high specific monoclonal antibody, the MDR1–Pgp was immunodetected in 34 out of 43 grade IV, 6 out of 10 grade III, 4 out of 7 grade II, and 1 out 3 grade I brain tumors. MDR1–Pgp resulted hyper-expressed, both in vessels and in neoplastic cells from the majority of tumors examined, compared to normal parenchyma. This study demonstrates that the MDR1 gene can be detected in all grade tumor brain malignancies and in endothelial cells of newly formed capillaries, thus, impairing drug access at the tumor cell level. Although the role of MDR1–Pgp in tumor blood vessels needs to be further examined and more clearly defined, drug resistance in malignant brain tumors may result from characteristics not only of tumor vasculature but also of neoplastic cells.

Keywords Brain tumors · Multidrug resistance (MDR) · MDR1–*P*-glycoprotein (MDR1–Pgp) · Immunohistochemistry · Monoclonal antibody (Mab)

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Background

The unresponsiveness of solid tumours to chemotherapy is a multifactorial phenomenon, which may include the overexpression of drug transporter mechanisms conferring the simultaneous resistance to a large array of anticancer compounds [19].

MDR1 gene encoding for *P*-glycoprotein (MDR1-Pgp), which is physiologically expressed in several human tissues by acting as an active (ATP-dependent) transporter, is capable of reducing drug bioavailability to target cells [10]. The presence of this drug efflux system on the cell membrane of tumor cells may play a significant role in the intrinsic or acquired resistance to chemotherapy [9].

Although a clear relationship between the expression level of MDR1–Pgp and the outcome of some pediatric tumors, including acute myelogenous leukaemia (AML), has been demonstrated, the role of MDR1–Pgp in the failure of chemotherapy of solid tumors is still a matter of discussion [20]. Drug resistance in brain tumors may

involve multiple mechanisms. For commonly used drugs, such as vinca alkaloids and epipodophyllotoxins, Pgp appears to be the major feature in the unresponsiveness of tumors to chemotherapy [11]. However, because of contradictory results on the expression of the MDR1–Pgp, its clinical significance on brain tumors requires further investigations. In fact, it is not rare that controversial indications about the presence or the expression level of MDR1–Pgp come out from different laboratories upon examination of identical tumor specimens.

Distinct aspects, including the use of weakly discriminating monoclonal antibodies (MAbs) and/or unsuitable techniques and procedures, contribute in generating differences in the multidrug resistance (MDR) phenotype evaluation of cancer cells [2]. In this context, several authors suggest that immunoreactivity detected with MAbs JSB1 [17], C494 [16], and C219 [21] extensively used for MDR1–Pgp typing of glioblastoma and other brain tumors could, therefore, reflect an artifact rather than a specific Pgp staining. As an example, it has been reported that MAbs JSB1 and C494 cross-react with a widely distributed cytoplasmic antigen, pyruvate carboxylase, which is present in abundance in normal astrocytes [1]. Thus, because the unexpectedly poor specificities of many of the antibodies thought to be specific for MDR1–Pgp, the role of Pgp in producing drug resistance in malignant astrocytoma is questionable [1]. Further, the MAb C219 is utilized worldwide for immunohistochemistry and biochemical characterizations of mammalian MDR cells, cross-reacting with non-*P*-glycoprotein molecules [4]. Hence, the use of MAbs JSB-1, C494, and C219 for the detection of MDR1–Pgp expression should be approached with caution [2].

For the evaluation of MDR1–Pgp expression in brain tumors, we have used the well-characterized MAb MM4.17 for the following reasons: (1) The MAb reacts with an external human-specific epitope that is mapped in the fourth external loop at a single amino acid level (TRIDDPET) [5]; (2) the MAb binds with very high affinity and specificity to MDR1–Pgp expressed in human tumoral MDR cells [3]; (3) it allows to detect even the very small level or variation of MDR1–Pgp, such as that usually expressed in circulating human lymphocytes [15]; (4) the MAb binding to target cells is abrogated by specific MM4.17 phagotopes mimicking external *P*-glycoprotein conformation [14].

The use of MAb MM4.17 can eliminate misleading interpretations of the presence and expression level of MDR1–Pgp in brain tumors. The results we obtained and reported in this article might well contribute in routine clinical determinations of MDR in tumor specimens, thus, contributing to our understanding of the basis of the mechanisms of brain tumors' resistance to anticancer compounds.

Materials and methods

This study analyzes formalin-fixed and paraffin-embedded tissue blocks from 83 patients with brain tumors between 2003 and 2006, from the Surgery Pathologic Anatomy Department III of the University of Study in Pisa. All these patients were treated with neurosurgical abscission of the brain tumor. Furthermore, the study also includes formalin-fixed and paraffin-embedded brain tissue from ten different regions of a normal adult brain autopsy and from ten fetal autopsy. Clinical and pathological information were obtained from medical records and surgical pathology reports.

Histopathology

Brain tumors are classified according to the World Health Organization (WHO) classification of the central nervous system tumors. This study analyzes 62 tumors of neuroepithelial tissue (39 malignant glioblastomas, grade IV; 2 gliosarcomas, grade IV; 1 ganglioneuroblastoma, grade IV; 8 anaplastic astrocytomas, grade III; 1 choroid plexus carcinoma, grade III; 1 anaplastic oligoastrocytoma, grade III; 5 oligodendrogliomas, grade II; 1 oligoastrocytoma, grade II; 1 ependymoma, grade II; 3 pilocytic astrocytomas, grade I), 1 embryonal tumor (1 medulloblastoma), and 20 tumors of meningothelials cells (10 meningothelial meningiomas and 10 transitional meningiomas).

Treatment

None of the 83 patients had received chemotherapy or radiotherapy before surgery.

Immunohistochemistry

Immunohistochemistry was performed on 5-mm thick formalin-fixed and paraffin-embedded sections mounted on slides. After conventional deparaffinization and rehydratation, endogenous peroxidase activity was quenched by incubation in 0.3% H_2O_2 (10 min) at room temperature. The following procedure was to unmask the antigen. Next, samples were washed in phosphate-buffered saline (PBS; pH 7.2 for 10 min) and were incubated (10–15 min) in normal bovine serum albumin. After that, the sample were incubated with the optimally diluted (5 ug/ml) specific antibody MM4.17 (30 min) at room temperature and then with the secondary antibody. As positive control, kidney tissue samples were used for MDR1–Pgp immunodetection.

Monoclonal antibody

For specific MDR1–Pgp immunodetection, a purified form of the MAb MM4.17 (IgG2a,k) directed to the fourth loop of Mdr1–Pgp extracellular domain was used [5]. Several studies including (1) the recognition of the mdr1 gene expressed in various human MDR cells [15], (2) the epitope mapping at the amino acid level using a PIN-synthesized mdr1 peptide library [5], (3) the abrogation of MAb staining to human tissues and MDR cell lines by an MDR1 phagotope [14], and (4) the specific staining of human tumors expressing MDR1–Pgp [3] have demonstrated that this rodent MAb utilized in a purified form react exclusively with Mdr1–Pgp. No cross-reactivity with other mammalian *P*-glycoprotein have been observed.

Immunoreactivity scoring

Three observers without knowledge of the clinical data independently assessed the expression of Pgp. The distribution of the Pgp expression was assessed by estimating the percentage of positively stained cells. Four staining categories were established: − (0–5% staining), +/− (6–25% staining), + (26–50% staining), ++ (51–75% staining), and ++ (76–100% staining).

Results and discussion

The poor prognosis of brain malignancies is partly based on the minor success obtained from chemotherapeutic treatments. Resistance mechanisms at the tumor cell level may be in addition to the blood–brain barrier involved in the intrinsic drug unresponsiveness of brain tumors [9, 10, 18, 19]. To determine the expression level of MDR1–Pgp in brain tumors, we have used the MAb MM4.17 that has been shown to react with unique specificity and affinity with a well-defined human epitope located on the fourth loop of the external domain of MDR1–Pgp.

Astrocytic tumors are the most common human brain tumors. They originate from astrocytic cell lineages and are classified, according to the WHO guidelines, into four categories of increasing malignancies: pilocytic astrocytomas (grade I), astrocytomas (grade II), anaplastic astrocytomas (grade III), and glioblastomas (grade IV).

In grade IV glioblastoma (n=39), we found that a considerable expression of MDR1–Pgp was observed in the majority of tumor samples (32 out of 39; 82%; Table 1). The MDR1–Pgp is mainly localized in the membrane and cytoplasm compartments of positive cells (Fig. 1), while its distribution was heterogeneous within the tumors. In MDR1–Pgp-positive glioblastomas, the specimens are arbitrarily classified as 7.5%+/−, 20.5%+, 18%++, and 36%++, according with the intensity of MAb MM4.17 immunostaining (Table 1).

Table 1 MDR1–Pgp expression in malignant glioblastomas (grade IV) as detected by the MAb MM4.17

Case	Age	Sex	Tumor cells	Endothelial cells[a]
1	59	M	++[b]	−
2	71	M	−	−
3	48	M	−	−
4	66	F	+	−
5	70	F	−	−
6	71	F	−	−
7	81	M	−	−
8	56	F	−	−
9	72	F	+	−
10	79	F	++	−
11	74	F	+	+
12	59	M	++	++
13	80	F	++	++
14	63	M	++	++
15	70	F	++	++
16	63	M	+/−	−
17	55	M	+	−
18	63	M	+	−
19	46	F	+	+
20	63	M	++	−
21	64	M	++	++
22	55	M	++	++
23	64	M	++	++
24	67	F	++	−
25	77	M	++	−
26	73	F	+	+
27	55	F	+	−
28	66	F	+	+
29	57	F	+/−	−
30	65	F	++	++
31	77	F	+	+
32	66	F	++	++
33	74	F	++	−
34	66	M	+	+
35	80	M	+/−	−
36	51	M	+	+
37	50	M	−	−
38	74	F	+	−
39	43	F	+	−

[a] From newly formed tumors
[b] Stained cells: − (0–5%), +/− (6–25%), + (26–50%), ++ (51–75%), ++ (76–100%)

Our findings seems in contrast with previous published data reporting different percentage of MDR1–Pgp positivity in neuroblastomas and gliomas [7].

However, these previously reported investigations have been conducted with non-MDR1–Pgp specific MAbs, such as JSB1 and C219 [22], or using non-histologically well-defined tumor samples [7].

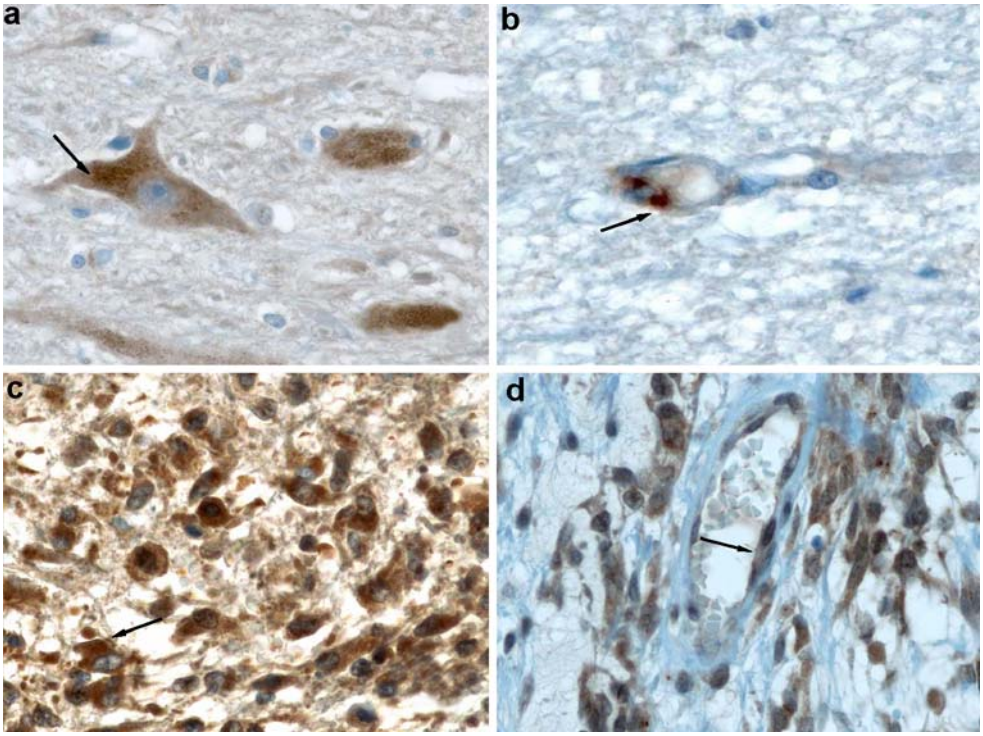

Fig. 1 MDR1–Pgp expression in normal and tumoral brain tissues. In the *upper part* of the panel, the *arrow* indicates the expression of MDR1–Pgp immunodetected by MAb MM4.17 in the cytoplasm of neuronal (**a**) and endothelial (**b**) cells of the mesencephalic nuclei. In the *lower part* of the panel, the *arrow* indicates the MDR1–Pgp expression in the cytoplasm of glioblastoma (**c**) and in the cytoplasm of endothelial cells of newly formed tumor vessels (**d**)

In 24 brain tumors showing various histological defined malignancies (Table 2), the MDR1–Pgp presents an identical cellular localization of the glioblastoma cells (Fig. 2). In these samples, MDR1–Pgp was detected in 2 out of 4 high grade IV tumors, 6 out of 10 grade III, 4 out of 7 grade II, and 1 out 3 grade I brain tumors.

The immunohistochemical investigation of 10 meningothelial and 10 transitional meningiomas, (both grade I in the WHO classification) shows that 19 out of 20 of these tumor samples express the MDR1–Pgp (Fig. 3). The intensity of the MAb MM4.17 staining was found etherogenous in cells of both meningioma histotypes: MDR1–Pgp-positive meningothelial meningiomas were classified as 30%++, 60%+, and 10%−, and MDR1–Pgp positive transitional meningiomas were classified as 10%++, 30%++, and 60%+ (Table 3). Differently from a previous report [22] where transitional meningioma were found to be more MDR1–Pgp positive in comparison to meningothelial meningioma, we found no clear relationship between meningioma histotype and MDR1–Pgp expression. Because none of the patients with meningiomas had received chemotherapy or radiotherapy before surgery, the high MDR1–Pgp levels in these tumors were not induced by any treatment. Meningiomas represent approximately 20% of the brain tumors [13]. WHO has subdivided meningiomas into 11 histological subtypes and classified them based on the cytological features of anaplasia as benign, atypical, or malignant. The molecular pathogenesis is poorly characterized, and only a few molecular markers have been associated with meningiomas [12].

To confirm and extend previously published observations on the expression of human MDR1–Pgp in the capillary endothelial cells of the central nervous system [6], ten adult and ten fetal autoptical samples from different brain sites of different gestational age were immunohistochemically investigated. The MDR1–Pgp expression was found in the endothelial and meningeal cells, in the choroid plexus epithelium of both the fetal and the adult autoptic brain tissues (data not shown). MDR1–Pgp positivity was also observed in the pyramidal neurons of different cortical areas, including hyppocampi Ammon's horn in the adult autoptic brain and in the neuronal cells of ponto-mesencephalic nuclei in fetal brain (data not shown). These results confirm that MDR1–Pgp may play an important role in the endothelial cells of the brain, pumping out xenobiotics from endothelial cells into the lumen of capillaries for the protection of the brain parenchyma and protecting fetal brain against toxic agents or maternal metabolic products during the intrauterine development.

To examine whether the endothelial cells of the newly formed capillaries during neoangiogenesis within malignant human brain tumors express MDR1–Pgp, 83 tumor specimens were investigated for MDR1–Pgp expression (Tables 1, 2, and 3).

The endothelial cells of the newly formed capillaries in 16 of 39 glioblastomas (41%) and in 2 of 24 other brain tumors (8%), including gliosarcomas, a medulloblastoma, a ganglioneuroblastoma, anaplastic astrocytomas, a choroid plexus carcinoma, an anaplastic oligoastrocytoma, oligodendrogliomas, an oligoastrocytoma, an ependymoma, and

Table 2 MDR1–Pgp expression in benign and malignant brain tumors as detected by the MAb MM4.17

Case	Age	Sex	Histology	Tumor cells	Endothelial cells[a]
1	80	F	Gliosarcoma (grade IV)	+[b]	+
2	75	M	Gliosarcoma (grade IV)	−	−
3	17	M	Medulloblastoma (grade IV)	+	−
4	13	F	Ganglioneuroblastoma (grade IV)	−	−
5	55	M	Anaplastic astrocytoma (grade III)	+	−
6	43	M	Anaplastic astrocytoma (grade III)	−	−
7	30	M	Anaplastic astrocytoma (grade III)	−	−
8	74	M	Anaplastic astrocytoma (grade III)	+	−
9	67	M	Anaplastic astrocytoma (grade III)	+	−
10	62	F	Anaplastic astrocytoma (grade III)	+	−
11	26	F	Anaplastic astrocytoma (grade III)	−	−
12	28	F	Anaplastic astrocytoma (grade III)	+	+
13	4 months	M	Choroid plexus carcinoma (grade III)	−	−
14	59	M	Anaplastic oligoastrocytoma (grade III)	+	−
15	57	F	Oligodendroglioma (grade II)	+	−
16	48	F	Oligodendroglioma (grade II)	++	−
17	34	F	Oligodendroglioma (grade II)	+	−
18	3	F	Oligodendroglioma (grade II)	−	−
19	1	F	Oligodendroglioma (grade II)	+	−
20	26	M	Oligoastrocytoma (grade II)	−	−
21	1	F	Ependymoma (grade II)	−	−
22	21	F	Pilocytic astrocytoma (grade I)	+	−
23	18	F	Pilocytic astrocytoma (grade I)	−	−
24	8	F	Pilocytic astrocytoma (grade I)	−	−

[a] From newly formed tumors
[b] Stained cells: − (0–5%), +/− (6–25%), + (26–50%), ++ (51–75%), ++ (76–100%)

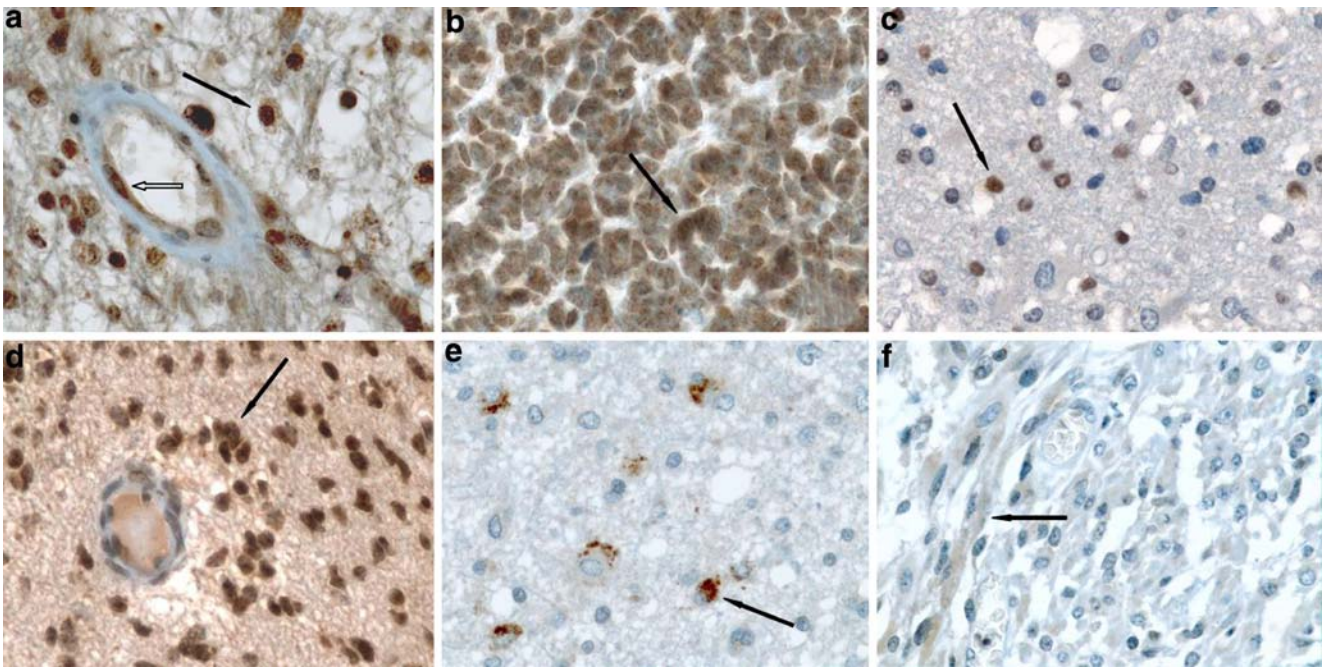

Fig. 2 MDR1–Pgp expression in brain tumors. The *arrows* (in *bold*) show that MDR1–Pgp is immunodetected in the cytoplasm of gliosarcoma (**a**), medulloblastoma (**b**), anaplastic astrocytoma (**c**), anaplastic oligoastrocytoma (**d**), oligodendroglioma (**e**), and pilocytic astrocytoma (**f**). MDR1–Pgp expression was also observed in the newly formed endothelial cells (*empty arrow*) of gliosarcoma (**a**)

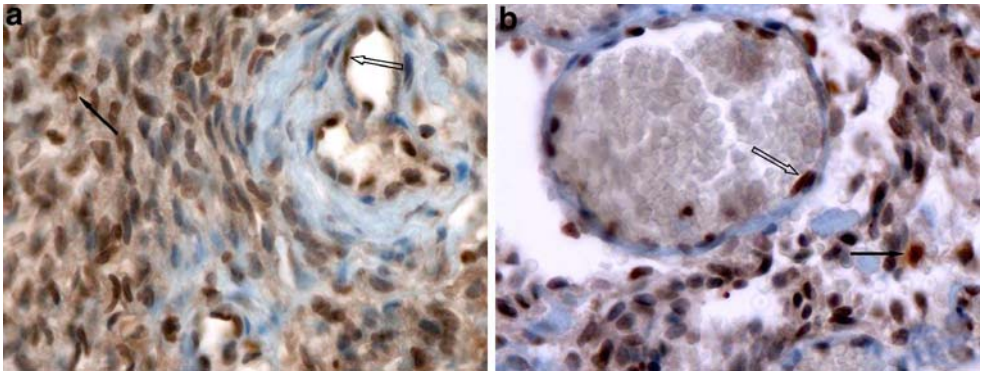

Fig. 3 MDR1–Pgp expression in meningioma. The *arrows* in *bold* show that MDR1–Pgp is immunodetected in the cytoplasm of transitional (**a**) and meningothelial (**b**) meningiomas. The *empty arrows* show that MDR1–Pgp is immunodetected in the endothelial cells of newly formed vessel in both tumors

pilocytic astrocytomas, immunostained positive for MDR1–Pgp. These results demonstrated that MDR1–Pgp is expressed not only in the capillaries of normal brain [6] but also in the majority of the newly formed capillaries of brain tumors. Our data are in good agreement with previous published data, in which MDR1–Pgp was biochemically identified by MAb C219 [8]. Similar percentage of MDR1–Pgp positivity was also observed by Toth et al. [22]. In this study, while the authors found 25 out of 29 cases of gliomas positive for MDR1–Pgp expression at the endothelial cell level, they found tumor cells in only 7 of 35 cases also positive for MDR1–Pgp. In contrast, we found that in glioblastomas and meningiomas (Tables 1 and 3), the MDR1–Pgp positivity of capillary endothelial cells of neo-vasculature correlates with that observed in cells of brain tumors. These putative differences in MDR1–Pgp detection may be due to the different discriminating ability of MAbs JSB1, C219, and MM4.17. However, it cannot be ruled out that unsuitable techniques and procedures or the use of nonhomogeneous histological classification may contribute in generating differences in the MDR–Pgp evaluation in brain tumors.

Summing up, our data indicate that the MDR of brain tumors may result not only from the expression of resistance markers in endothelial cells of tumor capillaries but also from the MDR1–Pgp expression in neoplastic cells. MDR1–Pgp in this special localization can remove anticancer compounds from tumor cells located around the capillaries. These observations suggest that, after careful evaluation in well designed preclinical studies, combined chemotherapy consisting of MDR1–Pgp reversing agents and specific anticancer compounds may represent a novel and promising clinical approach for the treatment of malignant brain tumors.

Table 3 MDR1–Pgp expression in meningioma as detected by the MAb MM4.17

Case	Age	Sex	Histology	Tumor cells	Endothelial cells[a]
1	56	F	Meningothelial	+[b]	+
2	71	F	Meningothelial	+	+
3	68	F	Meningothelial	+	+
4	45	M	Meningothelial	+	+
5	35	F	Meningothelial	+	−
6	55	M	Meningothelial	+	+
7	83	M	Meningothelial	+	+
8	84	M	Meningothelial	+	+
9	57	F	Meningothelial	−	+
10	28	M	Meningothelial	+	+
11	65	M	Transitional	+	+
12	77	F	Transitional	+	+
13	65	F	Transitional	++	+
14	47	F	Transitional	+	−
15	73	F	Transitional	+	+
16	64	M	Transitional	+	+
17	61	F	Transitional	+	+
18	76	F	Transitional	+	+
19	70	M	Transitional	+	+
20	73	F	Transitional	+	−

[a] From newly formed tumors
[b] Stained cells: − (0–5%), +/− (6–25%), + (26–50%), ++ (51–75%), ++ (76–100%)

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ORIGINAL ARTICLE

The spectrum of persistence of testicular blastema and ectopic testicular parenchyma: a possible result of focal delay in gonadal development

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Abstract Sex-cord formation and organization are important steps in testicular development and depend on adequate interactions between mesenchymal cells, pre-Sertoli cells, and germ cells. These elements form the testicular blastema, the precursor of the testicular parenchyma, morphologically characterized by poorly organized sex cords and mesenchymal components. Here, we study two uncommon testicular lesions, unrelated to other gonadal anomalies. In the first group, we describe the features of persistence of testicular blastema in three fetal autopsy cases, discussing its possible pathogenesis and clinical importance. In the second, we analyze 11 cases of ectopic testicular parenchyma in the tunica albuginea, an uncommon benign condition of uncertain clinical significance, whose main differential diagnosis is gonadal dysgenesis. Based on their similar topography within the testis, and on their possibly shared embryological origin, we propose that both lesions may represent the two extremes of a maldevelopmental spectrum resulting from a focal delay in testicular development.

Keywords Testicular development · Persistent blastema · Ectopic parenchyma · Tunica albuginea

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Introduction

Testicular blastema can be defined as the primitive tissue that gives rise to testicular parenchyma, characterized by immature sex cords, germ cells, and mesenchymal components. Persistence of blastematous tissue is described in many organs, such as lung, kidney, adrenal gland, and the rete testis, the latter three sharing similar embryonal origins.

Normal testicular development in mammals has been fully reviewed [5, 11, 12, 20]. Testis development in humans begins around week 4 of embryonal age, and should be complete by week 10, when one can clearly recognize its architecture [11, 17, 23]. Although there are few studies on testicular development in humans, the morphologic steps parallel those observed in murine models.

The fully developed testis is composed of germ cells and somatic cells. The somatic elements include Sertoli and interstitial cells (Leydig cells, endothelium and peritubular myoid cells). Normal gonadal development initiates through proliferation of the celomic epithelium adjacent to the mesonephros. This results in thickening of the epithelium, forming two protuberances on the ventro-lateral surfaces of each mesonephros, morphologically identical in both sexes, the gonadal ridges.

In males, somatic cells begin to condense into clusters, initiate basement membrane deposition forming cords, and polarize, acquiring epithelial characteristics. The primordial

sex cords form as a consequence of molecular interactions between the celomic epithelium and the mesonephros, and germ cells that migrated from the yolk sac colonize them, giving rise to the seminiferous cords. The tunica albuginea, a thick collagenized layer that separates the surface celomic epithelium from the cortical testicular parenchyma, forms after parenchyma differentiation is complete.

Testicular blastema is not expected to be present in testes after their complete development. We describe the persistence of testicular blastema (PTB) in three fetal cases, a finding not previously reported in the literature. We also describe 11 cases of ectopic testicular parenchyma (ETP) in the tunica albuginea, a rare condition. Although both lesions have an unexplored clinical significance, we believe it is important to distinguish them from features of testicular dysgenesis. Furthermore, we propose that in between the two lesions there exists a continuum in testicular maldevelopment, in which PTB represents the early stage of a series of changes that result in ETP. Here, we discuss their possible relationship and the biological mechanisms underlying this type of testicular abnormality.

Materials and methods

Fourteen cases were retrieved from the Pathology Department files at Hospital La Paz, corresponding to either autopsy examples (nine cases), orchiectomies (three cases), or testicular biopsies (two cases). Age ranged from 17 weeks of gestation to 53 years. Clinical data were reviewed, and information on age, laterality, and testicular location (intra-abdominal, inguinal canal, or scrotal testes) is summarized in Table 1.

Table 1 Clinical information of all PTB and ETP cases

Case	Age	Histology	Laterality	Situation
1	20 WGA	PTB	Bilateral	IA
2	17 WGA	PTB	Bilateral	IA
3	31 WGA	PTB	Bilateral	IA
4	24 days	ETP	Left	IC
5	27 WGA	ETP	Right	IA
6	4 months	ETP	Left	SC
7	53 years	ETP	Left	SC
8	34 years	ETP	Right	SC
9	59 years	ETP	Right	SC
10	3 years	ETP	NA	SC
11	28 years	ETP	Left	SC
12	5 years	ETP	NA	IA
13	46 years	ETP	Right	SC
14	29 years	ETP	Right	IA

WGA weeks of gestational age, *IA* intra-abdominal, *IC* inguinal canal, *SC* scrotal, *NA* nonavailable

Both autopsy and surgical cases were routinely fixed, paraffin-embedded, cut, and H&E-stained. Histological specimens were reviewed and slides containing lesions of interest were selected. Immunohistochemistry was performed in selected paraffin blocks using the streptavidin–biotin–peroxidase technique with antibodies against high-/low-molecular-weight cytokeratin AE1/AE3 (Signet™), collagen IV (DAKO™), placental alkaline phosphatase (PLAP) (DAKO™), inhibin (DAKO™), calretinin (DAKO™), and vimentin (Progen™). Appropriate quality control and quality assurance procedures were implemented, including positive and negative tissue controls with each assay. Histological and morphometric analyses were performed in the adjacent normal parenchyma (data not shown).

Results

Persistence of testicular blastema

In all three cases, the testicular lesions were incidental findings of fetal autopsies. In case 1, the patient was the product of a spontaneous abortion at 20 weeks of gestation due to severe chorioamnionitis, and showed no developmental abnormalities. Case 2 was the product of an elective abortion at 17 weeks of gestation due to a neural tube defect (lumbosacral rachischisis with associated myelomeningocele), associated with an abdominal wall defect (omphalocele) and skeletal abnormalities (asymmetrical arthrogryposis of lower limbs and ribs). No cytogenetic studies were performed, and a specific syndrome could not be diagnosed based on these findings. Case 3 had a cytogenetically proven trisomy 18 (Edwards syndrome), presenting with classical facial features, cardiac malformations, esophageal atresia with tracheoesophageal fistula, and skeletal abnormalities.

Light microscopy studies in cases 1, 2, and 3 showed well-defined foci of poorly differentiated testicular parenchyma located immediately beneath the celomic epithelium (Fig. 1a). In all cases, the lesions were bilateral and contiguous to the celomic epithelium, with no visible tunica albuginea formed in the involved areas (Fig. 1b). This poorly differentiated parenchyma resembled testicular blastema and consisted of ill-organized cords composed by polygonal to oval-shaped cells with scanty cytoplasm and prominent nucleoli. Another population of cells, characterized by cells with large, round, pale nuclei and abundant clear cytoplasm, resembling germ cells, was less frequently seen, sparsely distributed among the cords (Fig. 1c). The remaining testicular parenchyma, tunica albuginea, and epididymis were normal for the age.

Immunohistochemistry for AE1/AE3 antibody showed intense staining of the celomic epithelium and also of the

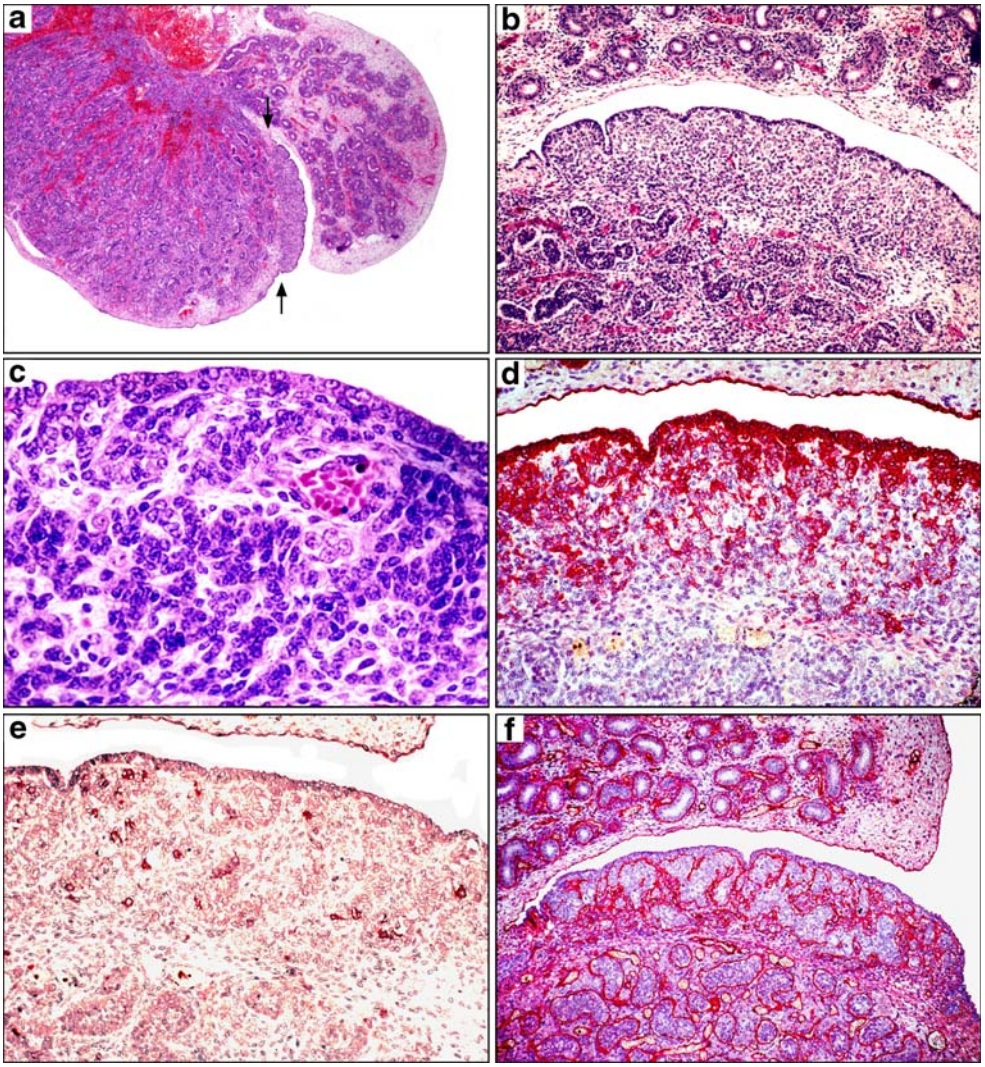

Fig. 1 **a**–**c** Microscopic appearance of PTB (H&E). **a** Focal and well-defined aspect of the lesion (*arrows*) (40×). **b** Poorly differentiated parenchyma adjacent to celomic epithelium, with absence of tunica albuginea (100×). **c** Ill-organized cords containing scattered germ cells (400×). **d**–**f** Immunohistochemical features. **d** Cytokeratin AE1/AE3 staining in celomic epithelium and superficial blastemal cells (200×). **e** PLAP staining of scattered gonocytes within the blastema (200×). **f** Collagen IV stain, highlighting the presence of a contiguous basement membrane between the blastemal cords and the celomic epithelium (100×)

adjacent polygonal cells observed in the testicular blastema (Fig. 1d). Immunoreactivity was less frequent in cells more deeply located below the celomic epithelium, and no staining was observed in the normal Sertoli cells of the well-formed seminiferous cords present in the rest of the testes. Strongly reactive cells in the epididymis and rete testis served as internal controls. In an opposite pattern, vimentin stained Sertoli cells in normal cords but was absent in both the persistent blastema and the celomic epithelium. Cells resembling germ cells were negative for AE1/AE3 and vimentin in all sections, and all were positive for PLAP (Fig. 1e). No PLAP immunoreactivity was observed in other cell populations.

Stains for collagen IV highlighted normal basement membrane deposition around the well-formed seminiferous cords, and also underneath the celomic epithelium layer. Collagen IV was present in a similar linear pattern within the nodules of persistent blastema, delineating irregular, large cords of cells in obvious continuity with the celomic epithelium basement layer (Fig. 1f).

Testicular parenchyma ectopy

Among the 11 cases, six were incidental autopsy findings with grossly and otherwise histologically normal testes (three children and three adults). There were two biopsy specimens; one biopsy was performed for evaluation of a small testis associated with hydrocele, and in the other, no clinical information was available. Of three orchiectomy specimens, two were due to cryptorchidism (one child and one adult) and one was due to a Leydig cell tumor.

Lesions observed histologically in cases 4 to 14 consisted of a variable number of seminiferous tubules, and testicular stroma containing Leydig cells, located inside the tunica albuginea. In all the cases, the tunica albuginea was well formed and collagenized, with normal thickness. Collagen fibers were always parallel to the surface, and there was no resemblance to ovarian-type stroma. The morphology of tubules, distribution of germ cells, and presence of Leydig cells inside the lesions were variable among the cases (Fig. 2a–c); however, all of them displayed

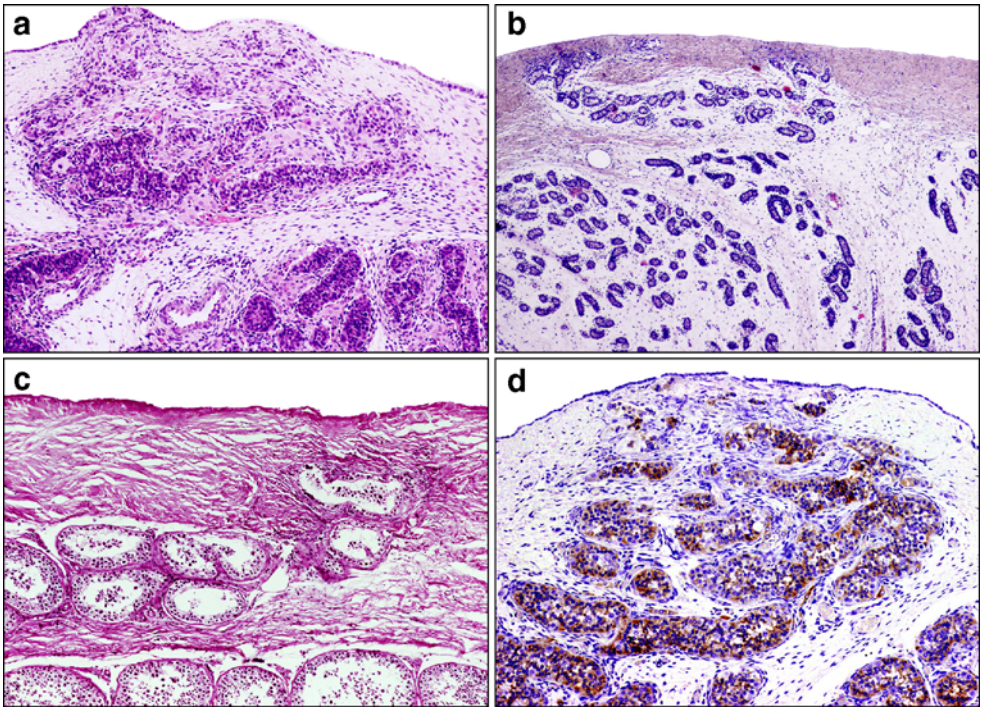

Fig. 2 **a**–**c** Histological aspect of ETP (H&E). **a** A childhood case, with the presence of infantile seminiferous tubules and Leydig cells within a normal tunica albuginea (200×). **b** ETP in a child with cryptorchidism (200×). **c** An adult case of ETP, with mature seminiferous tubules and Leydig cells inside a well-collagenized tunica albuginea (100×). **d** Immunohistochemistry for inhibin, showing both Sertoli and Leydig cells within the ectopic parenchyma (200×)

morphology features similar to the observed in the adjacent testicular parenchyma, including the morphological alterations observed in the cryptorchidism cases (*vide infra*).

Immunohistochemical studies were compatible with the pattern observed in normal, well-developed testicular parenchyma. Cytokeratin AE1/AE3 was positive in the celomic epithelium and negative throughout the parenchyma, including Sertoli cells. Inhibin and calretinin stains highlighted the presence of Leydig cells in both ectopic and adjacent normal parenchyma (Fig. 2d), while isolated gonocytes distributed within and outside the lesion stained strongly for PLAP.

Histological evaluation of the adjacent parenchyma revealed both normal histology and morphometric parameters (mean tubular diameter, tubular fertility index, and germ cell and Sertoli cell numbers) [13] in all childhood cases except in case 12, which showed the alterations commonly observed in cryptorchidism. The nonlesional parenchyma in adult cases showed slight alterations compatible with obstructive changes (cases 7 and 11), changes secondary to chronic disease/systemic therapy (cases 8 and 9), and features observed in cryptorchidism (case 14) [13]. All the observed changes were compatible with the clinical histories.

Discussion

PTB beyond the embryonal period has never been reported in the literature. In the three cases described here, the lesions were focal, well defined, and bilateral, contrasting with the well-developed adjacent parenchyma. The histological aspect was identical to the previously described findings in testes of less than 7 weeks of embryonal-age fetuses (corresponding to 9 weeks of gestational age) [17], with polygonal to oval-shaped cells with scanty cytoplasm resembling pre-Sertoli cells, poorly organized in primitive sex cords, and cells with clear cytoplasm, large nuclei, and prominent nucleoli resembling germ cells.

The more likely explanation for the PTB would be a localized delay in testicular parenchyma development because the remaining testicular components show normal maturation. Primordial sex cords are rudimentary, poorly formed tubular structures composed of pre-Sertoli cells and germ cells, and together with the adjacent gonadal stroma characterize the testicular blastema. Further tubular organization occurs as a consequence of interactions with the gonadal stroma [4, 5, 12, 19], and it happens in parallel with Sertoli cell differentiation, first at the central portion of the testis and later within the periphery [12]. The testicular stroma arises from mesonephric precursors that migrate into the gonad and further differentiate into endothelial and peritubular myoid cells. The processes of mesonephric cell migration, sex cord formation, and Sertoli cell differentiation all depend on the expression of the *Sry* gene and its downstream targets, mainly *Sox9*, at the gonadal ridge and mesonephros [5, 19]. Other proteins shown to be involved in sex-cord organization are adhesion molecules, such as integrins and cadherins [7, 10].

Vascular development is the earliest morphological feature of male gonadal differentiation, and developing testicular vessels play an important role in sex cord

development [2, 3, 16, 22]. Knockout animals for *Pdgfr-α*, a growth factor receptor responsible for sex-specific vessel development in the male gonad, fail to develop a normal vascular pattern and show delayed testicular development [16, 22]. The morphological pattern observed in *Pdgfr-α* −/− mice is very similar to that observed in the focal lesions of PTB, showing large and irregular primitive cords lacking Sertoli cell differentiation and an organized stroma. We could hypothesize that the pathogenesis of PTB might be a defective segmental branching of the developing celomic vessels, leading to a focal lack of interactions between testicular vessels and parenchyma.

The pattern of immunohistochemical staining observed in these lesions is interesting. The presence of AE1/AE3 cytokeratin staining in epithelial-type blastemal cells adjacent to the celomic epithelium, with loss of immunoreactivity in deeper cells, could indicate that these cells are arising from the superficial celomic epithelium and further maturating into Sertoli cells that lack cytokeratin expression, which is consistent with the normally observed pattern of cytokeratin expression in the developing testis [1]. The hypothesis of an origin in the celomic epithelium for epithelial blastemal [9] cells is reinforced by the presence of a continuous basement membrane between the celomic epithelium and the blastemal cords, as shown by collagen IV stains.

The clinical significance of PTB is unclear. The lesions in all three cases were incidental findings of postmortem examinations in previable fetuses and were probably unrelated to the cause of death. Although in two of the cases (cases 2 and 3, *vide supra*) there were associated malformations in other organ systems, the limited number of cases and lack of published data make it difficult to establish a relationship between the testicular lesion and the other phenotypic findings. We could not find in the literature any description of a similar testicular histology among other trisomy 18 (Edwards syndrome) cases. One can speculate whether these lesions could have a potential for malignant transformation in childhood, as occurs in neoplasms related to blastemal persistence in other organs, such as Wilms tumor in the kidney or pleuropulmonary blastoma in the lung. Further studies for genetic profiling of these lesions could identify molecular alterations and help to establish a possible relationship with malignant transformation.

The finding of ETP in the tunica albuginea, unrelated to other testicular lesions, has only been reported twice in the literature [14, 18]. Cases were described in both children (five cases) and adults (one case), ranging from 1 to 51 years of age. In four out of the five cases reported in children (ages ranging from 1 to 13 years), the testicular lesions were incidental findings of autopsy with grossly normal, descended testes; the fifth case was of a 6-year-old boy who underwent orchiectomy due to cryptorchidism [14]. The adult case was also an incidental finding following orchiectomy due to persistent pain secondary to a granulomatous epididymitis [18]. All reported lesions were unilateral, and their gross appearance characteristically showed small cysts measuring less than 1 mm at the testis surface. Histologically, the lesions were described as the presence of normally developed seminiferous tubules, some of them containing germ cells and stroma with Leydig cells, inside a well-collagenized, morphologically normal tunica albuginea, sometimes splitting it into two layers. Testicular histology outside the lesions was normal in all reported cases. In our series, none of the surgical specimens had a clinical suspicion of a lesion in tunica albuginea. ETP represented an incidental finding in both autopsy cases and surgical specimens obtained due to other clinical indications. Among our adult cases, slight alterations in the morphology were present in the adjacent parenchyma, such as changes secondary to obstruction, chronic disease, and cryptorchidism [13], but no causal relationship can be established between them and ETP due to the limited number of cases. As the lesions contain both testicular stroma and seminiferous tubules inside, we prefer to call them ETP rather than ectopic seminiferous tubules.

The focal character of the lesions, in addition to the presence of a normal tunica albuginea in all cases (displaying normal collagenization and absence of features of an ovarian-like stroma), absence of müllerian structures, and no clinical history of intersexual state, rules out the diagnosis of gonadal dysgenesis (GD) [6, 8]. This entity represents the main differential diagnosis in both ETP and PTB. Other clinical differential diagnoses for ETP include two lesions that result in cystic appearance of the tunica albuginea, mainly if the ectopic tubules present cystic dilatation: tunica albuginea cysts, a benign condition of probable mesothelial origin [15], and cystic teratomas. However, the histopathological aspect of both is very characteristic. Regarding PTB, another important differential diagnosis would be gonadoblastoma, which can also present with an aspect of disorganized tubules in the testicular peripheral portion, but shows other characteristic features, such as atypical gonocytes, Call–Exner bodies, and a nodular architecture. Furthermore, almost all gonadoblastomas are associated with an underlying dysgenetic testis [21].

We could speculate that ETP is the natural evolution of progressive development of PTB into mature parenchyma occurring inside the tunica albuginea. According to this notion, the ectopic parenchyma would have become “entrapped” in the tunica albuginea as a result of its delayed maturation, which probably occurred simultaneously with or after the formation of the tunica albuginea, which occurs under the influence of the anti-Müllerian hormone, secreted by differentiating Sertoli cells [12, 14]. In fact, it arises after the organization of sex cords is complete and

Sertoli cells already show some degree of maturation [23], possibly around the same time the primordial sex cords lose their connection with the celomic epithelium [9]. Another explanation for the pathogenesis of ETP could be a focal delay in tunica albuginea formation, allowing "penetration" of testicular parenchyma within it. However, one important difference between the two lesions described here is that all of our PTB cases showed bilateral involvement, whereas cases of ETP in both the reported cases and our series were unilateral. Nevertheless, as most of the cases of ETP were seen in orchiectomy or biopsy specimens, the contralateral testes were not studied to rule out bilateral lesions.

In conclusion, both PTB and ETP represent uncommon findings in testicular pathology, associated with a developmentally normal adjacent parenchyma. Although it is not clear whether these lesions may carry a potential for malignant transformation, their current clinical importance lies in their differential diagnosis with GD and also with other cystic lesions of the tunica albuginea, mainly in testicular biopsies, thus preventing these patients from unnecessary orchiectomy. Understanding of these findings may also shed light on our knowledge regarding biological mechanisms involved in testis development.

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CASE REPORT

Hepatoportal venopathy due to disseminated *Mycobacterium avium* complex infection in a child with IFN-γ receptor 2 deficiency

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Abstract The control of intracellular microorganisms such as mycobacteria is largely dependent on the adaptive immune response, specifically the interaction of T helper cells and antigen presenting cells such as macrophages. The interferon gamma (IFN-γ) pathway activation is crucial for containment and killing of mycobacteria, as evidenced by the fact that defects in this pathway often result in profound infections with both tuberculous and non-tuberculous mycobacteria. We herein report a case of a child with autosomal recessive IFN-γ receptor 2 (IFN-γR2) deficiency who developed hepatic venopathy secondary to disseminated *Mycobacterium avium* complex (MAC) infection.

Keywords Obliterative venopathy · Interferon · Mycobacteria

Abbreviations

IFN (R)	interferon (receptor)
MAC	*Mycobacterium avium* complex
NK	natural killer
TNF	tumor necrosis factor
IL-12	interleukin-12
NIH	National Institute of Health
GM-CSF	granulocyte macrophage-colony stimulation factor
STAT	signal transducer and activator of transcription

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Introduction

Interferon gamma (IFN-γ) is a cytokine critical for the management of intracellular organisms. Normally produced by T lymphocytes and natural killer (NK) cells, IFN-γ stimulates macrophages to elaborate IL-12 and TNF-α, which in turn stimulate helper T cells and NK cells. Infection with mycobacteria also stimulates macrophage production of IL-12. The IFN-γ receptor (IFN-γR) complex is composed of ligand binding IFN-γ receptor 1 chains and signal transducing IFN-γ receptor 2 (IFN-γR2) chains. Upon binding of IFN-γ, IFN-γR1 dimerizes and engages two chains of IFN-γR2, the limiting moiety. Aggregation of these chains apposes the constitutively associated Jak1 and Jak2 molecules, leading to their phosphorylation and activation of STAT1. This signal transduction pathway upregulates a multitude of genes involved in both the innate and adaptive immune systems, including TNF-α and IL-12. These actions culminate in macrophage activation and bactericidal activity [6]. Genetic defects found at multiple points along the IFN-γ/Jak/STAT pathway result in susceptibility to non-tuberculous mycobacterial infection [4, 9]. IFN-γR2 deficiencies have been described due to mutations in the extracellular and transmembrane domains [18].

Clinically, patients with IFN-γR2 deficiency present with non-tuberculous mycobacterial infections predominately due to *Mycobacterium avium* complex (MAC) infection. *Mycobacterium fortuitum*, cytomegalovirus, and herpes simplex virus (HSV) infections have also been reported [18]. However, only a few cases have been reported so far, thereby preventing a comprehensive description of the disease.

Disseminated MAC infections have been reported in patients with IFN-γR1 deficiency [14]. MAC peritonitis in the setting of disseminated MAC infection in an AIDS patient has been described [11]. Hepatic vein obliteration with subsequent portal hypertension has not been described in MAC infection. Liver findings in patients with disseminated MAC infection include ill-formed non-caseating portal granulomas formed by Kupffer cells. These macrophages are often distended by large numbers of MAC [13]. Liver involvement by systemic conditions and resultant portal hypertension can occur in association with a variety of conditions such as Gaucher's disease, sarcoidosis, Wilson's disease, and infections such as schistosomiasis [7, 17]. Clinically, patients can present with portal hypertension, congestive splenomegaly, esophageal varices, pancytopenias, and gastrointestinal bleeding. The underlying histopathogenesis of these conditions is generally agreed to be an obliterative venopathy of the portal system, with collapse, thrombosis, or occlusion of the portal veins. Suffocation and obliteration of the venous system occurs secondary to involvement of macrophages by storage products, as in Gaucher's disease, or through the formation of granulomas and fibrosis, as in schistosomiasis and sarcoidosis.

We report a child with autosomal recessive IFN-γR2 deficiency who developed hepatoportal venopathy due to disseminated MAC infection.

Clinical history

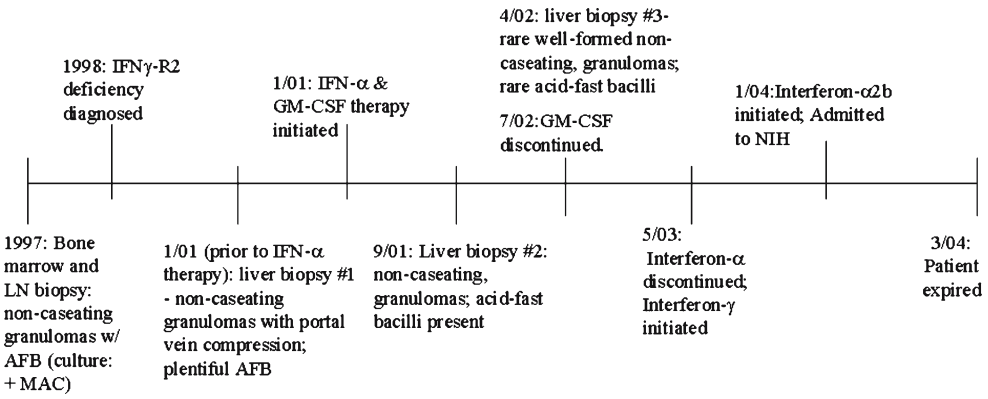
The patient was an HIV-negative 9-year-old boy born to unrelated American parents at 26 weeks. He did not receive the *Bacille Calmette-Guérin* immunization but did receive the usual childhood immunizations. At age 20 months, he presented with cough, cold symptoms, and pulmonary infiltrates. At 2 years, he developed lymphadenopathy, hepatosplenomegaly, and fevers; bone marrow and lymph node biopsies showed non-caseating granulomas containing acid-fast bacilli. Culture of the lymph node biopsy grew *M. fortuitum* and MAC. Despite improvement on antimycobacterial therapy including rifabutin, azithromycin, ciprofloxacin, ethambutol, and amikacin, the patient continued to have lymphadenopathy. He was referred to the NIH Clinical Center where complete autosomal recessive IFN-γR2 deficiency due to a two-base deletion in the extracellular domain leading to a frame shift and premature stop was identified [3]. The patient was managed for 4 years with antibiotics. A liver biopsy (1) was performed in January 2001 for elevation of liver enzymes. HSV1 esophagitis responded to acyclovir. Recurrent mild HSV1 of the thumb was suppressed by chronic administration of acyclovir. Splenomegaly was complicated by infarction leading to splenectomy. Recurrent ampullary stenosis due to mycobacterial infiltration required biliary stenting with frequent revisions. Chronic protein-losing enteropathy and malabsorption were ascribed to small bowel lymphangectasia. Between January 2001 (after biopsy 1) and May 2003, the patient received IFN-α and granulocyte macrophage-colony stimulation factor (GM-CSF) injections in an effort to induce macrophage antimycobacterial activity through activation of Jak/STAT pathways other than through the IFN-γ receptor. GM-CSF (100 mcg) was administered subcutaneously three times a week from January 2001 until July 2002. Liver biopsy 2 was performed in September 2001, after the patient had been receiving IFN-α and GM-CSF along with anti-mycobacterial therapy for 8 months. Liver biopsy 3 was performed in April 2002. Due to diffuse musculoskeletal pain, and sclerotic bony lesions in May 2003, IFN-α was discontinued, and IFN-γ was initiated.

The patient was readmitted in January 2004 for ascites, multiple ill-defined low-density liver lesions and painful, generalized lymphadenopathy. IFN-γ therapy was discontinued, and IFN-α 2B was restarted. Anti-mycobacterial therapy continued. His laboratory studies showed white blood cells $11.7\times10^3/\mu l$, hemoglobin 6.3 g/dl, hematocrit 21%, platelet count $58{,}000\times10^3/\mu l$, prothrombin time 13 s, partial prothrombin time 32.9 s, total protein 4 g/dl, albumin 2.1 g/dl, aspartate aminotransferase 63 U/l, alanine aminotransferase 28 U/l, alkaline phosphatase 1,082 U/l, total bilirubin 1.5 mg/dl, hepatitis B and C serology negative. Despite treatment, the patient died in March 2004. The patient's clinical course is summarized in Table 1.

Materials and methods

The patient underwent three liver biopsies at various time points as part of his clinical care while enrolled in the NIH protocol 93-I-0119. A full, unrestricted consented autopsy was performed on March 5, 2004. After formalin fixation, paraffin-embedded tissue sections were processed for hematoxylin and eosin (H and E) and Masson trichrome stained sections, as well as stained for acid-fast bacilli with Ziehl–Nielsen staining.

Table 1 Clinical summary timeline



Results

Liver biopsy 1 showed a moderate portal lymphohistiocytic infiltrate consisting of non-caseating granulomas with proliferation of bile ductules in the portal areas involving most of the portal areas in the biopsy. Early fibrosis, as determined by Masson trichrome stain, was present within the portal tract without evidence of bridging. The granulomas were ill formed and compressed the adjacent portal veins. Plentiful mycobacteria were demonstrated in the cytoplasm of the histiocytes (Fig. 1a and inset).

Liver biopsy 2 showed a mild granulomatous hepatitis involving isolated portal triads (Fig. 1b). Similar to biopsy 1, the granulomas were ill formed with inflammation spilling into the lobules. The portal tracts showed portal fibrotic expansion without bridging. Sinusoidal dilatation was present; however, the central veins were uninvolved.

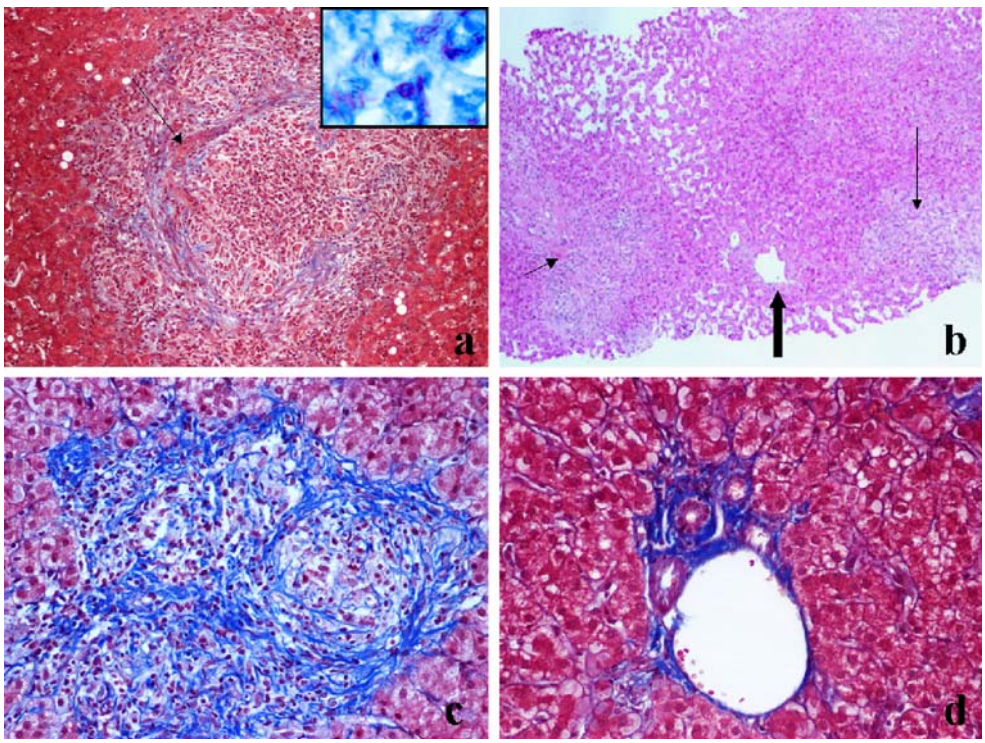


Fig. 1 **a** Liver biopsy 1, granulomatous portal inflammation with compression of the portal vein. This portal area shows a moderate mixed lymphocytic and histiocytic infiltrate that obliterates the central vein. Only a remnant portion of the collagen fibers (*blue*) and smooth muscle of the central vein is seen (*red*, *arrow*) (Masson trichrome, ×400). *Inset*, liver biopsy 1, numerous acid-fast bacilli (*arrow*) are present in the cytoplasm of the histiocytes (modified Ziehl–Nielsen, ×600) **b** Liver biopsy 2, isolated portal granulomatous infiltrates. Two portal areas are infiltrated by histiocytes and small lymphocytes (*thin arrow*). Fibrosis is seen extending out from the portal area (*short arrow*). The central vein is unremarkable (*thick arrow*). The sinusoids show dilatation. (H and E, ×40) **c** Liver biopsy 3, portal area with a well-formed granuloma and brisk chronic inflammatory infiltrate surrounded by early collagen formation (*blue*). The portal vein is compressed. (Masson trichrome, ×400). **d** Liver biopsy 3, an unremarkable portal triad with a patent portal artery and vein. (Masson trichrome, ×400)

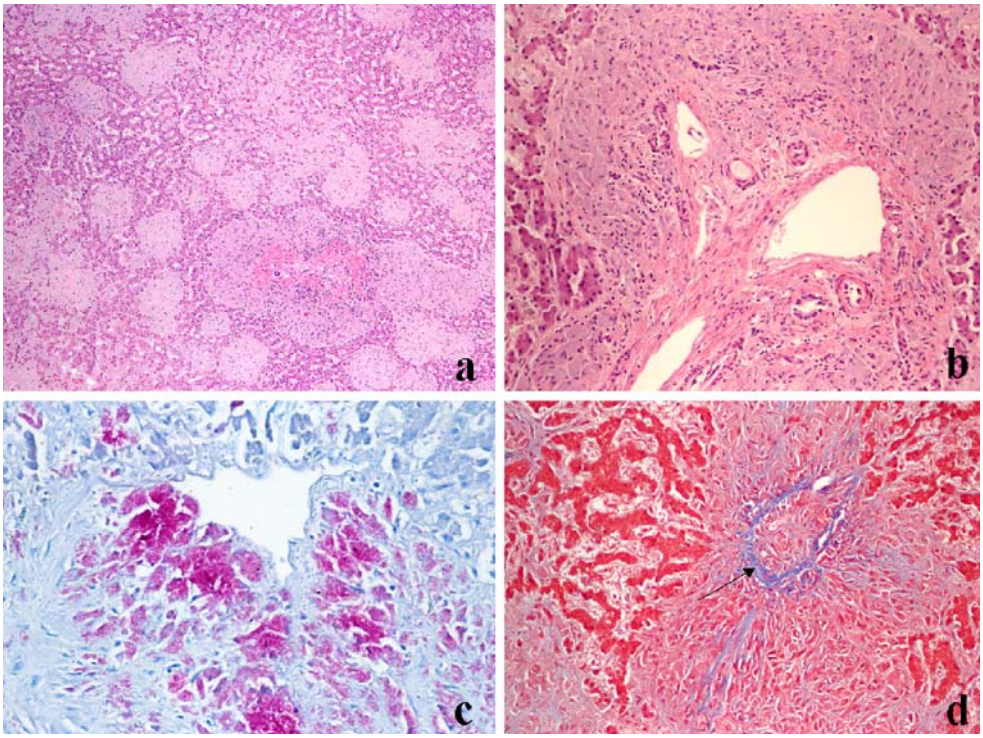


Fig. 2 **a** Autopsy liver section, multifocal well-formed non-caseating granulomas (H and E, ×40). **b** Autopsy liver section, a portal area expanded by histiocytes compressing the venous lumina (H and E, ×400). **c** Autopsy liver section, histiocytes distended by acid-fast bacilli compressing a central vein (Ziehl–Nielsen, ×400). **d** Autopsy liver section, fibrous obliteration of a central vein (*arrow*), the remnant collagen fibers are identified by *pale blue staining* (Masson trichrome, ×400)

Liver biopsy 3 was performed in April 2002 while the patient was still on IFN-α and GM-CSF therapy and showed a dramatic decrease in the number of granulomas. Most portal areas were unremarkable, and only a rare isolated well-formed non-necrotizing, granuloma was seen. A small number of acid-fast bacilli were found with special staining. This well-formed granuloma was associated with a brisk lymphocytic infiltrate (Fig. 1c). The majority of the portal areas appeared normal and were uninvolved by inflammation or fibrosis (Fig. 1d). As compared to the liver biopsies 1 and 2, the granulomas, which were seen during IFN-α treatment in biopsy 3, were rare, smaller, better formed, and had more lymphocytic infiltrate.

Autopsy findings

A full, unrestricted autopsy found that the peritoneal cavity contained 750 ml of serous ascites. The liver was 2,100 g (normal 560 g), with bile staining but without gross nodules. The cut surface of the liver showed diffuse stellate-shaped lesions, some with central degeneration, measuring from 1 to 2 cm in maximum dimension.

A diffuse, granulomatous infiltrate was present in all portal areas and also involved the lobules and central veins. The granulomas consisted of aggregates of pale-staining histiocytes distended by large numbers of acid-fast bacilli (Fig. 2a and c). Very few lymphocytes were present. The portal and central veins were either compressed or frankly obliterated by these non-caseating granulomas, which were associated with mild fibrosis (Fig. 2b). The hepatocytes showed atrophic changes with an increase in the nuclear/cytoplasmic ratio. Sinusoidal congestion was seen. The remnant of collagen around central veins could be demonstrated with trichrome staining (Fig. 2d).

In summary, all three pre-mortem liver biopsies showed the central veins to be relatively uninvolved by the granulomatous inflammation. The vascular blockage was predominately pre-hepatic involving the portal areas. At autopsy, there was a combination of pre- and posthepatic obstruction such that the hepatocytes in the lobule showed atrophic changes, but frank ischemic changes and/or necrosis was not seen.

Discussion

Veno-occlusive disease and hepatoportal venopathy have been described in patients with central and portal hepatic vein involvement due to storage diseases, sarcoidosis, primary biliary cirrhosis, primary sclerosing cholangitis, mastocytosis, cystinosis, schistosomiasis, and graft-vs-host disease [5, 7, 15]. Granulomatous disease associated with non-cirrhotic portal hypertension is rarely reported; primary biliary cirrhosis and sarcoidosis are the most frequent causes [12, 16]. Ascites is infrequently reported in portal venopathy and, in this case, may have been a complication of either the hepatoportal venopathy, the small bowel lymphangectasia/mesenteric lymphadenitis, or both. Non-

tuberculous mycobacterial infection has not been reported to cause portal venous obliteration in either HIV or non-HIV infected patients.

Patients with complete autosomal recessive IFN-γ2R deficiency show a total loss of normal IFN-γ signal activation and do not clinically respond to either endogenous or exogenous IFN-γ therapy. IFN-α shares with IFN-γ activation of the common downstream effector molecule, STAT-1, that served as the rationale for its use and may explain the clinical and histopathologic changes during IFN-α treatment. A similar use of IFN-α has recently been reported, and this also appeared to facilitate mycobacterial control [19]. IFN-γ has been used to treat patients with defects in the IL-12/IFN/Jak/STAT pathway with non-tuberculous mycobacterial infections who have residual IFN-γR activity [9].

The causes of the obliteration of the intrahepatic portal and central veins in the current case may be twofold. Firstly, the diffuse MAC infection with granulomatous inflammation may have compressed the venous lumina, mostly portal but both central and portal, as seen at autopsy (Figs. 1a and 2b and c). Secondly, the intermittent resolution of the granulomas seen during treatment may have spurred a reactive fibrotic response with resulting phlebosclerosis and fibrous obliteration, as observed at autopsy (Fig. 2d). Both processes would result in resistance to portal venous flow and portal hypertension. Clinically, we observed worsening of granulomatous inflammation when the patient was not undergoing treatment with IFN-α, contrasting with more well-defined granulomatous inflammation and clinical improvement during IFN-α therapy. The partial resolution and quality of the granulomas and MAC organism burden correlated with the duration of IFN-α and antibiotic treatment. This improvement was seen most dramatically in liver biopsy 3 (Fig. 1c,d), taken when the patient had been treated for 16 months with IFN-α and anti-mycobacterials. There was markedly decreased granulomatous inflammation and decreased MAC burden in biopsy 3 as compared to liver biopsy 1, taken before IFN-α therapy was initiated. The granulomas, which were seen during IFN-α treatment in biopsy 3, were smaller, better formed, and had more lymphocytic infiltrate than in liver biopsies 1 and 2. Acid-fast bacilli were rare and difficult to identify in biopsy 3 as well, in contrast to 1. Liver pathology from times of nontreatment showed ineffective granulomatous inflammation with abundant mycobacteria that involved the portal and central regions of the liver. These granulomas impinged on the venous lumina and obliterated some vessels. At autopsy, the patient had been receiving IFN-α for the second course for only 3 months and, before that, had received IFN-γ therapy for 7 months; anti-mycobacterials had been administered throughout. Although the granulomas were starting to appear well formed at autopsy, the MAC burden was quite high, and venous obliteration was observed. At autopsy, the vascular obstruction was both pre- and posthepatic, with atrophy of the hepatocytes and sinusoidal congestion.

Responding to IFN-γ stimulation is necessary for the containment of mycobacteria [10]. The IFN-γ response may also control the pro-fibrogenic milieu created by granulomatous inflammation. The anti-fibrogenic property of IFN-γ has been described in hepatitis C virus-infected patients [2]. IFN-γ and IFN-α have been used in other clinical settings for the prevention of fibrosis, such as in idiopathic pulmonary fibrosis [1]. The inability to respond to IFN-γ, as seen in patients with IFN-γR2 deficiency, may have contributed to the development of fibrosis after an inflammatory insult, as was seen in the current case.

Hepatoportal venopathy is an uncommon cause of non-cirrhotic portal hypertension in the Western world. Our case developed hepatoportal venopathy and veno-occlusive disease presumably secondary to disseminated MAC infection. The partial resolution of the granulomatous inflammation and MAC in response to IFN-α therapy reinforces the integral role that this cytokine pathway may play in controlling mycobacterial infections. This unusual clinical presentation may help better define the phenotype of patients with IFN-γR2 deficiencies as well as the importance of the IFN-α and IFN-γ pathways in controlling intracellular infection.

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CASE REPORT

Hodgkin's lymphoma in a patient with Jo-1 syndrome

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Abstract Jo-1 syndrome is an autoimmune disease with autoantibodies against the histidyl tRNA synthetase. Characteristic clinical findings include inflammatory myopathy and interstitial lung disease. We present the first case of a patient with Jo-1 syndrome (positive Jo-1 autoantibodies, myositis, interstitial alveolitis) who developed Hodgkin's lymphoma of nodular-sclerosing type. Thus, patients with Jo-1 syndrome and immunosuppressive therapy similar to other patients with autoimmune disease are at risk to develop lymphomas and should therefore be monitored carefully.

Keywords Anti-synthetase-syndrome · Jo-1 syndrome · Hodgkin's lymphoma · Myositis · Alveolitis

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Introduction

Patients with autoimmune disease have been suspected to develop malignancies with increased frequency. In particular, an association of non-Hodgkin lymphoma in patients with systemic lupus erythematosus, rheumatoid arthritis and primary Sjögren syndrome has been demonstrated. Reports about malignancies associated with anti-tRNA-synthetase autoimmune diseases are very rare. In Jo-1 syndrome, patients present with myopathy, interstitial pulmonary fibrosis and arthritis. Antibodies against the histidyl tRNA synthetase are diagnostic. In this paper, we report the first case of a patient with Jo-1 syndrome who developed Hodgkin's lymphoma.

Clinical history

A 47-year-old male patient presented with myalgia, exertional dyspnea (pO_2 40 mmHg), fatigue and a generalised erythema, predominantly affecting the proximal lower limbs and the face. Electromyography was normal, and biochemical myositis markers such as LDH [max. 565 U/l (104–248 U/l)] and creatine kinase [max. 334 U/l (<80 U/l)] were elevated. A myocardial source for CK elevation was excluded by isotyping and coronary angiography. Computed tomography (CT) scans revealed ground glass pattern opacity of both entire lungs compatible with interstitial lung disease (Fig. 1). Due to respiratory impairment, open lung biopsy was not attempted. Bronchioalveolar fluid analysis revealed a mixed neutrophilic and eosinophilic granulocytosis. Serologic detection of Jo-1- and SS-A-antibodies was finally sufficient to confirm the clinical diagnosis of Jo-1 antisynthetase syndrome. On treatment with corticosteroids and azathioprin, the respiratory symptoms improved. Three years

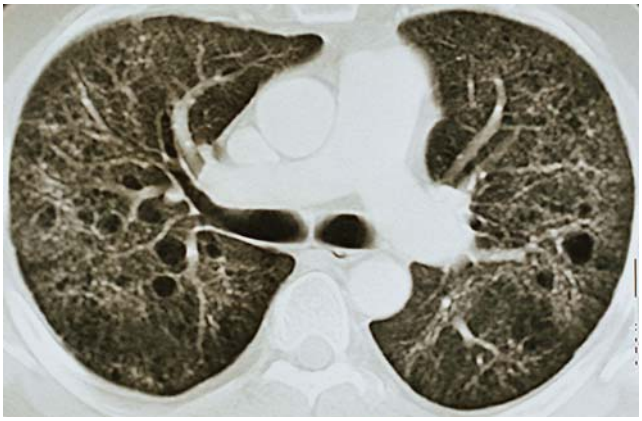

Fig. 1 CT scan of the chest. Both lungs show ground glass pattern opacity, interstitial scaring and bronchiectasis

later, fatigue and dyspnea began to worsen again. Treatment with methotrexate (MTX) failed to improve the clinical symptoms. During the following year, a severe respiratory insufficiency developed, necessitating the regimen to be changed to continuous cyclophosphamide and corticosteroids in a modified Fauci protocol. In addition, 15 courses of plasma apheresis were performed, and the endothelin receptor antagonist bosentan was added to the regimen. The course remained uneventful for the next 14 months. Subsequently, mediastinal lymphadenopathy was discovered by CT scan, and bilateral axillar and supraclavicular enlarged lymph nodes were noted on palpation. Right supraclavicular lymph nodes were removed for histological analysis.

Materials and methods

The study was performed in accordance with the guidelines of the local ethics commission. Histological material was fixed in 4% formalin and embedded in paraffin according to standard procedures. One to two micrometre sections were used for conventional and immunohistochemical staining procedures. The following antibodies were used: CD15 (1:50; Hölzel, Köln, Germany), CD20 (1:1,000; Dako, Hamburg, Germany), CD30 (1:10; Dako), CD3 (1:25; Menarini, Neuss, Germany), Ki67 (1:2,000; Dako), LMP-1 (1:200; Novocastra Laboratories, Newcastle Upon Tyne, UK), PAX5 (1:50; BD Biosciences, Heidelberg, Germany). For antigen retrieval, deparaffinised sections were heated in 10 mM citrate buffer pH 6.0 either for 5 min (LMP1, PAX5), respectively, 10 min (CD15, CD30) at 2 bar using a pressure cooker or for 30 min at boiling temperature in a microwave oven (CD20, CD3, Ki67). Immunohistochemistry was performed with diaminobenzidine as substrate. Epstein–Barr virus (EBV)-encoded RNA (EBER) in situ hybridisation was performed according to the previously published protocol (EBER probe Y-5200, PNA-ISH-detection kit K-5201, Dako) [7]. Slides were counterstained with hematoxylin. For T cell clonality analysis, the receptor gamma-chain was amplified following the standard procedures [5] and analysed using capillary electrophoresis.

Results

Morphology

The lymph node section showed an almost completely destroyed lymph node architecture and a mixed infiltrate consisting of spindle cells, plasma cells, eosinophilic granulocytes and lymphocytes. Classical Hodgkin and Reed–Sternberg cells (HRS) were detected besides sparse lacunar cells. Isolated mitotic figures were visible. The infiltrate was separated by broad bands of fibrosis and extended into the surrounding adipose tissue (Fig. 2a).

Immunohistochemistry and in situ hybridisation

Immunohistochemical staining demonstrated scattered single atypical cells with large prominent bilobed nuclei and nucleoli of the Hodgkin and Reed–Sternberg cell type which were positive for CD15 and CD30 (Fig. 2b, c), as well as for BSAP (PAX5; Fig. 2f) with the typical weaker staining of the HRS cells compared to reactive B cells [9]. CD20 staining was negative. Tumour cells were positive for EBV-related antigens as shown by LMP1 (Fig. 2e) [3]. Likewise, EBER in situ hybridisation (Fig. 2e) indicated prior EBV infection. Anti-CD3 showed diffusely scattered small bland T lymphocytes (not shown). There was up to 30% proliferative activity as detected by anti-Ki-67 staining (not shown).

T cell receptor gamma-chain PCR

The oligoclonal rearrangement pattern of the T cell receptor γ-chain was consistent with Hodgkin lymphoma, thus, confirming the diagnosis of Hodgkin's disease of nodular-sclerosing type.

EBV-specific antibodies

Antibodies directed against the Epstein–Barr viral capsid antigen (VCA) were detected in the serum at the time of diagnosis. Positive IgG and negativ IgM anti-EBV-VCA antibodies indicated a past, not acute, EBV infection.

Discussion

The Jo-1 syndrome belongs to the group of anti-tRNA-synthetase autoimmune diseases. These syndromes are

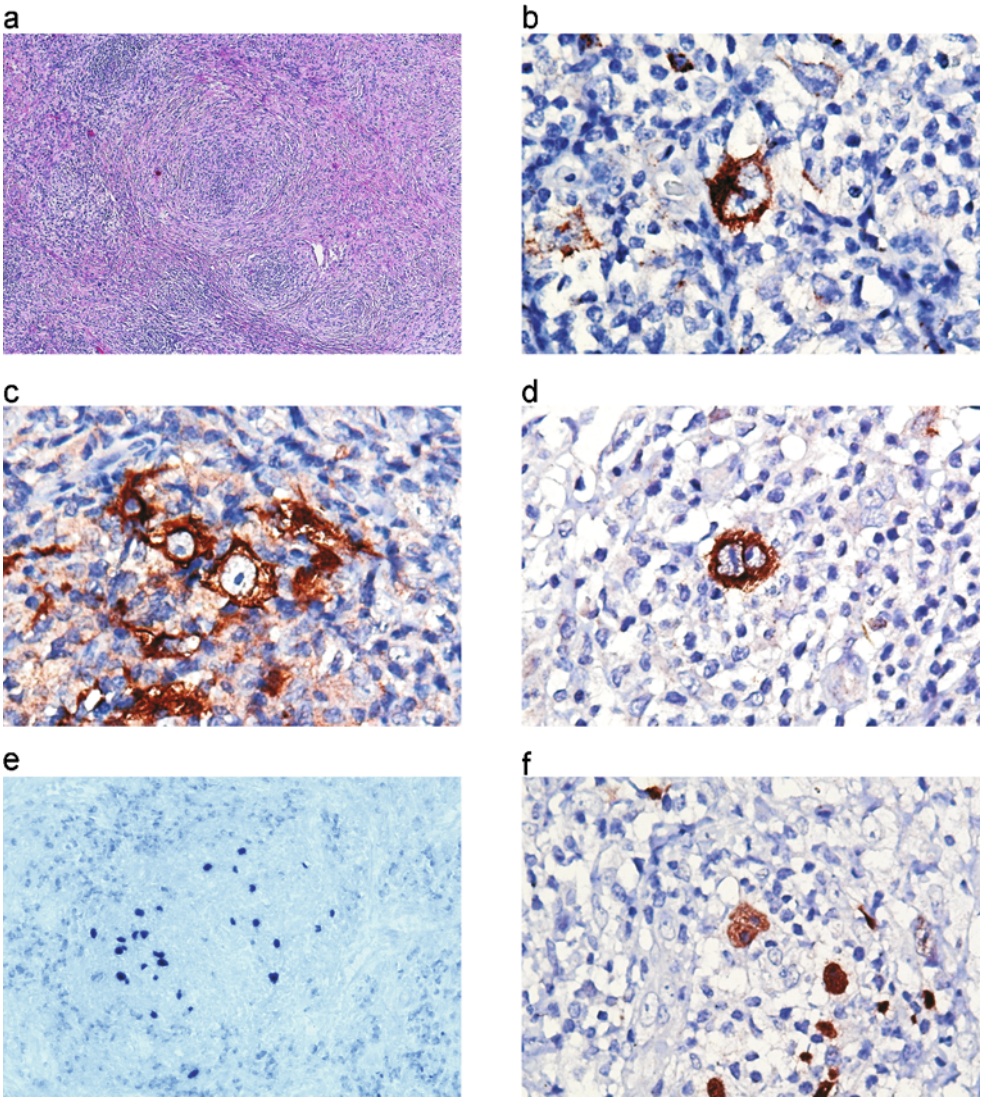

Fig. 2 **a** Hodgkin's disease, nodular-sclerosing type, with effaced lymph node architecture caused by a mixed inflammatory infiltrate consisting of eosinophilic granulocytes, lymphocytes, plasma cells and fibroblasts (HE, 50×). **b** Classical Reed–Sternberg cells expressing CD15 and **c** CD30 in a Golgi-like pattern (DAB, 400×). **d** EBV-associated LMP-1 expression in a RS cell (DAB, 400×). **e** EBER in situ hybridisation (100×). **f** BSAP (PAX5) expression of EBV-infected RS cells (weak staining) and reactive B cells (strong staining; DAB, 400×)

characterised by the presence of antibodies against different types of cytosolic aminoacyl–tRNA synthetases. In Jo-1 syndrome, antibodies against the histidyl tRNA synthetase are present in the serum. Inflammatory myopathy is found in nearly all cases, whereas other frequent clinical signs are interstitial pulmonary fibrosis, arthritis and elevated inflammation parameters [6, 8]. Fever, Raynaud phenomenon, mechanic's hands and carpal canal syndrome can be found in a subset of cases.

We report the first case of Hodgkin's lymphoma occurring in a patient with Jo-1 syndrome.

A linkage between autoimmune myositis and malignancies has frequently been observed. Dermatomyositis seems to be the most often linked entity [1], whereby the cause of this association is still unclear. Association of Jo-1 syndrome and malignancies is very rare. Watkins et al. [10] reported a case of poorly differentiated adenocarcinoma in a patient with Jo-1 antibody syndrome. Iwasaki et al. [4] presented a patient with polymyositis, interstitial pneumonitis and a positive Jo-1 antibody test in association with renal cell carcinoma. In this paper, we report a case of Jo-1 syndrome (Jo-1 autoantibodies, myalgia, interstitial lung disease, erythema) associated with Hodgkin's lymphoma. In our case, Hodgkin's disease was observed 3 years after onset of Jo-1 syndrome, first treated with MTX, and 14 months after intensified immunosuppressive therapy with cyclophosphamide.

Hodgkin's lymphoma in immunocompromised patients is rare and less frequent than B cell lymphomas [11]. But still, there is an elevated prevalence of Hodgkin's disease in immunosuppressed patients, including HIV patients, recipients of solid organ or bone marrow transplantation or patients with autoimmune disease treated with immunosuppressive drugs.

In our patient, Hodgkin's disease might be associated with the long-lasting immunosuppressive therapy rather than caused by the Jo-1 syndrome itself. However, a contribution to the pathogenesis by the underlying autoim-

mune disease is possible. One reason for the coincidence of the autoimmune disease and/or immunosuppressive treatment with lymphoma may be a deviation of the host's immune response leading to a defective immune surveillance to oncogenic viruses like EBV [2]. Interestingly, in our case, prior EBV infection could be demonstrated serologically by anti-EBV-VCA IgG type antibodies by positive LMP-1 immunostaining and EBER in situ hybridisation in the lymphoma cells.

Clearly, additional examples of occurrence of Jo-1 syndrome and malignant lymphomas need to be analysed to prove a significant association and study of mechanisms.

Acknowledgments We thank Prof. Hans H. Schild, director of the Department of Radiology, University of Bonn, for providing the CT scan image.

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LETTER TO THE EDITOR

Over-estimation of radiation-induced malignancy after the Chernobyl accident

Sergei V. Jargin

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Dear Editor,

The article by Romanenko et al. published in Virchows Archiv [6] is based on the study of renal tumours from the Chernobyl area and Kiev. Alterations of extracellular matrix (ECM) in renal cell carcinoma are reported. The main conclusion is that long-term low-dose ionising radiation (IR) "can promote malignant tumour progression" through the following mechanism: "significantly disrupted ECM with a lack of orchestrated communication between cells and between cells and ECM that leads to loss of cellular differentiation". It should be noted that the term "low-dose IR" would be relevant if radioactivity were counted from zero, but in fact it is a question of a minor increase of doses obtained due to the natural radiation background. Patients from contaminated areas around Chernobyl and Kiev are united in one group, although radioactivity in Kiev was elevated insignificantly [1], which can give rise to reasoning about radiation-induced malignancy in Kiev.

On the basis of limited material (contaminated area plus Kiev, 41 patients; control, 37 patients), statistically significant differences were found for 4 parameters: immunohistochemical expression of fibronectin, laminin, β-catenin and TGF-β1 (P values are 0.05, 0.008, 0.003 and 0.01, respectively); the integral level of statistical significance being very high for a medical study. At the same time, majority if not all tumours should have been spontaneous because cancer incidence (except for thyroid carcinoma) remained in the contaminated area within statistical error in agreement with the increase rate for Russia in general [2, 5]. The male/female ratio=27/14, approximately the same as for renal cell carcinoma in the general population, provides additional evidence in favour of spontaneous origin. The main features of spontaneous tumours must be on average the same as in the control group. Besides, comparison between the group from the contaminated zone plus Kiev and the control is performed without standardisation after the tumour grade: more than 50% of cases in the first group belong to poorly differentiated carcinoma (G3–G4), whereas in the control there is only 1 G3 case, all others being G1–G2. Without representative statistics about tumour grading, reported data can be misleading, making an impression that renal carcinoma in the contaminated areas and Kiev is on average less differentiated than in the general population. Reported differences between the groups could have been caused by differences in differentiation grade, which in its turn could have resulted from non-random case selection. In previous publications [3, 4], we criticised other studies by Romanenko et al. For further details, visit http://www.freewebs.com/ruspat1/

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LETTER TO THE EDITOR

Response from authors of "Extracellular matrix alterations in conventional renal cell carcinomas by tissue microarray profiling influenced by the persistent, long-term, low-dose ionizing radiation exposure in humans"

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The Chernobyl accident introduced for the first time the problem of chronic, persistent, long-term, low-dose exposure to ionizing radiation (IR) in humans. Currently, 21 years after the accident at the Chernobyl Power Plant in Ukraine (70 Km from Kiev), approximately 17 million people live in the radiocontaminated area and have been exposed to low-dose IR. Information regarding irradiation of the Ukrainian population is based on the official reports provided by the Ukrainian Government and the Ukrainian Academy of Medical Sciences. Morbidity of malignant renal tumors in adults during the period 1986–2006 increased from 4.7 to 9.9 per 100,000 of total population (from 6.0 to 12.6 per 100,000 of the male population and from 3.6 to 7.5 per 100,000 of the female population in Ukraine) [4].

The scientific collaboration between the Institute of Urology, the Academy of Medical Sciences in Kiev (Ukraine) and the Department of Pathology of the Valencia University in Spain has been in operation for approximately 9 years already. Recent studies by our group have shown that during the period subsequent to the Chernobyl accident, increases in morbidity, aggressivity, and proliferative activity of renal-cell carcinomas (RCCs) in Ukrainian patients were recognized [2]. At the beginning of our study, we did separate patients with RCCs from the city of Kiev and patients with RCCs from radiocontaminated areas. Our recent studies have shown that RCCs (their histological and molecular biological features) of both these groups were identical (it is therefore not necessary to distinguish them); however, they significantly differ from the analogous tumors from clean (without radio-contamination) areas of Ukraine as well as the Spanish RCCs.

It must be noted that the tumors (RCCs) were randomly selected (successive cases) from the laboratories of Kiev and Valencia. No one doubts the spontaneous origin of these RCCs in both geographical areas. The main differences are not in reference to their origin but to their biological, histological, and clinical behavior, which is clearly more aggressive in the Ukrainian population in comparison with the Valencian cases. Furthermore, "chronic low-dose irradiation toxicity" (CLDIT) exerts its effect as a cocarcinogen or within a pluri-carcinogenetic context. We have never proposed that CLDIT was the only cause of this cancer.

The development of "radiation sclerosing proliferative atypical nephropathy" in the peritumoral kidney tissue then followed and demonstrated a good correlation with the duration of radiation exposure [3]. In addition, the influence of chronic, regular, and sustained low-dose IR on renal carcinogenesis in the population living in Cesium 137-contaminated areas of Ukraine has been confirmed [2].

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There is no dose–response relationship in the range 2–12 Gy, suggesting that an unstable phenotype may be induced by quite low radiation doses [1]. Moreover, the "bystander" or nontargeted effects of very low doses of IR were recently described as important genetic consequences of IR, which may arise in cells that in themselves received no direct nuclear exposure [1]. Thus, the actual target for radiation effect in a cell population may be much larger than the irradiated cell itself [5].

Research during the last decade has focused on cellular and molecular mechanisms for the effects of low-level IR in mammalian cells as well as in mammalian carcinogenesis. Our group follows the same scientific direction.

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LETTER TO THE EDITOR

TTF-1 staining in glioblastoma multiforme

Malcolm Galloway · Rosalind Sim

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Dear Editor,

Agaimy and Wunsch recently reported potentially misleading thyroid transcription factor 1 (TTF-1) immunoreactivity in a primary ovarian malignant mullerian mixed tumour [1]. This is one of several recent papers highlighting the danger of relying on TTF-1 expression in isolation as a marker of either lung or thyroid origin for a malignant tumour. Comperat et al. reported that 5% of primary colorectal adenocarcinomas expressed TTF-1 when assessed with Novocastra's SPT24 clone [2]. Penman et al. also found that the SPT24 but not the 8G7G1 TTF-1 clone showed nuclear positivity in some colorectal adenocarcinomas [3].

We would like to highlight the observation that immunocytochemical TTF-1 positivity may be found in glioblastoma multiforme, which to our knowledge has not previously been reported. Usually, the distinction between glioblastoma multiforme and metastatic carcinoma is relatively easy to make on morphological grounds; however, there are some cases in which the tumour is poorly differentiated, and for such cases, immunocytochemistry may be of value in making this distinction.

Following our observation in routine diagnostic practice of two cases of glioblastoma which showed positivity with antibodies to the SPT24 clone of TTF-1 (Fig. 1), a further 26 cases of glioblastoma multiforme and one case of gliosarcoma were assessed. In all cases, there were morphological, immunocytochemical, radiological and clinical features supporting the diagnosis of glioblastoma, and none had any evidence of metastatic disease or of any pre-existing lower grade cerebral neoplasm.

Immunocytochemical staining for the SPT24 (Novocastra) and 8G7G3/1 (Dako) TTF-1 clones, MNF116, CK7, CK20 and glial fibrillary acidic protein (GFAP) was performed in all cases. Staining for the TTF-1 clone SPT24 (Novocastra) was performed with 2 min of pressure cooking pretreatment in 2 l of 10 mM citrate buffer at pH 6.0. The primary antibody was diluted at 1:100 and incubated for one hour at room temperature. Standard ABC method was performed using Vector's RTU Universal Elite ABC kit PKY200. The staining was developed for 10 min in diaminobenzidine. The technique for TTF-1 clone 8G7G3/1 (Dako) immunocytochemistry was identical, other than that the primary antibody was diluted at 1:50. To calculate percentages of positive cells, at least 500 presumed neoplastic nuclei per case were counted from the areas in which staining appeared most abundant.

In total, fourteen glioblastomas (50%) showed some degree of nuclear positivity when stained with the SPT24 TTF-1 clone. This staining was patchy and variable between cases (ranging from 0.1 to 62.2% of nuclei). Nuclear positivity was seen in under 2% of cells in seven cases, between 2 and 10% of cells in three cases, and over 30% of cells in the other four cases. Positive neoplastic nuclei were not detected in the other fourteen cases of glioblastoma or in the gliosarcoma. All cases were negative when stained with the 8G7G3/1 TTF-1 clone. Staining for GFAP was positive in the cytoplasm of at least a proportion of the neoplastic cells in all cases. MNF-116 and cytokeratin 20 staining was negative in all cases. Cytokeratin 7 staining was negative in all cases of glioblastoma, but showed focal positivity in the spindle cell element of the gliosarcoma.

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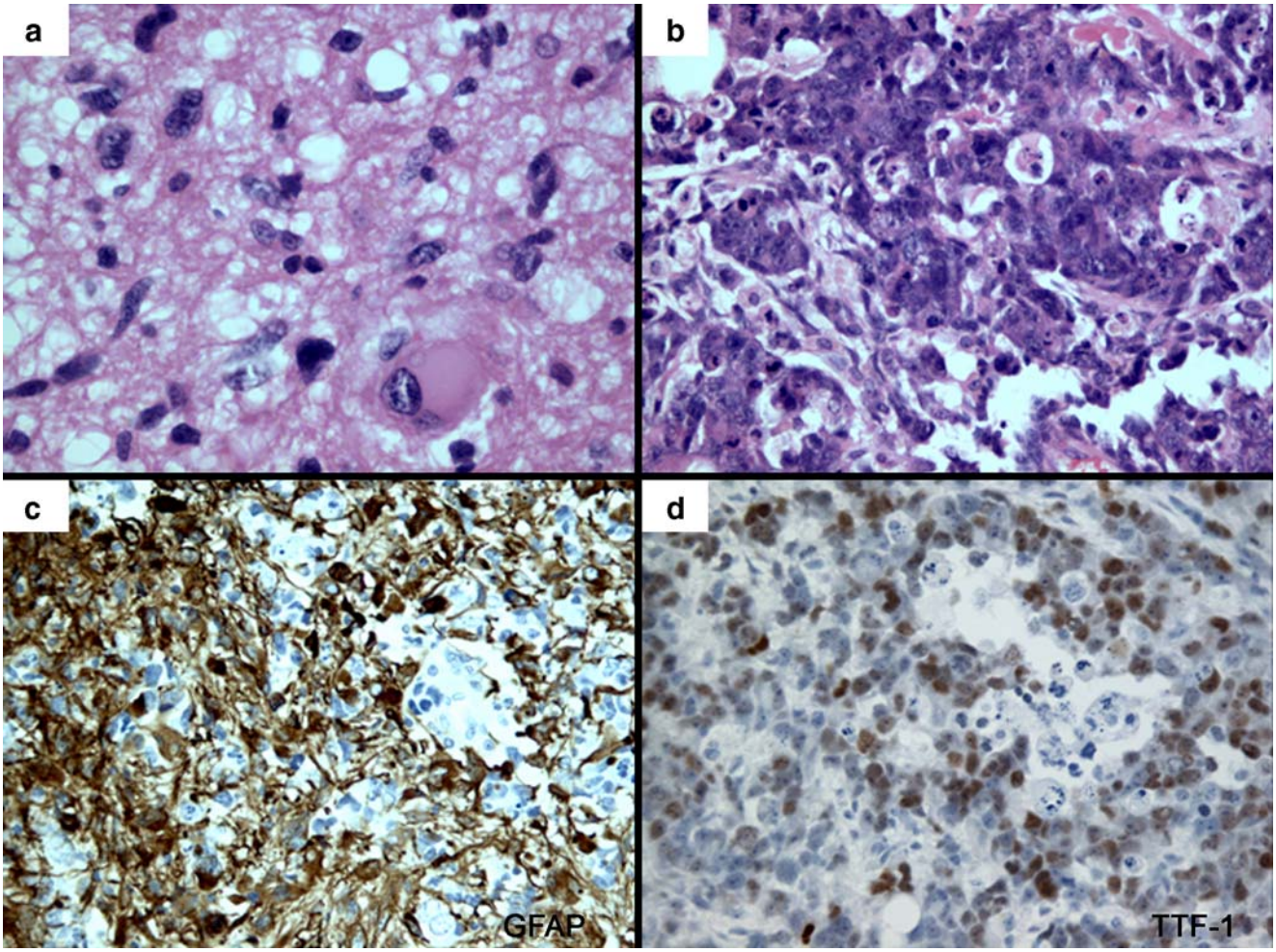

Fig. 1 Photomicrographs of the tumour from the first patient in whom we had noted TTF-1 positivity. The patient is an elderly man with glioblastoma multiforme (WHO grade IV) involving the temporal lobe. Mitotic activity, necrosis and vascular endothelial hyperplasia were present. **a** An area of tumour with typical glioblastoma multiforme morphology. **b** In this area the neoplastic cells are cohesive, poorly differentiated, and resemble metastatic carcinoma. **c** Many of the neoplastic cells are GFAP-positive. **d** There is widespread nuclear positivity with SPT-24 clone of TTF-1

It is unclear whether the TTF-1 positivity with Novocastra's SPT24 TTF-1 clone seen in a high proportion of cases of glioblastoma reflects cross-reactivity or whether this clone is detecting genuine expression of TTF-1 of uncertain significance. It is known that TTF-1 is expressed during development in areas of the basal forebrain; however, a previous study that examined TTF-1 expression in 73 primary brain tumours (including astrocytomas, glioblastomaa, ependymomas, oligodendrogliomas, medulloblastomas, and gangliogliomas) with clone 8G7G3/1 only showed positivity in two ependymomas of the third ventricle [4]. It is of interest that a recent study assessing the value of TTF-1 staining in identifying brain metastases of pulmonary origin using the 8G7G3/1 TTF-1 antibody found no positivity in 50 glioblastomas, but the SPT24 clone was not assessed in this study [5] (the antibody clone used has been confirmed via personal communication with Dr. Prayson).

These cases highlight the danger of misleading results when using TTF-1 clone SPT24 in the diagnosis of brain tumours. Although TTF-1 nuclear positivity in metastatic carcinoma of the lung is usually more widespread than was seen in our cases of glioblastoma, it should be noted that some cases of carcinoma of the lung express TTF-1 in only a minority of the neoplastic nuclei [5]. Focal nuclear positivity was seen in 50% of our series of glioblastomas, and to avoid the risk of diagnostic confusion, we suggest that the 8G7G3/1 clone may be more suitable than the SPT24 clone for use in diagnostic neuropathological practice.

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FREE PAPERS (Oral Presentations)

0P1-1

THE ACCURACY OF IMPRINT CYTOLOGY OF SENTINEL LYMPH NODE IN BREAST CANCER: AN ANALYSIS OF CAUSES OF DISCREPANCIES

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Background: Axillary lymph node status in breast cancer provides important information for staging, prognosis, and decision making for adjuvant therapy. However, less than half of the patients are likely to derive therapeutic benefit from complete axillary lymph node dissection (ALND), and most of them may suffer from chronic morbidity after the procedure. Intraoperative evaluation of axillary sentinel lymph node (SLN) provides information of status of regional lymph nodes, and may prevent ALND if SLN is negative. Touch imprint cytology (TIC) is a rapid and effective method without sacrifying valuable tissue. The aim of this study is to clarify the possible causes of the discrepancy between TIC and histopathology results. Method: A total of 1455 SLNs from 574 patients with breast cancer during the period of May 2002 to July 2006 were reviewed. The SLNs were detected either by isotope or blue dye method. The fresh lymph nodes were rapidly stained with Hematoxylin and Eosin (H&E) and examined immediately. The specimen then underwent standard histopathological processing. At least 3 levels of routine H&E-stained sections and one level for pankeratin immunostaining were obtained. Intraoperative TIC results were correlated with final histopathology results. Cases with discrepancy between TIC and permanent sections were reviewed by experienced cytopathologist. Results: The sensitivity, specificity, negative predictive value and accuracy were 57.1%, 98.7%, 94.6%, and 93.8%, respectively. Among 17 false positive cases, 9 were interpretation errors and 4 were due to tumor cells exhausted in processing. Four cases were contaminated during the process. Sampling error was the major cause in false negative cases (54/73; 74%). The remaining 19 cases were interpretation error. The median size of metastatic focus in false negative cases with interpretation error and with sampling error was 2.30mm and 1.05mm, respectively (p=0.0004). Conclusion: Sampling error is the major cause of discrepancy in false negative cases and their metastatic foci are smaller compared with those with interpretation error. Contamination may cause false positive in benign lymph nodes, and patients will undergo unnecessary ALND. More caution should be paid in order to prevent interpretation error and contamination.

OP1-2

THE VALUE OF FLUORESCENCE IN SITU HYBRIDISATION (FISH) IN THE DIAGNOSIS AND FOLLOW UP OF THE TRANSITIONAL CELL CARCINOMA

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Introduction: Transitional cell carcinoma (TCC) comprises about 90% of the urinary bladder tumors with about 50% recurrences. All of the nowadays diagnostic techniques are limited by low sensitivity. Therefore, it's very important to find highly sensitive diagnostic clues for early detection of TCC. Fluorescence In Situ Hybridisation (FISH) is a high sensitive technique, providing rapid examination of cytogenetic abnormalities in urothelial cells even in the early stage of their neoplastic transformation. Since February 2005 the UroVysiov Kit was FDA approved for using as for diagnostic purpose for primary tumors, as well as for follow up of the patients with TCC . The kit is multicolor and multitarget, providing visualization of the cytogenetic rearrangements in the chromosomes 17, 3 and 7 and in the Locus-Specific Gene 9P21 (Tumor Suppressor Gene). The aim of the study is to check correlation between the results of FISH for both diagnostic and follow up purposes of TCC patients, with the results of conventional urine cytology and urinary bladder biopsies. Results: 67 urine samples from the patients with primary TCC or suspected for recurrence were examined using FISH UroVysiov Kit. The distribution of the cases according to cytological findings and the results of FISH are represented in the table 1 (The distribution of the cases according the results of cytology and FISH). Cystoscopic diagnostic biopsies were also performed in about of 40 cases and the results are represented in the table 2 (The correlation between suspected and/or malignant cases by cytology and FISH with histological findings). It's important to emphasize that in all 17 cases which were diagnosed as malignant by cytology, correlation with results of FISH was 100%. In the cases which were diagnosed as malignant by the biopsy, the correlation with FISH was 94%. Conclusion: UroVysion FISH assay is an easy and rapid technique, providing more specify and sensitivity for the conventional urine cytology for the diagnosis and follow up of the patients with TCC with high correlation rate with cytology and histology (94%). So, in the borderline cases by cytology, use of UroVysion FISH assay is recommended.

OP1-3

CYTOPATHOLOGIC DIFFERENTIAL DIAGNOSIS OF MALIGNANT MESOTHELIOMA, ADENOCARCINOMA AND REACTIVE MESOTHELIAL CELLS: A LOGISTIC REGRESSION ANALYSIS

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Background: Distinguishing malignant mesothelioma, adenocarcinoma and reactive mesothelial proliferation in both cytologic and surgical pathologic specimens is diffucult despite the application of new diagnostic techniques. Traditional cytomorphologic criteria are still important in the differential diagnosis of these entities. Method: The pleural effusion cytologies from 40 cases of malignant mesothelioma, 40 cases of adenocarcinoma and 30 cases of reactive mesothelial proliferation diagnosed at Ankara Ataturk Chest Disease and Chest Surgery Education and Research Hospital during 1997-2007 were reviewed. 27 cytologic features which are regarded as useful in the differential diagnosis of mesothelioma, adenocarcinoma and benign mesothelial proliferation were assessed. These cytologic features were subjected to a stepwise logistic regression analysis. Results: Three features were selected to distinguish malignant mesothelioma from adenocarcinoma: giant atypical mesothelial cell, nuclear pleomorphism and acinary structures, the latter two being characteristics of adenocarcinoma. The other variables selected to differentiate malignant mesothelioma from reactive mesothelial cells were: cell ball formation, cell in cell engulfment and monolayer cell groups, the latter being a feature of benign proliferations. When these selected variables were used the logistic model correctly predicted %90 of cases of benign mesothelial proliferation versus %97.5 of malignant mesothelioma and %92.5 of malignant mesothelioma versus %92.5 of adenocarcinoma. Conclusion: Conventional cytomorphologic assessment is the first step to establish an accurate diagnosis in pleural effusions. Several cytologic features are of predictive value in seperating malignant mesothelioma from adenocarcinoma and reactive mesothelial proliferations.

OP1-4

PULMONARY BRONCHIOLOALVEOLAR CARCINOMA. REVIEW OF 43 CASES OF CYTOLOGICAL DIAGNOSIS

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Background: Bronchioloalveolar carcinoma (BAC) represents a clinic, radiological and pathologic entity of malignant pulmonary carcinomas. It has got a well known biologic behaviour, an established treatment and a good prognosis, compared with other subtypes of non-small-cell lung carcinoma. Cytological study is an useful and an invasive method in the diagnostic practice. We propose a cytological review and immunohistochemical (IHC) analysis of these tumours. Material And Methods: We collected 43 cases from 1983 until 2007, diagnosed by cytological analysis. Samples were obtained from fine needle aspiration (FNA), sputum, bronchial washing and bronchoalveolar lavage. We evaluated 13 morphologic parameters. We performed IHC techniques (p53, MUC 1, MUC-5 and CK20) on 15 of 30 cases with available cell block, or histological samples, evaluating intensity of staining and percentage of positive cells. Results: 57% of cases presented high cellularity and 43% mucinous and haematic background. Cells were arranged in flat sheets or 3-dimensional clusters. Nuclei were eccentric with nucleoli and grooves (62%) and inclusions (45%). We observed neither mitosis figures (10%) nor high grade atypia. Cytoplasm were cylindrical and clear in mucinous BACs and polygonal and dense in non mucinous BACs. Psammoma bodies were found in 24% of cases. As IHC study is concerned, 67% showed weak staining of p53, 67% of cases expressed MUC-1, MUC-5 was not valuable. CK20 was only positive in the 2 mucinous BAC cases. Discussion: BAC represents 4% of pulmonary tumours, with predilection for non-smokers women. These tumours grow along the alveolar septae without evidence of stromal invasion. Two histological subtypes exist: mucinous and non-mucinous. The differential diagnosis must be made with bronchial hyperplasia, conventional adenocarcinoma or metastasis. Cytological characteristics (highly mucinous material and mucosecretor cells) allow to identify mucinous type (31% of cases in our series). Non mucinous type was diagnosed as well differentiated adenocarcinoma because the absence of specific features. The IHC profile obtained was according with the literature data and could be useful for the diagnosis of this entity.

OP1-5

ENTROPY OF THE CHROMATIN TEXTURE IN ROUTINELY STAINED CYTOLOGY IS A PROGNOSTIC FACTOR IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Age, peripheral leukocyte number at diagnosis, and specific cytogenetic abnormalities are well known risk factors in acute lymphoblastic leukemia (ALL). Recently it has been shown that nuclear texture in Giemsa-stained smears reflects the DNA methylation pattern. Entropy derived from the gray level co-occurrence matrix is a measure of heterogeneity of an image and therefore may be used for the objective description of the chromatin texture of nuclei in cytologic smears. The aim of this study was to evaluate the prognostic value of the gray-level histogram entropy measured in cytologic smears of patients with B-ALL. Material and Methods: In newly diagnosed patients with B- ALL of two institutions, clinical and laboratory data, as well as those of quantitative flow cytometric analysis were obtained. Routinely May-Grünwald -Giemsa stained smears from diagnostic bone marrow aspirates were digitalized (100 objective magnification), and 100 randomly selected nuclei analyzed. Shannon´s entropy of the gray level histogram was calculated for each nucleus. Overall survival was evaluated using uni- and multivariate Cox proportional hazard models stratified for therapy. Results: 66 patients entered the study with a mean age of 32.3 years (range 2 - 74 years) and a mean peripheral blood leukocyte count of 50.0 billion /l (range 0.6 billion/l – 300.0 billion/l). There were no significant correlations between the entropy values of the chromatin texture and age or leukocyte count of the patients. In the univariate analyses, higher entropy values were an unfavorable prognostic factor ($p=0.0045$) as well as age ($p=0.001$) and the logarithmated peripheral leukocyte count ($p=0.028$). In the multivariate Cox analysis all three variables remained independent prognostic factors. Conclusions: The present study demonstrates that subtle alterations of the chromatin structure, not detectable by an observer, but measured objectively by the entropy values of the gray level histogram of routinely stained bone marrow smears provide important additional prognostic information. Increased heterogeneity of the chromatin structure is associated with higher risk and may reflect a different methylation pattern as well as genetic modifications.

OP1-6

IMMUNOHISTOCHEMICAL EXPRESSION OF GALECTIN-3, CYTOKERATIN 19 AND P27 IN CYTOHISTOLOGICAL DIAGNOSIS OF THE THYROID NODULES

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Introduction: Fine-needle aspiration(FNA) is a well established diagnostic technique for the preoperative approach of the solitary or dominant thyroid nodule. The different thyroid lesions can be displayed like nodules, representing different entities with different antigen expression. Aim: To evaluate the diagnostic value of the immunohistochemical markers: cytokeratin-19(CK19), Galectin-3(Gal-3) and p27, we correlate their expression in the material of cell block with respect to surgical specimen and to validate their preoperative value. We investigate whether the differences in antigen expression could be improve the accuracy of conventional FNA and provide the histological diagnosis Material and method: Immunohistochemical analysis was performed on 91 thyroid specimens (24 nodular hyperplasias-NH-, 28 follicular adenomas-FA-, 16 follicular carcinomas-FC- and 23 papillary carcinomas-PC-) from selected patients with thyroid nodules and their preoperative cytological samples. We analysed CK19(b170), Gal-3(9C4) and p27(1B4) expressions in both cell block and their histological counterparts, using semi quantitative valuation. Results: The immunohistochemical expression was: in 24 NH: Gal-3 in 12,5% histological samples (hs)/4,16% cytological samples (cs); CK19 in 16,66% hs/4,16% cs; p27 in 58,33% hs/50% cs; in 28 FA: Gal-3 in 7,14% hs/3,57% cs; CK19 in 17,85% hs/3,57% cs; p27 in 64,28% hs/50% cs; in 16 FC: Gal-3 in 25% hs/18,75% cs; CK19 in 18,75% hs/31,25% cs; p27 in 43,75% hs/43,75% cs; in 23 PC: Gal-3 in 82,6% hs/78,26% cs; CK19 in 91,30% hs/78,26% cs; p27 in 21,73% hs/4,34% cs. Conclusions: Our findings show that Gal-3($p<0.001$) and CK19($p<0.001$) are useful to differentiate follicular variant of classic CP from other thyroid lesions, in both cell block and surgical specimen. A tendency to express p27 was observed in benign thyroid lesions. However, heterogeneous positive pattern expression is not useful for preoperative diagnosis of conventional cytology. Gal-3 does not discriminate between benign follicular lesions (FA) and their malignant counterpart (FC). The data presented indicate that Gal-3 and CK19 are reliable presurgical molecular markers allowing a better selection of patients requiring surgery.

OP1-7

A COMPARISON OF FINE NEEDLE ASPIRATION CYTOLOGY AND CORE BIOPSY IN THE DIAGNOSIS OF BREAST MASSES

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BACKGROUND: Fine needle aspiration cytology (FNAC) is a fast, cost-effective and reliable method of diagnosing clinically suspicious breast masses. In the recent years, some studies reported that core biopsy (CB) has a higher rate of adequacy and a higher accuracy compared to FNAC and proposed CB as the first step of evaluating breast lesions. METHOD: We reviewed a total of 522 FNAC and 331 CB samples performed in our institution from the years 2000-2006. Either FNAC or CB were obtained from patients. Material adequacy was evaluated on site with modified toluidine blue stained wet films during fine needle aspiration procedure and aspiration was repeated if the sample was found to be inadequate. Although samples obtained with both methods had specific diagnoses in the original reports, for sake of comparison, diagnoses were classified into malignant, benign, suspicious, inadequate, less than optimal but benign categories. FNAC and CB diagnoses were compared with those of the corresponding resection materials, if available. RESULTS:Invasive carcinoma was reported in 153(29%) of FNAC and in 67(20%) of CB samples. FNAC had a high inadequacy rate (12%), while CB had none. All of the acellular/hypocellular FNAC samples were found to be inadequate during on-site evaluation. The majority of these were cyst contents with no or sparse epithelial cells, a minor group was fibrotic/schirrous lesions that did not yield enough diagnostic cells. Cystic lesions were not sampled by CB. Suspicious category was remarkably low (<1%) when compared to other reported series of FNAC. When the FNAC and CB diagnoses were compared to those of corresponding excision materials, the sensitivity of FNAC was 92%, and that of CB was 97%. Both methods had a specificity of 100%. CONCLUSION: FNAC is a highly accurate method for the diagnosis of suspicious breast lesions. On-site evaluation is important to guarantee material adequacy. When cysts are excluded, FNAC has a comparable adequacy rate to CB, with the exception of fibrotic lesions. Regarding its reliability, ease and speed of use, we suggest FNAC as the first step of evaluating the majority of suspicious breast masses. CB should be the preferred method for the diagnosis of fibrotic/schirrous lesions.

OP1-8

URINE CYTOLOGY FOR THE IDENTIFICATION OF INCLUSION BEARING "DECOY CELLS": THE INCREASED SHEDDING OF CLUMPED DECOY CELL VARIANTS IDENTIFIES PATIENTS AT VERY HIGH RISK FOR POLYOMAVIRUS ALLOGRAFT NEPHROPATHY

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BACKGROUND: The risk assessment of renal allograft recipients for the development of polyomavirus allograft nephropathy (PVN) includes the detection and quantitation of polyomavirus intranuclear inclusion bearing decoy cells (DC) by urine cytology. Greater than 10 decoy cells per ThinPrep slide have a positive predictive value for PVN of 27%. Different DC phenotypes can be recognized at the light and electron microscopic level: Type 1 (classic variant with ground glass type inclusions) and Types 2-4 (cells with various irregular and clumped intranuclear inclusions). AIMS: 1) to evaluate potential differences in the shedding of different DC phenotypes in patients with biopsy proven PVN versus patients with no diagnosis of PVN (i.e. non-diseased viral activators); 2) to study changes in the shedding of different DC phenotypes over time in patients with persistent PVN; 3) to correlate differences in the shedding of various DC phenotypes with the histologic stages of PVN (A-early; B-florid; C-sclerosing). DESIGN: 102 urine samples from 14 patients with biopsy proven PVN and 57 urine samples from 34 patients with asymptomatic viral activation and no PVN were retrospectively reviewed (ThinPrep slides, Papanicolaou stain, count of DC types 1-4 per slide). Per definition, all urine samples showed a total of ≥10 decoy cells/ThinPrep slide with no significant differences in the total DC numbers between patients with PVN (median: 36; range: 10-318) and without PVN (median: 31; range:10-150). RESULTS: Shedding of DC phenotypes 2-4 was significantly increased in the PVN patient group at time of initial diagnosis versus asymptomatic viral activators without PVN (DC phenotypes 2-4: PVN group = 5 cells (median), 31% of total DC count per slide, range = 3-70%; group with viral activation and no PVN= 0 cells (median), 3% of total DC count per slide, range = 0-32%; $p<0.002$). Shedding of DC phenotypes 2-4 remained increased over time during persistent PVN. It was already detected in 28 urine samples from 10 patients with PVN up to 8 weeks prior to the initial biopsy diagnosis. The shedding of increased numbers of DC phenotypes 2-4 did not correlate with the histologic disease stages of PVN (stage A: n = 8 patients; stage B: n=5 patients; stage C: n=1 patient). CONCLUSION: increased numbers of DC phenotypes 2-4 (accounting for ≥25% of the total DC number per slide) in voided urine specimens from renal allograft recipients indicate a high risk level for PVN (= "presumptive PVN"; positive predictive diagnostic value for PVN of > 75%).

OP1-9

FNA CYTOLOGY OF PRIMITIVE NEUROECTODERMAL TUMORS

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OBJECTIVE: Primitive neuroectodermal tumors/extraskeletal Ewing's sarcoma is a round/small-cell tumor that manifests most commonly in the paravertebral and pelvic regions/ It occurs predominantly in adolescents and young adults, and follows an aggressive course with a high recurrence. Fine-needle aspiration (FNA) is a diagnostic technique, especially effective with the help of immunocytochemical (ICC) and ultrastructural studies on the FNA material. MATERIAL&METHODS: Fine-needle aspiration biopsy specimens from 11 histologically proven cases of extraskeletal Ewing's sarcoma were studied by both light microscopy, immunocyto/histochemistry and electron microscopy (EM). RESULTS: All cases had cellular smears. The cells occur mostly as loose sheets, but also dispersed as single cells, with rare mitoses and few apoptotic bodies. The small tumor cells have round (or molded) nuclei with somewhat coarse granular chromatin, and inconspicuous nucleoli. Six cases had rosette-like structure. The rosette-like structures lack the typical fibrillar material at their centre, as seen in cases of neuroblastoma. Numerous naked nuclei and focal crush artifacts were seen. Mitosis were rare. All eleven tumors with abundant glycogen vacuoles. Ultrastructural studies shows characteristic features, such as: intracytoplasmic glycogen, lipid droplets and primitive cell junctions. CONCLUSIONS: Primitive neuroectodermal tumors/extraskeletal Ewing's sarcoma shows cytologic features similar to Ewing's sarcoma of bone. We would like to emphasize high diagnostic value presence of so-called 'punched-out clear spaces' in the cytoplasm in primitive neuroectodermal tumors/extraskeletal Ewing's sarcoma smears and CD99/O13 immunocytostaining showed strong membranous reactivity. We also comment the role of EM and ICC as ancillary technique in cytologic diagnosis of these tumors.

OP2-1
THE AMAZON TELEMEDICINE PROJECT: AN AFFORDABLE TELEPATHOLOGY SYSTEM FOR REMOTE ACCESS AREAS
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BACKGROUND: Telemedicine can provide support for healthcare in extremely remote areas of the planet through the use of satellites, low and wide-band internet transmission. But there are some key points of utmost importance to establish a reliable and continuing telemedicine system such as the development of software with user-friendly interface and short learning curves and adequate training of non-skilled communitarian staff, reference hospitals and attending physicians in the remote area. This project aims to develop and implement an efficient and cost-effective system for the Amazon Indian native population and extremely impoverished migrants of other Brazilian regions. The areas of teledermatology, telecardiology, teledentistry, teleradiology and telepathology and videoconferences for continuing education of these populations. Telepathology and teledermatology were the first to be implemented in January, 2007. METHOD: 181 patients consulted during a one week period in January, 2007 in the city of Tabatinga, close to the border of the Amazon Brazilian State with Colombia and Peru. The Internet was used as the primary telemedicine tool. A second visit is scheduled for October, 2007. RESULTS: Of all patients, 37 were Indians and the remaining were migrants from various areas of Brazil. Seventy patients had their diagnosis made by teledermatology and received medical treatment by a physician on site. Twenty two patients had skin or oral mucosal biopsies sent to the Hospital Sao Lucas of Pontifica Universidade Catolica do Rio Grande do Sul in Porto Alegre, Southern Brazil. The onsite attending physician received the final diagnostic reports encrypted by e-mail. Of these biopsies, 9 were dignosed as dematoses, one as deep mycosis, 4 epidermoid carcinomas and 8 basocellular carcinomas. Most neoplastic lesions had maximum diameters over 4 cm, demonstrating a population without access to health assistance. CONCLUSIONS: This ongoing project is feasible, cost-effective and extremely relevant for non-assisted populations in remote areas in the world. The main problems that need further studies and tests are related to adequate training of onsite healthcare assistants, permanent involvement of the staff at the reference institutions and the development of teleconference material for continued education of these populations adapted for their cultures. Several endpoints such as epidemiologic measures like infant mortality rates among others need to be evaluated to assess the impact of telemedicine in these situations.

OP2-2
UTILIZATION OF COLLEGE OF AMERICAN PATHOLOGISTS CHECKLIST AS A QUALITY IMPROVEMENT TOOL FOR ACCREDITATION
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Background: Accreditation of Pathology Departments is a process which requires meticulous quality improvement efforts. We utilized College of American Pathologists (CAP) Checklist for benchmarking during this process in our department. Initially a Quality Improvement Committee (QC) was established to guide and monitor the whole process. QC met every three months for evaluation. Here we present the goals established and the unresolved issues at the end of a year. Methods: QC started its activities by reviewing the 94 criteria on the CAP Checklist and decided that 48 of these criteria were already met in the department. In the second phase QC analyzed the reasons of failure in the remaining 42 criteria to initiate quality improvement activities. Priorities were determined by the QC. A physician satisfaction survey took place and the medical staff was encouraged to contribute to the quality improvement activities by filling a problem record book. Most of the activities were dedicated to improve the surgical pathology reporting turnover times. Results: At the end of the first year a reevaluation disclosed that 87.2% of the CAP criteria were met. When priority activates were analyzed it was seen that the average turnover time for pathology reports which was 6.8 days was reduced to 5 days at the end of the year. One of the QC strategies was to establish a new frozen section unit within the floor of operating theaters since the original one was located in another building within our department. Before this unit the yearly number of frozen sections was 876 and it increased to 1171/year afterwards. This measure reduced the frozen section turnover time to less than 20 minutes in 63% of cases. Problem record book enabled the staff to express their ideas for improvement freely and the problems reported increased in a linear fashion. QC decided that two major topics remaining for further quality improvement are; an efficient consultation system within the pathology department and a system for consulting specimens referred from other institutions. Discussion: This process proved that CAP Checklist can be successfully utilized for benchmarking during the preparation period before an external accreditation. These criteria can be used as a reference to find solutions for both intra and extradepartmental obstacles for improvement in a pathology department. Personal support and reconciliation of the staff, as well as a consensus proposal between the hospital directory and the pathology department is critical for the enhancement of the achievement.

OP2-3
TISSUE PATHWAYS AND CANCER DATASETS; AN OVERVIEW OF THE PROGRAMME OF THE ROYAL COLLEGE OF PATHOLOGISTS
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The cancer dataset programme of the UK Royal College of Pathologists was initiated in 1996-7 with the publication, of datasets for the histopathology reporting of breast and colorectal cancers. By the end of 2007, over 30 datasets will be available to view on, and download from, the College website [www.rcpath.org.uk]. The cancer datasets are being supplemented by a series of Tissue Pathways Guidelines for the handling and report of non-cancer specimens. The aims of the dataset programme include education, through providing a succinct literature review of current acceptable practice, improving clinical management by providing consistent prognostic information, providing information to assist Cancer Registries, and providing a set of data to allow national audits of practice. The tissue pathways aim to provide a succinct evidence-based description of the optimal macroscopic description, trimming, staining and reporting of non-cancer specimens. Achieving these aims presents significant challenges to the College as a commissioning organisation, to the authors of datasets and to the users. The authority of the datasets relies on a robust consultation process with clinical stakeholders and the College membership before the datasets are published, and the promise of regular revision. This has been reinforced by clearer guidelines for authors, a standard format for the datasets, and an internal audit process according to international standards. The datasets describe core data that are regarded as those data that are most important for clinical management based on published evidence; these are incorporated into the national cancer dataset and are used for national cancer audits. Tissue pathways have been constructed in a similar manner to the cancer datasets with clear guidelines on format and content. The challenges for users are to adapt to the continually increasing amount of information required for optimal clinical management and the need to use the datasets flexibly in conjunction with free text reports when

assessing complex specimens. Future challenges include the widespread incorporation of the datasets into laboratory IT systems, facilitating access to the datasets through improvements in the College website and in decision support software within the laboratory, and the continuing engagement with specialist pathology groups to incorporate new prognostic data for the management of patients with non-malignant diseases and cancers.

OP2-4
IS DIGITAL SLIDE MICROSCOPY LIMITED TO SPECIFIED MORPHOLOGICAL APPLICATIONS?

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BACKGROUND: Digital slide microscopy is enabled by amazing rapidity of hardware and software development after the millennium. Following some skepticism of whole slide imaging (WSI), growing expectations can be detected among surgical pathologists. Disappointment of earlier applications have been followed by WSI systems, which enables many specified examination in quantitative morphometry, image processing for signal detection, etc. While these progress on high way, question of routine surgical pathology, how digital microscopy supports pathology reporting, is open. METHODS: In a 6-month period in 2006, we have selected randomly 60 cases of various diagnostic entities. All type of specimens has been included. We have scanned routine case slides using Mirax Scan Desk scanner (Zeiss) than stored them on local network connected to Windows server. We have examined the 'digital cases' on two way: 1.) via local area network, 2.) via Internet. E-slides were uploaded on slide server, Pathonet (3DHISTECH) on Internet, so reachable for remote consultant. After remote evaluation we have discussed some cases using VoIP phone system, some in personal conversation as well. RESULTS: Experiences could be classified be more aspects, as technical and human parameters. Considerable diversity could be discovered between LAN and Internet based consultation, caused by bandwidth limitation at case upload on slide server. However, no such differences could be detected et remote case evaluation using e.g. ADSL line of 3 MB/sec transfer rate. Whole slide (although not 'all slide' of the entire case yet) consultation including immunohistochemical slides, special staining, TMA specimens may satisfy clinical expectations of the routine reporting in average hospitals as well. Appling on line VoIP phone in helped to an approximately 99% consensus diagnostic concordance. We found that WSI tele-reporting enables proper judgment of 'digital case': remote consultant may adjudicate all expected diagnostic factors as tumor grade, stage, free excision margins, immunohistochemical and lymph node status, morphological multiplicity, etc. CONCLUSIONS: Although the digital histology microscope technology is spreading worldwide it seems to support two main area: teaching and morphometric investigations: technology remains clinically separated, closed in specified labs for specified applications. Extensive studies are required how can we integrate routine laboratories in this progress to support daily decision via cyber corps specialists' collaboration.

OP2-5
HONEY AS A SUBSTITUTE FOR FORMALIN?

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Background. In cellular pathology, fixation preserves tissues by preventing autolysis and putrefaction. Formalin is the most widely used tissue fixative,as the result of increasing concerns about its effect as a potential carcinogen,attempts have been made to find safer alternatives.Alcoholic formalin is useful as a fixative on the tissue processors,because in addition to fixation,the dehydration process is also begun. For centuries, honey has been shown to be a successful antibacterial agent,having the potential to preserve compounds without any harmful effects on users.In addition,studies have shown that honey has acidic and dehydrative properties.Pine honey is a particular type of that the honey bees produce,not on the basis of nectar or pollen,as is the case for other types of honey,but by using the honeydew excreted by an insect, an aphid named Marchalina hellenica, which lives by sucking on the sap of certain pine species, and leaves the honeydew on the trunks of these trees.Pine honey is produced in western (mainly southwestern) Turkey,in a number of Greek islands and in in New Zealand. The aim of this study is to determine honey properties that influence tissue fixation and compare the other routine fixative in histopathology. Method. In this study,different fresh tissues were obtained from the Department of Pathology at Marmara University Hospital Same tissue pieces were dissected into small segment using normal laboratory protocol and immediately placed into numbered universal tree containers.First containers was 10% honey diluted with distilled water,second container had 10% neutral buffered formalin (NBF) and last container was alcoholic formalin.All tissues were fixed 24h and processed to paraffin wax, sectioned and stained by hematoxylin-eosin and Gomori One Step Trichrome.Ki-67 and Vimentin were stained by streptavidin-biotin peroxidase immunohistochemical method.All slides were examined by light microscopically. Results. The histomorphology of tissue sections were rated according to cellular outline, cytoplasmic and nuclear detail,erythrocyte integrity, overall morphology and staining based on a 1-4 grading system.Tissues fixed in alcoholic formalin and NBF have similar histomorphology.Honey fixative has minor histomorphologic difference, however,in no way interfere with establishing the correct diagnostic conclusion.In three groups, among the expressions of Vimentin and Ki-67 was not a significantly difference ($p>0,05$). Conclusion: Low concentrations of honey can be a safe alternative fixative to formalin in histopathology laboratories.

OP3-1
EURO-FISH: A CONCERTED ACTION TOWARDS STANDARDIZED TRANSLOCATION DETECTION IN PATHOLOGY

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Background: Lymphomas are cancers originating from the lymphatic system. This specific group of cancers can be divided into two major categories; Hodgkin versus non-Hodgkin lymphomas. Their definition is based on clinical, morphological, immunophenotypic and genetic features and is generally considered difficult and prone to mistakes. Since non-random chromosomal translocations are specifically involved in the different lymphoma types, the detection of these aberrations will be an increasingly important step in the correct identification. For that reason, the Euro-FISH program aimed at a validation of a robust, standardized FISH protocol. When implemented this procedure will be an aid in discriminating the most difficult to diagnose cases. Method: The Euro-FISH protocol is a useful tool in discerning the different types of lymphoma and available treatment options. The Euro-FISH project, organized in three stages therefore has established a robust standardized FISH protocol, to be used in routine laboratories for diagnostically difficult lymphoma cases. For the evaluation of the protocol 16 split-signal FISH probes were tested on 10 different lymphoma categories (MCL, splenic MZL, gastric MALT, CLL/SLL, DLBCL, FCL, LPL, BL, ALCL, T-LBL) supplemented with reactive tissues. The chromosomal localization of every probe was confirmed by using metaphase slides of healthy donors, before the probes were tested on the selected entities. Results and conclusions: Based on a shared protocol FISH testing was standardized (Stage 1) in all eight participating laboratories. The initial stage (Stage 0), testing on metaphase slides, confirmed that all 16 available probes were correctly localized. Stage 1, testing of laboratory specific TMA's, made with the laboratories own samples, indicated that a laboratory specific digestion might be recuired to obtain obtimum FISH results. However, Stage 1 resulted in general digestion time appropriate for more than 90% of the samples, which thereby allowed the exact same procedure to be used in Stage 2 in all participating laboratories In Stage 2, 4 TMA's of 36 cones each were prepared with 10 entities and reactive tissue orriginating from the eight laboratories. The results show that TMA's are usable in this setting and that standardization is feasible in the great majority of cases. From our results we conclude that the split-signal FISH technique provides reliable results in at least 90% of the cases tested and can in principal be implemented for routine use in every pathology laboratory with access to a fluorescence microscope.

OP3-2

TOPOISOMERASE IIα EXPRESSION IS AN INDEPENDENT PROGNOSTIC FACTOR IN HODGKIN'S LYMPHOMA

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Background: Topoisomerases are enzymes involved in the formation of transient single or double-stranded DNA breaks, relaxing DNA supercoiling. The isophorm IIα (topoIIα) is a target for several cytotoxic agents used in the treatment of hematologic malignancies. These agents appear to stabilize the DNA-topoIIα complex and inhibit DNA relegation, resulting in an accumulation of lethal double-strand DNA breaks, leading the cells to apoptosis. Furthermore, topoIIα is mainly expressed in proliferating cells, being identical to the proliferation-associated antigen KiS1 and can be used as a proliferation marker in normal and neoplastic cells. Reports in the litterature are conflicting and it is not clear whether high levels of topoIIα are beneficial, increasing tumor sensitivity to topoIIα inhibitors, or they are associated with worse prognosis reflecting increased proliferative activity. In order to elucidate this issue, we correlated the immunohistochemical expression of topoisomerase IIα (topoIIα) with clinicopathological parameters, the expression of Ki-67 and the outcome in a large series of Hodgkin's lymphoma (HL) patients, who had been homogenously treated with ABVD or equivalent regimens. Method: Immunohistochemistry using the monoclonal antibodies Ki-S1 (topoIIα) and MiB1 (Ki-67) was performed in 238 HL patients and their expression was evaluated in approximately 100 neoplastic Hodgkin Reed-Sternberg (HRS) cells. Results: The mean±SD percentage of topoIIα- and Ki-67-positive HRS cells was 64±19% (5%-98%) and 73±19% (8%-99%), respectively. The median percentage of topoIIα-positive HRS cells was 64% (interquartile range, 51-78%). There was no correlation between topoIIα expression and patient characteristics. TopoIIα and Ki-67 expression were correlated (Spearman's Rho 0.255, $p<0.001$). TopoII expression within the highest quartile of this patient population was predictive of failure free survival (FFS) (10-year rates 82±3% vs 68±7%, $p=0.02$ for patients falling into the quartiles 1-3 and 4 respectively). In multivariate analysis topoIIα expression was independently predictive of FFS and HL-specific survival. Conclusion: TopoIIα was expressed in all cases of HL showing a correlation with Ki-67 expression. Under current standard therapy including drugs inhibiting its activity, topoIIα was an independent adverse predictor of FFS and HL-specific survival with no statistically significant correlation with other established prognostic factors.

OP3-3

REDISTRIBUTION OF CD4 AND FOXP-3 POSITIVE TREG CELLS FROM LYMPHOID TISSUES TO PERIPHERAL BLOOD IN EARLY STAGE HIV INFECTED PATIENTS.

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Background: The suppression of immune responses by CD4 regulatory T-cells (Treg) may play a role in the spreading of viral chronic infectious diseases. Tregs are involved in suppression of immune cells.The purpose of this study is to analyze the distribution of Tregs in HIV infection, before and after HAART. Methods: Paired samples from lymphoid tissues (LT) and peripheral blood lymphocytes (PBL) were simultaneously obtained from 27 patients with chronic asymptomatic HIV-1 infection, before and after HAART. Six HIV- individuals were used as controls. Fresh tissue was divided in two pieces, one was immediately snap frozen and used to determine the LT viral load and to extract mRNA. A formalin-fixed-paraffin-embedded tissue block was obtained in each case for immunoarchitecture analysis and immunohistochemical studies. We analyzed FOXP3 expression by RT-PCR in LT and PBL. We studied the number and distribution of FOXP3+ Treg in LT by immunohistochemistry/computer image analysis and in PBL by flow cytometry. Clinical data, CD4 counts and viral load were obtained from each patient. Results: We observed a reduction in FOXP3 expression in LT of HIV patients, both before and after treatment compared to healthy donors at mRNA ($p<0.001$) and protein ($p=0.018$) levels. The expression of FOXP3 was even lower after treatment (mRNA,$p=0.041$; protein,$p=0.041$). These changes were positively correlated to the changes in LT viral load ($r=0.671$;$p=0.009$). To the contrary, a high number of FOXP3 positive cells was observed in PBL from HIV patients, both before ($p=0.014$) and after treatment ($p=0.002$). FOXP3 mRNA levels in PBL were not influenced by therapy and they remained

constant after HAART although still higher than controls.The patients treated with NNRTI showed a lower FOXP3 mRNA expression when compared to the group treated with PI in both compartments analyzed, PBL (p=0.008) and LT (p=0.015). Conclusion: HIV patients had low number of FOXP3 Treg cells in lymphoid tissues compared to HIV- individuals due to a redistribution of these cells from LT to peripheral blood compartment in chronic HIV infected patients. This finding correlated with the immunological recovery observed after treatment. For the first time, we associate a particular HAART regimen with alteration in Tregs, showing an increased FOXP3 gene expression in the group of patients treated with PI compared to other modalities of HAART.

OP3-4

GCET1 EXPRESSION IN ENDEMIC BURKITT LYMPHOMA. CORRELATION WITH EBV STATUS AND IGH MUTATION PATTERN

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BACKGROUND: Gcet1 (centerin, serpin A9), a gene that belongs to the serpin family of proteins, is located on chromosome 14q32. It is induced when B cells are stimulated with CD40L and it may play an important role in GC-B cell physiology and survival. Previous studies have shown that the expression of GCET1 is primarily restricted to GC B-cells (centroblasts and large centrocytes but not small centrocytes) and also to lymphoid malignancies with GC B-cell maturation. The BL exhibit a heterogeneous staining for Gcet1 that probably reflects the heterogeneity of cell of origin of this lymphoma. The normal counterpart of the neoplastic B cells in Burkitt lymphoma (BL) is still unclear. Based on immunoglobulin gene rearrangement studies, some authors suggest an origin from germinal center cells and others from memory B cells. METHOD: To better clarify the origin of BL we analysed the GCET1 expression on 38 endemic BL, and we correlated the GCET1 expression with the EBV status and with the immunoglobulin mutation pattern of these cases. For that purpose we have performed immunohistochemical analysis using a newly generated monoclonal antibody reactive in paraffin embedded tissues and EBV RNA in situ hybridization (ISH) using. A semi nested polymerase chain reaction (PCR) to amplify the VDJ rearrangements of the immunoglobulin heavy chain (VH) genes was performed and the resultant amplificates were sequenced for comparison with known germline VH segments RESULTS AND DISCUSSION: We found that GCET1 expression was significantly correlated with EBV status (Table 1). Table 1 EBV+ EBV- A9+ 2/31 5/7 A9- 29/31 2/7 Interestingly the mutation pattern of Ig genes differed between EBV-/A9+ cases and EBV+/A9- cases as EBV-/A9+ cases had a significantly lower average mutation frequency that EBV+/A9- cases. All together these results again suggested that EBV-positive and EBV-negative BL may originate from distinct subsets of B cells, pointing to a particular role for the germinal center reaction in the pathogenesis of these tumors. The immunoglobulin gene mutation patterns of single GC A9 + and A9- cells in the GC will be analysed and compared to that of BL.

OP3-5

ALTERED EXPRESSION OF MIRNAS IN C-MYC NEGATIVE BURKITT LYMPHOMA CASES

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Background: The hallmark of Burkitt lymphoma (BL) is the translocation t(8;14) that place c-Myc under the control of immunoglobulin gene regulatory elements, resulting in the over-expression of this gene. Variant translocations also occur into either ? or ? light chain locus on chromosome 2 and 22 respectively. From the EUROFISH (www.euro-fish.org) project, aiming at the standardization of FISH procedures in lymphoma diagnosis, emerged that 5 cases out of 26 classic endemic BLs were negative for c-Myc translocation with split signal probes. These results were also confirmed by the lack of alternative translocations as demonstrated by means of either IgH and IgL split signal probes as well as a t(8;14) specific fusion probe. These findings suggest that a different pathogenetic mechanism, other than c-Myc translocation, may be involved in malignant transformation in those cases. We aim therefore to investigate alternative oncogenic pathway. Several studies have reported miRNAs involvement in cancer and their association with fragile sites in the genome. MicroRNAs are a class of small non-coding RNAs that are able, in their mature form (?22nt), to regulate gene expression by mRNAs cleavage or translational inhibition. Methods: By using web available softwares, we searched for miRNAs directed against the key regulators of B cell proliferation and differentiation. Expression of these miRNAs was then analyzed by Real-time PCR in BLs both positive and negative for traslocation. Results and Discussion: We selected hsa-mir125a, Hsa-mir125b, Hsa-mir9, Hsa -mir127 and has-mir34b. We found a different pattern of expression between c-Myc negative and c-Myc positive cases for Hsa-mir9, Hsa-mir127 and has-mir34b. The specific effect of these miRNAs are under investigation by transfection of synthetic miRNAs and their inhibitors in a human lymphoblastoid cell line immortalized by EBV.

OP3-6

EXPRESSION OF BTK IN NORMAL AND NEOPLASTIC LYMPHOID TISSUES

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Background: Non-receptor protein tyrosine kinases (PTKs) are important for many signal transduction such as T-cell receptor and B-cell receptor signals. They also play an important role in the cellular growth and differentiation. Tec family are protein tyrosine kinases and comprises Tec, Bruton's Tyrosine Kinase (BTK), Itk, Bmx and Txk proteins. BTK is crucial for the development and differentiation of B-cells. Mutations occurring in BTK gene are responsible for X-linked agammaglobulinemia (XLA) which is the most common disorder among primary immunodeficiency diseases. The purpose of the present study was

to examine the expression of BTK protein in normal and neoplastic lymphoid tissues since no previous stusies have been reported. Method: Expression of the Btk protein was assessed by immunohistochemistry in paraffin-embedded tissue sections of various normal tissues, and in several types of tumor cell lines. The material investigated consisted of paraffin-embedded sections of normal and neoplastic lymphoid tissues, cytospin preparations of lymphoma-derived cell lines and normal non-lymphoid samples (e.g. heart muscle, breast, thyroid, liver, stomach, pancreas, kidney, adrenal gland, endometrium, placenta, ovary, prostate, and testis). The diagnosis of lymphoma was made following the criteria of WHO classification. Double immunoenzimatic staining was also performed on some tissue sections of human tonsil. Results: In human tonsil, BTK was expressed strongly in germinal centre and mantle zone B-cells, marginal zone cells of the spleen and monocytoid B cells (in a case of lymph node infected by Toxoplasmosis) were also BTK-positive. In bone marrow, BTK was detected in some of the lymphoid cells, granulocytes and megakaryocytes. Among lymphomas, BTK was found in the majority of non-Hodgkin's B cell lymphomas, (T–cell lymphomas were negative) and in many cases of lymphocyte predominant Hodgkin lymphoma (in the investigated cases of classical Hodgkin lymphoma the tumour cells were BTK-negative). In normal non lymphoid tissues, glandular epithelial cells of stomach, acinar cells of pancreas and tubular cells of kidney were BTK-positive. Conclusions: Our results suggest that BTK can be considered as a new marker for marginal zone B cells, a molecule to be added to the panel of B cell markers for study of B cell lymphomas and another useful marker to distinguis the two forms of Hodgkin lymphomas.

OP3-7

ANALYSIS OF LYMPHOCYTE SUBPOPULATIONS, BONE MARROW HEMATOPOIESIS AND HISTOPATHOLOGY IN RITUXIMAB-TREATED LYMPHOMA PATIENTS WITH LATE-ONSET NEUTROPENIA

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Late-onset neutropenia (LON) is a complication of Rituximab of yet unknown pathophysiology. We investigated potential underlying mechanisms in 12 patients with lymphoma who developed LON after completion of the intended treatment with Rituximab±chemotherapy. Ten of 12 patients developed B-cell depletion and inverted CD4/CD8 cell ratios; absolute CD8+ lymphocytosis and T-large granular lymphocyte (T-LGL) proliferation were identified in 8/12 and 7/12 cases, respectively. TCR Vβ repertoire flow cytometric analysis revealed expanded Vβ subsets in three cases, who displayed significantly increased proportions of Vβ3 or Vβ22 or Vβ16 expressing T8 cells. Multiplex PCR analysis in all patients with expanded T-LGL populations did not detect monoclonal TCRβ or TCRgamma gene rearrangements. Bone marrow (BM) biopsy samples from 10/12 patients of the present study were examined morphologically and immunohistochemically at onset of neutropenia. Mild (5-10%) to moderate (10-15%; one case: 30%) lymphocytic infiltration with predominantly nodular and/or interstitial growth was observed in all cases. Immunohistochemical analysis showed that the lymphoid aggregates lacked CD20+ and CD79a+ B-cells and were composed entirely of CD3+, CD45RO+ (CD3>>CD45RO) and CD43+ T-cells. Moderate-to-significant reduction of the granulocytic series and pronounced shift to the left in the form of abnormal localization of immature precursors (ALIP) with concomitant dysplastic changes of mature forms, mimicking MDS, was observed in 7/10 cases. The remaining cases (3/10) showed hyperplasic granulocytic series with ALIP-like lesions as well as dysplastic changes of mature forms, also mimicking MDS. All cases were either negative or had <2% CD34+ immature precursors. Hyperplasia of the erythroid and megakaryocytic series with abnormal paratrabecular localization as well as concomitant dysplastic changes was identified in all cases. Patients also displayed: (i) low reserves of granulocytic progenitors associated with increased apoptosis and increased proportion of Fas-expressing cells within the CD34+/CD33+ cell compartment; (ii) Fas-Ligand and Interferon-gamma mRNA expression within BM CD3+ cells; (iii) accelerated apoptosis in all stages of BM granulocytic development. All patients were negative for granulocyte-reactive antibodies. We conclude that T-cell mediated immune myelopathy associated with MDS-like changes and suppression of myeloid progenitor cell growth is critically implicated in the pathophysiology of LON in a number of Rituximab-treated patients.

OP3-8

EVALUATION OF ANGIOGENESIS IN THE BONE MARROW OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA BY A NOVEL MORPHOMETRIC METHOD

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Background: Although it is well known that angiogenesis plays a key role in the growth and metastasis of solid tumors, its role in hematological malignancies is still unclear. The use of anti-angiogenic factors in the therapeutic strategy of such diseases is promising, making mandatory the investigation of angiogenesis contribution in disease evolution and prognosis. The aim of this study was to estimate morphometrically, by a novel method, angiogenesis in childhood B-Acute Lymphoblastic Leukemia at the onset of the disease. Method: Tissue sections from formalin-fixed, paraffin-embedded trephine bone marrow biopsies from newly diagnosed, untreated, children with B-Acute Lymphoblastic Leukemia (n=25) were stained immunohistochemically with anti-Factor VIII antibody. Angiogenesis was estimated by two morphometric methods. In the one method it was expressed as microvessel density by evaluating "hot spots", a widely used method of microvessel density, and in the other method -that has been applied previously in breast carcinomas-it was expressed as volume corrected angiogenesis index (VCAI), by taking into account the bone marrow cellularity; thus providing a more accurate, although time consuming, method. The formula [V1/(NBMC1/Tot1) + V2 / (NBMC2/Tot2) + Vn / (NBMCn/Totn)]/N was applied; where V is the number of vessels per optical field, NBMC is the number of nucleated bone marrow cells coinciding with the knots of the grid, Tot is the total number of grid points projected in one viewing optical field coinciding with NBMC and fat cells, and N is the number of optical fields scored. Control group comprised free of disease bone marrow biopsies obtained for staging of children with solid tumors (n=26). Results: Leukemic bone marrows samples were found to have increased vascularity (microvessel density: 2.32 + 0.3 vs. 1.47 + 0.16 for the controls, p = 0.025) and increased but not statistically significant different VCAI (1.009 + 0.19 vs. 0.77 + 0.19 for the controls, p=0.29). Conclusion: These findings suggest that for the evaluation of angiogenesis in B-cell lineage Acute Lymphoblastic Leukemia of childhood, the parameter of bone marrow cellularity has to be taken into consideration, using the volume corrected angiogenesis index. Patients with increased angiogenesis, as studied with this novel method, may prove candidates for anti-angiogenic treatment, and should be followed for the outcome of their disease.

OP3-9
INVOLVEMENT OF THE BREAST BY HEMATOLOGICAL MALIGNANCIES: A STUDY OF PRIMARY AND SECONDARY INVOLVEMENT
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Background: Involvement of the breast by hematological malignancies is rare, in spite of the fact that secondary lymphomas represent the most common metastatic lesions of the breast. Non-Hodgkin Lymphomas (NHLs) make up the majority of these malignancies. Methods: We reviewed the clinical features and morphology of 21 cases of hematological breast malignancies diagnosed in our department over the past 15 years. The cases were reclassified according to the WHO 2001 classification after examination of H&E slides and available immunohistochemical stains. Additional immunohistochemical stains were performed when necessary. Results: The age of the patients ranged between 17 and 78 (mean 48), 20 were female, 1 was male. The presenting complaint was generally the presence of a breast mass, although most patients were seen to have accompanying multiple lymphadenopathies. Several of the patients were misdiagnosed during their initial evaluation at another institute. Histomorphological analysis revealed 2 cases of granulocytic lymphoma, both patients had a history of acute myeloid leukemia. There were 16 cases of Non-Hodgkin Lymphoma (NHL), 6 of which were primary. Of the 6 primary lymphoma cases 4 were diffuse large B-cell lymphoma and the other 2 Marginal zone lymphomas. The clinical data of 2 patients was not accessible and hence we could not evaluate the primary versus secondary nature of these cases, both were diffuse large B-cell lymphomas. The distribution of the remaining 8 cases of NHL representing secondary involvement were as follows: 2 cases of Diffuse large B-cell lymphoma , 2 cases of chronic lymphocytic lymphoma/leukemia, one with Richter transformation, 2 cases of lymphoblastic lymphoma and 1 case each of Burkitt lymphoma and mantle cell lymphoma. There were 3 cases of secondary breast involvement by classical Hodgkin lymphoma, an exceedingly rare finding to our knowledge. Conclusion: Keeping in mind that several of our cases were misdiagnosed, either as inflammatory lesions or nonhematological malignancies, hematological malignancies should be considered in the differential diagnosis of breast lesions, especially in incisional biopsy specimens, to avoid unnecessary operations or delays in treatment.

OP3-10
EXPRESSION AND ACTIVATION STATUS OF THE GENOTOXIC STRESS-INDUCED 53BP1 PROTEIN IN NON HODGKIN AND HODGKIN LYMPHOMAS
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Background: The tumor protein p53 binding protein 1 (53BP1) is involved in DNA damage response after induction of DNA double-strand breaks (DSB). Recent studies in 53BP1 and p53 double knockout mice show that 53BP1 and p53 synergize to suppress genomic instability and lymphomagenesis suggesting o potential role of 53BP1 loss in lymphoma pathogenesis. However, expression and activation status of 53BP1 in reactive lymph nodes and lymphomas are unknown. Methods: The specificity of 53BP1 antibodies used in this study was previously tested in our laboratory by immunoprecipitation and Western blot analysis in lymphoma cell lines. Using immunohistochemical methods, we assessed for total 53BP1 protein expression in reactive lymph nodes and in 167 B-cell or T-cell non-Hodgkin lymphomas of various types, as well as in 48 Hodgkin lymphomas. In a subset of 130 53BP1-positive lymphomas activation (phosphorylation) of 53BP1 (p-53BP1) was also assessed. Results: 53BP1 immunoreactivity was primarily nuclear in normal or tumor cells. In normal reactive lymph nodes, 53BP1 was predominantly expressed in centroblasts and centrocytes of reactive germinal centers and less frequently in lymphocytes of the mantle and marginal zones or interfollicular areas. Using a 10% cutoff for positivity, 53BP1 was expressed in 9 of 10 (90%) Burkitt lymphomas, 15 of 19 (76%) chronic lymphocytic leukemias/small lymphocytic lymphomas, 36 of 40 mantle cell lymphomas (MCL) (90%), 20 of 20 (100%) follicular lymphomas (FL), 42 of 43 (98%) diffuse large B-cell lymphomas (DLBCL), 30 of 35 (86%) anaplastic large cell lymphomas (ALCL), and 42 of 48 (87%) Hodgkin lymphomas (HL). Using the same cutoff, p-53BP1 was expressed with variable staining intensity in 27 of 29 (93%) DLBCL, 25 of 26 (96%) FL, 24 of 24 (100%) MCL, 26of 28 (93%) ALCL and 20 of 23 (86%) HL. Conclusion: Loss of 53BP1 expression is found only in a small subset of Hodgkin and non-Hodgkin lymphomas. When expressed, 53BP1 is frequently phosphorylated (activated) indicating activation of DNA damage response pathways in lymphomas.

OP3-11
DIFFERENTIAL EXPRESSION OF ACTIVATED (PHOSPHORYLATED) C-JUN N-TERMINAL KINASE (JNK) IN B-CELL NON-HODGKIN LYMPHOMAS
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Backround: c-Jun N-terminal Kinase (JNK), member of the mitogen activated protein kinase (MAPK) superfamily, has been shown to play a role in cell transformation, proliferation and apoptosis. JNK is activated through phosphorylation by upstream MAPK kinases (MKK), MKK4 and MKK7, in response to cytokines, growth factors or ultraviolet (UV) radiation. In addition, JNK is positively regulated by c-myc oncoprotein. The activation status of JNK in B-cell non-Hodgkin lymphomas (B-NHL) is unknown to date. Methods: Using tissue microarrays, immunohistochemistry and an antibody highly specific for phosphorylated JNK (p-JNK), JNK activation was assessed in 144 B-NHL tumors including 12 Burkitt lymphoma (BL), 39 diffuse large B-cell lymphoma (DLBCL), 21 follicular lymphoma (FL), 48 chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), 18 multiple myeloma (MM), and 6 marginal zone lymphoma of MALT type (MZL-MALT). Nuclear staining of p-JNK present in stromal and endothelial cells in all tissue specimens served as an internal positive control. Results: Using a 10% cutoff, p-JNK was expressed 10 of12 (83%) BL, 9 of 39 (23%) DLBCL, 4 of 21 (19%) FL, and 1 of 6 (18%) MZL-MALT. By contrast, none of the 48 CLL/SLL and 18 MM cases included in the study group expressed p-JNK ($p<0.001$, Kruskal Wallis test). Staining intensity and number of positive tumor cells reflecting the expression level of pJNK varied considerably among the positive cases; 5 of 10 (50%) BL, 3 of 9 (33%), and 1 of 4 (25%) of FL expressed p-JNK strongly in a high proportion (>50%) of tumor cells. Conclusion: p-JNK is differentially expressed among B-NHL subtypes. BL tumors, which are characterized by c-myc over-expression, show the highest level of JNK activation suggesting a potential role of JNK in myc-induced tumor cell proliferation and apoptosis of these tumors.

OP4-1
HISTOPATHOLOGICAL CHARACTERISTICS OF PRIMARIES AND PELVIC LYMPH NODE METASTASES PREDICTING SURVIVAL IN SURGICALLY TREATED NODAL POSITIVE PROSTATE CANCER PATIENTS
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Background: The current TNM classification has only one category for nodal positive prostate carcinomas implying similar outcome for these patients. However, there is accumulating evidence that surgically treated nodal positive prostate carcinoma patients have a variable clinical course. This encourages identification of tumor characteristics predicting survival. Methods: We evaluated the prognostic potential of various histopathological features in primaries (volume, stage, Gleason score) and nodal metastases (quantity of tumor burden, Gleason score, extranodal extension) of 102 prostate carcinoma patients (median age: 65 years, range: 45–75; median follow-up 7.7 years, range: 1.0–15.9 years). All had clinically non-metastasizing cancer, underwent radical prostatectomy and standardized extended lymphadenectomy and had nodal metastases upon histological examination. They had no immediate androgen deprivation. Results: A median of 21 nodes (range: 9-68) and 2 positive nodes (range: 1–24) per patient were detected. A significant stratification was possible with the Gleason score of the primary and virtually all nodal parameters favoring patients with better differentiated primaries and metastases, lower nodal tumor burden, and without extranodal extension of metastases. In multivariate analyses diameter of the largest metastasis (≤1 cm vs. >1 cm) was the strongest independent predictor for biochemical recurrence free (bRFS; P<0.001), disease specific (DSS; P<0.001) and overall (OS; P<0.001) survival with a more than quadrupled relative risk of cancer related deaths for patients with larger metastases (HR: 4.2, CI: 2.0–8.9; 5 year bRFS/DSS/OS: 18%/57%/54%). The highest 5 year survival probabilities had patients with exclusively micrometastases (bRFS/DSS/OS: 47%/94%/94%). Conclusion: The TNM classification with only one category for nodal metastases for prostate carcinomas is unsatisfactory since subgroups with significantly different prognoses can be identified. The diameter of the largest metastasis per patient (≤1 cm vs. >1 cm) should be used for substaging because of its independent prognostic value. Extranodal extension of metastases provides further prognostic potential which is particularly important in case of limited lymphadenectomy without adequate estimation of nodal tumor burden. Finally, survival probabilities of patients with only micrometastases indicate a chance of cure by surgery alone for some of them.

OP4-2
ABERRANT PROTEIN EXPRESSION IN MICROPAPILLARY BLADDER CARCINOMA

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Introduction: Bladder cancer affects approximatively 10000 patients in France each year. More than 90% are conventional urothelial carcinomas. Several histological variants exist. One rare variant, micropapillary carcinoma (MPC), with male predominance is from great clinical importance, because of its aggressiveness. The exact incidence is unknown, as these tumors are not always exclusively pure forms. We explored, whether MPC display special protein expression, such as overexpression of markers known to be predictive for unfavourable clinical outcome. M&M: We chose p53, MIB-1, Aurora-A and survivin. Immunoexpression of micropapillary carcinomas was evaluated by two experienced pathologists. Normal urothelial tissue never expressed any of these markers. Results: 13 patients, all male, aged between 46-85 years were included in this retrospective study. Staging, according to the WHO classification 2004, showed 1Cis, 2 pT1, 2pT2, 3pT3 and 5pT4 tumors. The micropapillary contingent in tumor specimen was between 30-100%. Ten patients were treated by cystoprostatectomy, 3 underwent transurethral resection. Nine patients died of disease, one patient was lost, 3 patients are alive with follow-up between 8 -26 months. All micropapillary carcinomas overexpressed p53 and MIB-1 in 39% weakly and in 61% in a strong way respectively. Aurora-A was strongly overexpressed by all micropapillary carcinomas. Survivin displayed weak staining in 46% and strong staining in 54%. Conclusion: p53 is a prognostic factor of bad clinical outcome. Studies have shown a sensitive reduction of overall survival or/and progression free interval for p53-positive tumors. MIB-1 protein is strictly associated with cell proliferation. Urothelial carcinomas with overexpression of MIB-1 have a less favourable outcome, a higher risk of progression and significant association with distant metastasis. Aurora-A plays a role in cell division and in maintaining genomic integrity. Increased expression of Aurora-A is linked to clinical aggressiveness and poor overall survival of the patients. Major implication of survivin in bladder cancer development has been demonstrated. Survivin inhibits apoptosis and acts in downstream signaling pathways. Elevated urinary levels of survivin are associated with cancer presence, higher tumor grade and advanced pathological stage. Our study clearly shows overexpression in all MPC of markers of bad clinical outcome. These cancers seem to act in several pathways of genetic deregulation, which might explain their clinical aggressiveness.

OP4-3
EFFECT OF TAMOXIFEN ON GROWTH OF BLADDER CANCER CELLS IS INDEPENDENT OF ESTROGEN RECEPTORS BUT INVOLVES UPREGULATION OF BAX AND CYCLIN D1 EXPRESSION

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Introduction:- Tamoxifen is an estrogen receptor alpha (ERα) antagonist widely used in the therapy of breast cancer. However, it can inhibit growth of neoplastic cells other than breast including transitional cell carcinoma (TCC) of bladder. Many TCC cells express ERα and/or ERβ hence it is likely that tamoxifen may prove to be an effective therapeutic agent for selected bladder cancers. This study was performed to detect any relationship between expression of individual ER isoforms and effects of estrogen and tamoxifen on proliferation of bladder cancer cell lines, and to identify genes modulated by these agents. Materials and Methods:- Growth curves were prepared for 10 bladder cancer cell lines exposed to 10-fold increments of concentration (0.001-10.0 μmol) of β-estradiol either alone or in conjunction with tamoxifen (0.01-10.0 μmol). Total RNA was extracted from all cell lines during linear growth and reverse transcribed for PCR using primers specific for the two isoforms of ER. Messenger RNA prepared from total RNA was amplified, labelled, and hybridised to custom-designed DNA oligonucleotide arrays (MetriGenix, Gaithersburg,USA). Results:- ERα was present in 8 bladder cancer cell lines but ER βwas identified in only 6. One cell line contained neither isoform. B- estradiol at an optimum dose of 1.0 μmol significantly increased growth in 6 cell lines, especially TCCSUP. Tamoxifen at 10.0 μmol was lethal to all cell lines but at lower doses (optimum 1.0 μmol) it stimulated growth in two including TCCSUP. In two cell lines, tamoxifen had little effect, and in 6 it inhibited growth. Inhibition was greater than that due simply to antagonising estradiol, especially in line HT1197, and was not related to ER status. For example, TCCSUP and HT1197 both contained ERα but not ERβ. Analysis of data from the arrays showed heterogeneity of gene expression between cell lines with no apparent relationship to ER status. Of the 80 genes on the array, 14 were highly expressed when related to control genes and, of these, 3 were significantly upregulated by tamoxifen but

not estradiol. Two of these,Bax and CCND1 (cyclin D1), are involved in promoting apoptosis and the cell cycle respectively. Conclusion:- Modulation of Bax and Cyclin D1 expression may explain, in part, how tamoxifen influences cell growth. It also provides objective evidence that tamoxifen could be successfully employed in treatment of TCC with fewer side effects than current regimes.

OP4-4
POSSIBLE ROLE OF CYCLOOXYGENASE-2 IN SCHISTOSOMA-AND NON-SCHISTOSOMA ASSOCIATED BLADDER CANCER

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Background and purpose: Cyclo-oxygenase (COX) is an angiogenic factor, which is strongly related to inflammatory diseases and the development of cancer and metastasis in several cancers. It is overexpressed in a variety of premalignant and malignant conditions, including urinary bladder cancer. Our aim is to investigate and compare the expression of cyclooxygenase-2 (COX-2) enzyme in patients with bladder cancer, chronic cystitis and normal bladder. The results were correlated to the classical prognostic factors, mainly tumor stage and grade in a trial to determine the prognostic significance of COX-2 marker. Materials and methods: Seventy-five bladder samples, including 50 cases with bladder cancer (31 were bilharzial associated and 19 non bilharzial associated), 20 samples from cases with chronic cystitis (7 were non specific and 13 were bilharzial cystitis) as well as 5 samples from normal bladder tissue taken as control.The specimens were stained by streptavidin biotin immunohistochemistry protocol, with COX-2 monoclonal antibody. Results: Although no notable expression of COX-2 was observed in the normal bladder, it was slightly expressed in chronic cystitis notably in areas of dysplasia and squamous metaplasia, whereas there was a significant increase in COX-2 ($P < 0.001$) with moderate to strong granular cytoplasmic expression in all malignant histological types. The COX-2 reactivity was higher in transitional cell carcinoma (TCC) than in squamous cell carcinoma (SQCC) ($P < 0.001$). As well as COX-2 expression was significantly higher in schistosomal TCCs than in non schistosomal TCC ($P < 0.05$). There was a statistically significant positive correlation between COX-2 expression and tumor grade ($P = 0.0052$). COX-2 expression was significantly higher in Grade 3 bladder transitional cell carcinoma than in Grades 1 and 2 ($P < 0.05$, $P < 0.01$). Also the correlation between COX-2 expression and progression of bladder transitional cell carcinoma was observed ($P = 0.001$), there was significant difference in COX-2 expression level between the bladder transitional cell carcinomas at different clinical stages ($P < 0.01$). Conclusion: COX-2 is over-expressed in schistosoma-associated bladder cancer. COX-2 might be of significance to the development and proliferation of bladder transitional cell carcinoma, consistent with a potential role for COX-2 inhibitors in the prevention and management of this disease.

OP4-5
DIFFERENTIAL DIAGNOSTIC OF RENAL CLEAR CELL CARCINOMA USING PLOIDOMETRY

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Background. The problem of differential diagnostics of renal clear cell carcinoma in various stages of disdifferentiation is particularly important in the light of patient's prognosis determination. Aim: to use ploidometry research for studying the epithelial cell clones in the clear cell renal tumors to determine objective criteria of different stages of cancerogenesis. Methods. Computer ploidometry test was carried out for nuclei of epithelial cells in renal operative specimens, taken from 50 patients with clear cell carcinomas in different grades. Renal epithelium nuclei taken from 10 healthy individuals operated in the case of traumas were used as control. Histological preparations of 8 micron thickness, stained by Feulgen's technique, were subject of the research. Nuclei's ploidy was defined by means of the computer image analyzer "Imager-CH" ("Avtan-San" software version). The "tissue ploidy standart" (2c) was used for obtaining the data of 3024 renal epithelium nuclei. Inter-phase cell nuclei of clones of normal renal epithelium, clear cell carcinomas in different grades were the subjects of investigation. Results: In comparison with the average ploidy value of the normal renal epithelial cells nuclei, the average ploidy index increases 1,8 times in well differentiated clear cell carcinomas (1 grade), 2,1 and 2,4 times in middle differentiated (2,3 grade) and 2,8 times in poorly differentiated clear cell carcinomas (4 grade). The study results showed that tumor cells proliferative activity and nuclei ploidy increase in parallel with the increased disdifferentiation of clear cell carcinomas. Retrospective analysis of 45 cases of renal clear cell carcinomas was provide in routine diagnostics and using ploidometry, the mistake in carcinomas grade was fined in 24%. The average ploidy of nuclei significantly differs between various grades of cancers (4,6 c versus > 6,8 c) which makes the ploidy index a useful differential diagnostic criteria. Conclusions: Computer ploidometry may be used in differential diagnostics of clear cell renal carcinomas. Use of ploidometry data in morphological diagnostics of renal tumors can be an objective tool for taking justified clinical decisions.

OP4-6
INVASION FRONT PHENOMENA IN PROSTATIC CANCER

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Background: Intratumoral heterogenitiy is a common phenomenon in prostatic cancer, leading to different architectural and cytological classifications (Gleason, Mostofi, AK-Uropath).In most studies however, no correlation was made with the intratumoral distribution of these markers in respect to centrally located carcinoma cells and tumor-cells in the invasion front (which are probably more important for the biological behaviour of the tumor). We therefore analysed various markers in the epithelial-mesenchymal transition zone (EMT) of prostatic carcinomas. Materials and Methods: Tumor cells of the EMT-Zone of 40 intermediate grade prostatic carcinomas were analysed in respect of their expression of chromosome 7 aneuploidy (Zytolight CEN 7 Probe; FISH), E-Cadherin (Biologo, Dr.Schultheiss, Kronshagen, Klon 5H9, IgG1, IHC), N-Cadherin (Biozol, Eching GeneTex.Inc; IgG, polyclonal, IHC) and hepsin (Cayman Chemical, via Biozol Eching; affinity puified IgG, Rabbit; IHC) in comparision to adjacent non-tumor epithelia and carcinoma- cells in well-defined patterns. Results: E-Cadherin: Good expression in basal and secretory cells of non-neoplastic gland and basal cells of PIN. Low expresssion in the tumor-cell-membranes of Gleason Pattern 3 and 4 ; good expression in peripherial parts of cribriform areas and in small tubular and solid parts of the tumor. Loss of E-Cadherin-expression in ETMs adjacent to prostatic stromal cells. N-Cadherin: No N-cadherin in non-neoplastic glands; high cytoplasmic and membranous expression in tumorcells of EMTs. Hepsin.Low expression in non-tumor glands; medium intracytoplasmatic expression in PINs and cancer cells of well defined patterns; very high expresssion in epithelias of small-glandular areas and in most EMTs; Some (regressing?) EMTs show low levels of hepsin, corresponding to their loss of E-Cadherin. Aneuploidy of chromosome 7: (high grade PIN and cancer cells adjoinig ton the EMT showed three to five chromosom 7 centromers by CEN7 analysis. Conclusions:

Tumorcells in EMTs show extensive remodelling of shape and function. Especially the loss of intercellular connections (cadherins) and the abundance of a serin-protease (hepsin), fostering invasion by dissolving collagens and dissolving tight cell-adhesions in the invasion front might influence the tumor's biology more than architectural tumor patterns alone.

OP4-7

TMPRSS2:ERG GENE FUSION EXPRESSION AND PROSTATE CANCER PROGRESSION

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Background. Recent studies have reported functional fusion of the TMPRSS2 and ERG genes with consequent ERG overexpression in a large proportion of prostate cancers. Some data suggest that ERG expression is higher in less aggressive prostate carcinomas while others show an association between TMPRSS2:ERG gene fusion and more aggressive disease. Materials and Methods. The TMPRSS2:ERG fusion status was assessed in tumour samples from 84 patients undergoing radical prostatectomy between 1998 and 2000. Sixty patients (group A) had surgery alone, while 24 patients (group B) received androgen ablation therapy for 3 months before surgery. Results. The fusion of TMPRSS2 and ERG was demonstrated by reverse transcription polymerase chain reaction (PCR) in 84% of patients in group A and in 54% of patients in group B (p=0.01). The levels of TMPRSS2:ERG and ERG, measured by real-time quantitative PCR, did not show significant association with clinical and pathological characteristics of the tumours, except for a negative correlation of ERG overexpression with Gleason score (R=0.457; p=0.0001), only observed in group A. The lower proportion of patients harbouring TMPRSS2:ERG fusion in group B suggests that androgen ablation inhibits the expression of TMPRSS2:ERG, underscoring the key role of androgen-mediated transcription control of the gene fusion. Biochemical (PSA) recurrence-free survival was not significantly different in group A patients both with and without TMPRSS2:ERG expression. Conclusions. In this specimen set, we observed that the presence of a TMPRSS2:ERG fusion expression did not correlate with recurrence-free survival following radical prostatectomy. Further studies in larger cohorts of tissue are under way to confirm these observations.

OP4-8

THE EFFECT OF CHRONIC ARSENIC EXPOSURE FROM DRINKING WATER ON THE PATHOLOGIC CHARACTERISTICS OF UROTHELIAL TUMORS IN A NON-SELECTED PATIENT POPULATION WITH BLADDER CANCER

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Background: In the United States, among the highest incidence and mortality rates for bladder cancer are found in northern New England. Several lines of evidence suggest a possible environmental explanation for this excess. Arsenic, a known cause of bladder cancer in parts of Taiwan, Bangladesh, and other countries where drinking water concentrations are markedly elevated, was considered a plausible explanation in northern New England because of known elevations of arsenic in the ground water and the fact that over 33% of residents obtain their drinking water from drilled wells. We reported in a large, population-based case-control study (Karagas MR, et al. Cancer Cause Cont 2004;15:465) that there is an elevated odds ratio for bladder cancer among patients in the uppermost category of arsenic exposure only in smokers. The current study explores whether there are differences in the pathologic characteristics of bladder cancers in patients with the highest and lowest chronic arsenic exposures. Method: All residents of the state of New Hampshire with bladder cancer newly diagnosed from July 1, 1994, to June 30, 2000, were identified through the state cancer registry. Individual exposure to arsenic was determined in toenail clippings using instrumental neutron activation analysis. All slides were reviewed by a single pathologist. Tumors were classified according to both WHO (1973) and WHO/ISUP (1998) criteria. From a total of 690 patients with pathologically confirmed cancer and toenail arsenic results, tumor classification, grade, and stage were compared between patients with the highest decile (>0.205 μg/g) and the lowest quartile (<0.061 μg/g) of toenail arsenic levels. Results: Tumors from individuals with high arsenic concentration tended to have lower grade, non-invasive tumors, as well as a higher proportion of stage T.i.s. (carcinoma-in-situ) tumors. There was no difference in the proportion of non-urothelial tumors between individuals with high versus low arsenic exposure; non-urothelial tumors comprised a minority of cases in both groups. Conclusion: In this preliminary analysis of a population-based study from the United States, we noted some histopathologic differences between those with higher and lower arsenic exposures. Further investigation of these differences is underway.

OP4-9

TISSUE MICROARRAY STUDY OF THE RELATIONSHIP BETWEEN CLUSTERIN EXPRESSION AND HORMONE THERAPY EFFECT IN PROSTATIC ADENOCARCINOMA

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Background: Clusterin is a secretory glycoprotein that is present in many tissues. It has been identified as one of the main prostasomal proteins. It has dual functional properties related to both apoptotic and antiapoptotic signals. Clusterin expression is upregulated in prostatic tumor tissue after neoadjuvant hormone therapy (NHT). On the other hand, increased expression of clusterin has been associated with shorter biochemical recurrence-free survival. We conducted the present tissue microarray (TMA) study to further elucidate the relationship of clusterin expression with the effect of NHT in tumor cells, as well as with several clinico-pathological variables. Design: For this study, two TMA blocks with 233 prostatic adenocarcinoma samples selected from radical prostatectomy specimens were constructed. They included 175 cases with and 58 without previous NHT. Sections were stained with an antibody for Clusterin. Intensity of Clusterin staining was recorded (0-3), as well as the severity of the hormone blockade-induced changes in tumor cells (also scored 0 to 3). ANOVA tests were performed to study relationship of Clusterin expression with treatment, with treatment-induced changes, pathologic stage and biochemical recurrence-free survival. Result: The intensity of Clusterin expression was significantly related to previous NHT (Chi-square 34.042, p=0.0001). There was no statistically significant relationship between Clusterin expression and pathologic stage or biochemical recurrence-free survival, regardless of previous NHT. Conclusion: The strong association between intensity of

Clusterin expression and the presence and effect of NHT in tumor cells suggests that Clusterin may be involved in the mechanisms that are responsible for this effect. Alternatively, Clusterin expression could be the consequence of such changes. The biological role of Clusterin in prostatic adenocarcinoma needs to be further elucidated, because of the potential therapeutic implications of the availability of Clusterin antisense oligonucleotides.

OP4-10
FISH ANALYSIS OF PTEN GENOMIC DELETION IN PATIENTS WITH RENAL CELL CARCINOMA

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Introduction: Recent advances in the understanding of the molecular biology of renal cell carcinoma (RCC) have identified new prognostic factors. The PTEN (phosphatase with tensin homology deleted in chromosome 10) is a tumor suppressor gene which deletions and/or mutations have been reported in several solid tumors and associated with poor prognosis. Material And Methods: 110 patients were analyzed from 1980 up to 2005. Clinic-pathological features were reviewed according the WHO/TNM criteria. FISH analysis for PTEN was performed in a TMA block. PTEN genetic abnormalities were correlated to overall survival and disease free survival, and to the established RCC prognostic factors. Results: There were 68 men (61.8%) and 42 women (38.2%), and 55 years-old in median. The average follow-up was 42.3 months. The Fuhrman's grade 1 was observed in 18 patients (16.4%),G2 in 47 patients (42.3%), G3 in 27 (24.5%), and G4 in 18 patients (16.4%). According the 2002 TNM, the following distribution was observed: 54 patients (49.1%) EC I, 14 patients (12.7%) EC II, 26 patients (23.6%) EC III and 16 patients (14.5%) EC IV. There were 71 patients (64.5%) with clear cell subtype, 12 patients (10.9%) with papillary subtype, 14 chromophobe subtype (12.7%), 10 (9.1%) unclassified renal cell tumor, 2 two patients with TTE3 gene subtype and one collecting duct carcinoma. FISH was available in 47 cases. Eighteen of them (40%) had PTEN hemizygous deletion, 3 PTEN homozygous deletion, and in 16 patients (32%) there was no PTEN deletion. Monossomy of chromosome 10 was found in 9 (8%) and trissomy in 2 patient (1.8%). The overall survival (OS) and cancer specific survival (CSS) for the 110 patients were 70.3% and 71.8%, respectively. There were no statistically significant differences in OS (p=0.182) and CS (p=0.273) when patients with or without deletion were compared. There was no differences when compared PTEN status and Fuhrman grade (p=0.109), TNM staging (p=0.220) and tumor subtype (p=0.132). CONCLUSION: The PTEN deletion detected by FISH was not associated with OS, although a trend was observed. Also, there is no association to the traditional prognostics factors for RCC.

OP4-11
CLINICAL SIGNIFICANCE OF NF-κB LEVELS IN UROTHELIAL CARCINOMA

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Background: Nuclear transcription factor (NF)-κB is a family of proteins which regulate the expression of immune genes that mediate cellular apoptosis, proliferation, invasion and angiogenesis. NFκB has been reported to be constitutively activated in various neoplasms and its immunoexpression has been regarded as a marker of poor prognosis in several carcinomas. However, its clinical significance in urothelial carcinoma remains an unresolved issue. We therefore conducted a retrospective investigation in order to address the role of NFκB in urothelial carcinoma and to explore its potential prognostic significance. Method: The levels of p65/RelA protein expression were quantified immunohistochemically in paraffin-embedded tissue from 116 patients with urothelial carcinoma, using a polyclonal rabbit antibody against the p65 subunit. Nuclear and cytoplasmic immunoreactivity was assessed separately. Basic statistical analysis was performed using appropriate non parametric tests, whereas survival analysis by plotting survival curves according to the Kaplan-Meier method and comparing groups using the log-rank test, as well as by using multivariate analysis (Cox's model). Results: Positive nuclear p65/RelA staining was detected in 92/116 cases (79.3%) and cytoplasmic staining in 95/116 cases (81.89%). Concurrent nuclear and cytoplasmic expression of NFκB was seen in 96.7% (89/92 cases). Nuclear p65/RelA expression was associated with tumour grade and stage in the entire cohort as well as in invasive carcinomas ($p<0.0001$ in the former, $p=0.0019$ and $p=0.0196$ in the latter relationships). Also, cytoplasmic p65/RelA expression was lower in advanced stage (T2/T3 versus T1, $p=0.0197$) and high grade tumours (I/II versus III), although the latter was significant only when analysis was restricted to invasive carcinomas. p65/RelA expression adversely affected survival in both univariate and multivariate analysis in invasive ($p<0.0001$ and $p=0.001$ respectively) as well as in muscle invasive carcinomas ($p=<0.0001$ and $p=0.01$ respectively). Conclusion: The results of our investigation demonstrate that NF-κB activation is correlated with tumour histologic grade and invasive potential in urothelial carcinomas, probably supporting the crucial role of this molecule in tumour agressiveness. Moreover, our study, directly implicates NF-κB nuclear expression as an independent prognostic marker of adverse significance, conveying information beyond that obtained by standard clinicopathological prognosticators.

OP5-1
LEFT VENTRICULAR MECHANICAL SUPPORT IN CONGESTIVE HEART FAILURE: CHANGES OF PLOIDY STATUS, NUMBER OF BINUCLEATED CARDIOMYOCYTES AND CELL-CYCLE REGULATORY PROTEINS

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Aims: In chronic heart failure (CHF) left ventricular assist devices (LVAD) lead to cardiomyocyte hypertrophy regression and numerous molecular changes. Besides hypertrophy, cardiomyocyte nuclei undergo polyploidy in CHF. Given the significant decrease of cardiomyocyte diameter after LVAD treatment, we investigated the cardiomyocyte ploidy status after left ventricular unloading. Methods: In 24 paired myocardial samples the Feulgen stain was applied and computed DNA cytometry both before LVAD and after unloading was performed. Moreover, the number of binucleated cardiomyocytes and cardiomyocyte diameters were determined. The expression of cell cycle regulatory proteins was immunohistochemically demonstrated and morphometrically quantified. Results: There is a significant reduction of the cardiomyocyte ploidy status with a significant increase of di- and tetraploid cardiomyocytes after LVAD. Moreover, a significant correlation between the ploidy status reduction and hypertrophy regression was outlined. The number of bi- and multinucleated cardiomyocytes was significantly increased, whereas expression of cyclin D1 was significantly decreased after LVAD. Conclusions: There is a significant reduction of the cardiomyocyte ploidy status after ventricular unloading, that significantly correlates with

hypertrophy regression, suggesting that cardiomyocytes shift from polyploidy to di- and tetraploidy upon volume- and pressure unloading by LVAD, which might be regulated by cell cycle regulatory proteins.

OP5-2
IMMUNE INFLAMMATION PECULIARITIES IN ATHEROSCLEROSIS

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Introduction: The aim of this study was to evaluate the role of immune inflammation in human atherosclerosis. Immunomorphological investigation was directed on CD-4, CD-8, TNF-α,HSP- 60 and CRP level in atherosclerotic lesions. Material and methods: This study has been performed on the human aorta, coronary heart vessels and cerebral arteries collected from 86 early performed autopsies. The patients with cardiovascular insufficiency on atherosclerosis background from 30 to 69 years of age were taken into study group. CD-4, CD-8, TNF-α, HSP- 60,CRP implication was studied trough immunohistochemical reaction using monoclonal antibodies. This material was fixed in 4% phormaldechyde solution. Results: T-cells are believed to act as orchestrator of atherogenesis. The increase of CD-4, CD-8 lymphocytes-T and HLA–DR mononuclears' count in intima and adventitia of the vessels was releved in humans aged 31-59. CRP is an important component of the plasma proteins that insudates the arterial wall preceding the so called initial atherosclerotic lesions. Among the inflammation mediators TNF-α have been detected in increased quantity, being localized on the surface and in depth of atherosclerotic plaque. Identification of HSP-60 cross-reactive T-lymphocytes within atherosclerotic tissue indicates that cell-mediated responses are also involved. Because human HSP-60 have been detected on the surface of endothelial cells in the arterial wall, it could be a putative autoantigen involved in eliciting cell-mediated and humoral immune responses against the vessel wall causing its injury and that leads to atherosclerosis development. The most quantity of the inflammation's markers has been detected at the early stages of atherogenesis, in young persons. In advanced lesions and in old persons their incidence decrease. Conclusions: Our outcomes demonstrate the toxic and immune factors' existence which are considered to be decisive in atherosclerosis pathogenesis. These data give us the reason to elaborate other diagnosis and treatment strategy.

OP5-3
SUDDEN CARDIAC DEATH – A VIEW OF FORENSIC PATHOLOGIST

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In Switzerland the cases of sudden unexpected death and especially of relatively young people are reported to the judge who orders a forensic autopsy. In some cases, the cause of death remains however unexplained after a classical autopsy and toxicological analyses. In these cases, the cause of death may be related to potentially inherited arrhythmias. According to the literature, the cases of unexpected arrhythmogenic death are estimated to represent 5-10 % of all sudden deaths. The advances in molecular biology have opened new diagnostic perspectives in forensic pathology. In fact, with the appearance of molecular autopsy, it becomes possible to detect the cause of death related to genetic origin and without morphological substrate diagnosed at autopsy as for example cardiac channelopathies. To illustrate this concept, two cases with no evident cause of death found at classical autopsy are presented, one of a 6-month-baby and a second one of a 24-year-old woman. For both victims SCN5A mutations have been detected. One of these mutations has been already described in the literature, whereas the pathogenicity of the second one was determined by analysis of the mutation-induced alteration in cardiac sodium current. However, the molecular diagnosis concerns not only cardiac diseases. Indeed, since the apparition of pharmacogenetics it is considered that both drug disposition and response are individually variable and some genetically vulnerable victims may die with extremely low drug levels found at toxicological analyses. It is also evident that chronic drug abusers may have increased tolerance for high levels of substances detected in their body. Thus, the autopsy findings as well as the results of toxicological and genetic analyses need to be interpreted together in order to decide which case could be really considered as a sudden cardiac death. The molecular diagnosis is important to clarify a case, especially in cases without morphological substrate and makes also possible the detection of the genetic predisposition to sudden deaths of relatives. In this respect, the information provided by genetic analysis places the forensic pathologist in an interdisciplinary collaboration. At the University of Lausanne a multicenter team has been created between departments of forensic pathology, cardiology, and genetics in order to diagnose and prevent the cardiovascular diseases in relatives. The role of the forensic pathologist in this team as well as the legal aspects of post-mortem genetic analyses will be discussed.

OP5-4
ENDOMYOCARDIAL BIOPSY GUIDED BY ELECTROANATOMIC VOLTAGE MAP IN ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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Objective: To evaluate the sensitivity of right ventricle (RV) electroanatomic (EA) mapping with CARTO system (Biosense-Webster) for arrhythmogenic right ventricular cardiomyopathy (ARVC) diagnosis. Background: Despite currently used standardized criteria represent a useful clinical tool, the diagnosis of ARVC remains a clinical challenge, particularly in its early stages. Endomyocardial biopsy (EMB) may be used for a definitive in vivo histopathological demonstration of the typical fibrofatty infiltration of RV myocardium but its sensitivity is low. Recent data support CARTO system as an innovative approach to identify low-voltage regions reflecting fibrofatty myocardial replacement. Methods: Twenty patients (pts) (9 males, 33.2 + 18.4 years) with frequent symptomatic drug refractory ventricular ectopies or sustained/unsustained ventricular tachycardia of right ventricular origin were enrolled for the study. Noninvasive standardized ARVC criteria were fulfilled in 10 pts (50%). An EA reconstruction of RV was performed in all pts, sampling multiple endocardial sites (202 + 61) during sinus rhythm. Bipolar voltage map analysis, documented low-voltage areas (< 0.5 mV) in 17 pts (85%). In 14 pts (70%) with pathological EA map (6 males, 31.9 + 17.7 years) an EMB focused on low-voltage areas was attempted (study group). For each patient, up to 6 specimens were picked up from low-voltage regions. Endomyocardial biopsies were fixed in 10% phosphate-buffered formalin and embedded in paraffin. A minimum of 15 sections were obtained from each sample and stained with Hematoxylin & Eosin and Masson Trichrome. Immunohistochemical stains for CD3, CD20 and CD68 were also performed to eventually characterize inflammatory infiltrates. Slides were examined under light microscopy by two observers, unaware of voltage mapping results. Results: EMB guided by RV voltage map was diagnostic for ARVC in 11 pts (79%), while only non specific findings (mild hypertrophy and interstitial fibrosis) were documented in

the remaining 3 pts (21%). Among pts with bioptic evidence of ARVC, 9 pts (82%) did not fulfill standardized noninvasive criteria and 7 pts (64%) presented only minor RV morpho-functional alterations. Conclusions: EMB guided by bipolar RV voltage mapping seems to have a high diagnostic sensitivity for ARVC, particularly in patients presenting in the early stages of the disease. Nevertheless, the real clinical impact of this innovative approach needs to be confirmed in a greater patient population.

OP5-5

ANGIOGENIC MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN THORACIC AORTAS

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Background.Rapid revascularization of ischemic organs is essential to restore tissue function. In experimental models,bone marrow derived-endothelial progenitor cells are able to mobilize to ischemic sites and complement neo-angiogenesis in vivo; however, their clinical use is yet hampered by difficulties in obtaining an adeguate number of healthy and functional progenitors.Recently vasculogenic progenitors were demonstrated in the vascular wall of human muscular arteries; this study aimed to establish whether human thoracic aortas from healthy multiorgan donors contain mesenchymal stem cells (MSC) with angiogenic property. Methods.Fresh thoracic aortas from 10 heart beating multi-organ donors were processed for IHC staining (c-kit,CD-34,ASMA,vWF,ki-67,CD-45,CD-68) and used to isolate putative progenitor cells. Cells taken at passages 3-5 were characterized with flow citometry (CD133-PE, CD117-FITC, CD34-PE, CD44-FITC, CD90-PC5, CD105-PE, CD166-PE, CD45-APC, CD146-PE, HLA class I-FITC,HLA-DR-FITC). IF studies on cells plated in collagen biocoated slide chambers were done in parallel. Total RNA was extracted from cells and the expression of the following mRNAs was investigated:OCT4,BMI-1,BCRP-1,IL-6, c-kit, CD-133, KDR. For the in vitro angiogenesis assay, cells were cultured for 7 days in DMEM plus 2% FBS with and without 50 ng/ml VEGF. The expression of endothelial cell markers (vWF, KDR) was performed using flow cytometry, IF and RT-PCR."Capillary formation was investigated on Matrigel; cell seeded on Matrigel were also processed for TEM."Results.IHC studies of human thoracic aortas showed distinct CD34+ and c-kit+ cell populations in between the media and adventitia;"ki-67+ cells were found in corresponding areas."Cells expanded from thoracic aortas had characteristics of MSC:CD44+,"CD90+, CD105+, CD166+, CD45-; they showed a transcript expression of stem cell markers: OCT4, c-kit, BCRP-1,"IL6, and BMI-1.Cells cultured in the presence of VEGF acquired characteristics of endothelial cells, i.e., increased expression of KDR, vWF expression as documented by flow cytometry, IF and TEM. The matrigel assay revealed that cells were able to form capillary-like structures within 6 hours of seeding. Conclusions:This study demonstrates that thoracic aortas from multi-organ donors yield MSC with the ability to differentiate in vitro into endothelial cells.These cells can be expanded rapidly, providing numbers which are adequate for therapeutic neovascularization; again, being recovered from young and healthy donors, they can be cryostored in appropriate cell banking facilities for later use.

OP5-6

LYMPHATIC VESSELS, LYMPHATIC VASCULAR ENDOTHELIAL GROWTH FACTORS AND THEIR RECEPTORS IN HUMAN HEART VALVES

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Background: Despite the importance of myocardial vasculature in many pathological conditions very little information is available about cardiac lymphatic vessels in normal and pathological conditions. Recent generation of lymphatic specific antibodies enables accurate studies of lymphatic vasculature. Additionally we studied the expression of lymphatic vascular endothelial growth factors and their receptors in valves. Methods: We evaluated bioptic samples of 6 aortal and 3 mitral valves from 8 patients (age range 50-77 years, 7 males, 1 female). The histopathological diagnosis was as follows: infective endocarditis, postrheumatic fibrohyalinosis, myxoid degeneration and calcified stenosis. Vasculature was assessed by immunohistochemical staining with the panel of antibodies: CD31, LYVE-1, D2-40. The expression profiles of vascular endothelial growth factors VEGF-C and VEGF-D and their receptors VEGFR-2 and VEGFR-3 were additionally evaluated. Results and conclusions: Highest amount of lymphatics was found in infective endocarditis (up to 70 per section) and they formed up to 100% of all vessels. The increase was found also in degenerative calcified stenosis. The myxoid degeneration was accompanied by lowest increase. The majority of vessels in fibrotic areas were CD31-positive blood vessels. Lymphangiogenesis was accompanied by an increase in the lymphatic vascular growth factors expression. The profound knowledge of cardiac lymphatic system has an impact on further therapeutic vascular growth studies and improvement of new therapeutic approaches.

OP5-7

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF A SERIES OF 33 CONGENITAL VASCULAR MALFORMATIONS – A SINGLE CENTRE EXPERIENCE

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Background: Congenital vascular malformations (CVMs) are the result of abnormal blood vessel development. Whilst some lesions are self-limiting, the sub-class arteriovenous malformations (AVMs) persist and in many instances with potential implications for disfigurement, functional disability, morbidity and rarely mortality. Different clinical categories of CVMs are not distinguishable histologically and features described in the literature are largely non-specific. This is partly responsible for the current lack of understanding and subsequent sub-optimal management of CVMs, especially of AVMs. We have analysed the histological morphology in comparison with control tissues in 33 patients with CVMs (including 9 AVMs). The significant findings were related to clinical confounding parameters (sex, high or low flow categorisation, location of the CVM, previous embolisation / sclerotherapy and associated surface ulceration. This study was performed to elucidate the histology of these uncommon but often locally aggressive lesions and to gain insight into possible mechanisms involved in their

aetio-pathogenesis. Methods: 33 consecutive CVM lesions excised from patients presenting between 1992 - 2006 were analysed morphologically using routine techniques, histochemical (H&E, Masson Trichrome, Elasic Von Gieson and Toluidine blue) and immunohistochemical (CD68, CD3, CD20, Factor VIII, actin 1-alpha, Ki67 and S100). 10 control specimens were similarly analysed. As 10 outcomes have been analysed on the same small dataset, a Bonferroni correction was applied such that $p<0.005$ indicates significance. Results: Macrophages ($p<0.0001$), mast cells ($p<0.0001$), vessel number ($p=0.0004$) and vessel diameters ($p=0.0005$) were significantly higher in the CVM group compared to controls. There was no correlation between the statistically significant morphological parameters resulted in this study, and any of the clinical parameters. Discussion: Macrophages and mast cells have recognised roles in angiogenesis. Our findings suggest that the inflammatory cellular infiltrate may be implicated in the development of CVMs. Increased vessel diameter may be the result of abnormal local concentrations of angiogenesis mediators. Further insight into the role of inflammatory and angiogenic cytokines in the aetio-pathology of these lesions may provide novel criteria for more clinically appropriate classification and accurate diagnosis of CVMs. This enhanced clinico-pathological correlation should improve therapeutic options and efficacy.

OP6-1

MORPHOLOGIC, PHENOTYPIC AND CYTOGENETIC CHARACTERIZATION OF THREE ESTABLISHED EWING/PNET CELL LINES

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Background: Cell lines are of great importance in the study of the genetic changes in tumors, and some reveal several aspects of tumor oncogenesis. Several Ewing/PNET cell lines are available, usually isolated from the original tumors and only on few occasions from the xenotransplanted tumors. Method: The aim of this report is to characterize and describe the evolution of three Ewing/PNET cell lines, primarily isolated and cultured (in vivo and in vitro cell lines) from the original tumor. Subsequently, xenotransplanted cell lines were cultured in vitro, and analyzed and characterized by morphologic (phase contrast microscopy), immunohistochemical (CD99, FLI-1, Vimentin, HNK-1, NF, c-kit, EPO, EPO-R and SCF), cytogenetic (EWSR1 break apart, p16 and p53 status) and molecular biology (gene fusion type) studies. Subsequently, in vivo re-inoculation of these cell lines in nude mice was performed and all studies including the ki-67 index, p53 and p16 immunohistochemical analysis were made; the results were then compared with the initial in vivo xenotransplanted tumors. Results: CD99 was the most-expressed immunohistochemical marker in the three xenotransplanted cell lines, the original tumors and the nude mice tumors. The FISH breakapart (EWSR1) studies showed positive translocation of the EWS gene in all the material studied, as well as the molecular biology studies which revealed the fusion gene EWS/FLI1 in the three xenotransplanted cell lines. The growth of the tumors in nude mice was more accelerated when the inoculation was performed from the in vitro xenotransplanted cell lines; in these cases, the proliferation index measured by the value of ki67, increased progressively in the passages. This could be related or associated with the genetic changes that occur in cells lines; p16 deletions and/or p53 mutation can immortalize the cells lines and promote a high proliferation rate of the cells in vitro and in vivo. Conclusions: There was heterogeneity in the results of the p16, p53 and ki67 in the three cell lines, the original tumors as well as in the nude mice tumors. * Performed with support of EUROBONET VI FW.EU Contract Nº 018814 and FISS Madrid: PI 04/0882 and RD 06/0020/0102

OP6-2

POTENTIAL PROGNOSTIC IMPORTANCE OF CHOSEN MORPHOMETRIC CHARACTERISTICS OF THE CELLS OF WILMS' TUMOR AND NEUROBLASTOMA IN CHILDREN

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Currently new technologies based on the computer image analysis are used in the histopathological evaluation. Application of these technologies make possible more detailed and precise morphological diagnosis. The aim of the study was the evaluation of the MVf (morphometric discriminat of the function MV) and its potential prognostic importance in the group of children with Wilms' tumor and neuroblastoma. The MVf value was calculated on the basis of the age of the patients and also on the basis of surface area, circumference and diameter of the neoplastic cells. Our material consisted of 51 cases of Wilms'tumors and 19 cases of neuroblastomas from the files of the Department of Pathology of the Age of Development Medical University of Lodz. The MultiScanBase CSS Ltd. Company system was used for scoring the morphometric characteristics (surface area, circumference, diameter and maximum and minimum Feret diameter) of the neoplastic cells. According to the article "Nuclear Morphometry and Prognosis in Favorable Histology Wilms' Tumor. A Prospective Reevaluation" the MVf value > - 0.35 was related to unfavorable prognosis. Our date showed that the higher MVf value were observed in neuroblastomas than in Wilms' tumors. We also proved that the lower MVf value was related to favorable prognosis in children with nephroblastomas. We noted that the higher value of this parameter in children with neuroblastomas characterized tumors with benign clinical course. In conclusion we believe that the MVf value is an independent useful prognostic marker in the group of Wilms' tumor and neuroblastomas in children.

OP6-3

GENETIC ALTERATIONS IN NEPHROBLASTOMAS AND CORRESPONDING NEPHROGENIC RESTS

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Background and Aims: Nephroblastomas are thought to develop from nephrogenic rests (NR) by accumulating genetic alterations. All subtypes of nephrogenic rests can potentially develop into nephroblastoma. In a few single studies identical genetic changes have been identified at chromosome 11p15, 11p13 and 16q. In this project we want to establish the molecular relationship between NR and nephroblastomas. Methods: For our study we have selected several different tumor suppressor candidate genes (nm23, p63 and PTEN), which also either play a role in nephrogenesis like WT1 gene or in the development of renal cancer. Expression analysis of these genes was performed by routine immunohistochemistry in combination with the DAKO ENVISION detection system. Nephrogenic rests, nephroblastomas and the corresponding normal tissue were manually microdissected under the microscope followed by routine extraction of genomic DNA in order to evaluate loss of heterozygosity (LOH) of our candidate genes. We investigated polymorphic gene loci by different PCR-based methods. Results: 30 cases of nephroblastomas with associated NR from the paediatric tumor registry, Kiel, will be included in the study. So far samples of 11 cases of perilobar NR, 5 cases of intralobar NR and one case showing a perilobar and an intralobar NR have been investigated. p63 protein expression showed predominant negative staining except for some tubular structures and squamous elements within the tumour. The expression pattern of

metastasis suppressor gene nm23 was only cytoplasmatic. It was strongly expressed within the tumour and NR in seven cases. The remaining cases showed predominant partial positive staining of tumour and NR. The cases so far investigated with antibodies against PTEN showed expression in normal renal tissue and weak expression in the blastemal part of the tumour. Analysis of LOH of the PTEN gene has revealed so far non-informative cases. Conclusions: In order to interpret our results of the PTEN gene we need to investigate the yet remaining cases. The mixed staining pattern of nm23 needs to be clarified in connection with clinical data and outcome. So far we have been able to exclude a possible role of p63 in the pathogenesis of nephroblastomas.

OP6-4

LUNG DEVELOPMENT IN PATIENTS WITH SEVERE HYPOPHOSPHATASIA (HPP): SEVERE EFFECTS DUE TO POOR MECHANICAL FUNCTION

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Background: A better understanding of the mechanisms of lung morphogenesis and lung plasticity will improve our ability to treat and or prevent a variety of neonatal and adult lung diseases. Growth and maturation of fetal lungs are regulated by both humoral and physical factors. But most of the studies on lung development have been done in animal models. “Experiments in nature” will help us to better understand the molecular, biochemical and biophysical events involved in producing normal human lung. Mechanical stretch that stimulates fetal lung proliferation is inhibited by mechanical forces (i.e. congenital diaphragmatic hernia), or a lack of amniotic fluid (oligohydramnios); all lead to a reduction in airway number in fetuses. The critical period for alveolarization begins during the canalicular period (16–26 weeks post conception [pc]) of lung development. But alveolarization continues on with impressive growth during the post natal period (38 weeks pc–2 years postnatally). Hypophosphatasia (HPP) is a rare inborn error of metabolism which features deficient activity of “tissue non-specific” alkaline phosphatase. Patients with the perinatal (p-HPP) form die in utero or shortly after birth. Patients with infantile HPP (i-HPP) present before 6 months of age with rickets, failure to thrive, or vitamin B6-dependent seizures. Approximately 50% succumb to respiratory failure. Radiographs of both p-HPP and i-HPP reveal a generalized demineralization of all bones with narrowing of the rib cage and fractures. Collagen and the extracellular matrix are normal in HPP. Reports on the pathogenesis of respiratory failure in these patients and others with severe and fatal skeletal abnormalities are deficient. Methods and Results: We examine four children with HPP including two with i-HPP who lived beyond 6 months. A lung biopsy at 14 months, revealed a decreased number of alveolar ducts, marked medial hypertrophy, intimal proliferation and fibrosis in pre-acinar and intra-acinar pulmonary arteries with luminal narrowing. Alveoli were lined with Type II pneumocytes with absent tubular myelin. No Type I cells were identified. Patients with p-HPP who died at birth had normal radial alveolar counts but a paucity of blood vessels (alveolar:arterial ratio > 20) Conclusion: Despite postnatal survival, mechanical factors must be corrected in the critical period of alveolarization before these changes become irreversible. Delays in the current strategies to correct the mineralization defect in the skeleton after birth may not be sufficient.

OP6-5

FOCAL ADHESION KINASE EXPRESSION IN MOST COMMON PEDIATRIC SOLID TUMORS

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Background: Focal Adhesion Kinase (FAK) is a nonreceptor protein kinase widely expressed in various tissues and cell types, playing important role in integrin signaling. In vitro studies showed that tumor cells present increased FAK signaling, inducing uninhibited proliferation, protection from apoptosis, invasion, migration, adhesion, spreading and tumor angiogenesis. Several in vivo studies supported the above observations and further correlated FAK expression with clinicopathological parameters in several types of human malignancies. The aim of our study was to characterize FAK expression in the most common solid pediatric tumors (neuroblastoma, nephroblastoma and rhabdomyosarcoma) and estimate clinical significance. Method: Ultrasound-guided Fine-Needle Aspiration (FNA) aspirates from 30 patients with neuroblastoma, 15 with nephroblastoma and 10 with rhabdomyosarcoma were obtained at initial diagnosis and maintained as ThinPrep preparations. Tumor cells were immunostained with the streptavidin-biotin immunoperoxidase technique to evaluate FAK expression. FAK positivity, overexpression (positivity in more than the mean percentage value) and intensity of immunostaining (mild, moderate, intense) were correlated with the clinical stage of disease and tumor cells´ proliferative capacity (Ki-67 labeling index). Results: FAK positivity was noted in 20 out of 28 (71,4%) neuroblastoma, in 10 out of 16 (62,5%) nephroblastoma and in 7 out of 8 (87,5%) rhabdomyosarcoma cases, while FAK overexpression in 8 out of 20 (40%) positive neuroblastoma, in 4 out of 10 (40%) positive nephroblastoma and in 3 out of 7 (42,8%) rhabdomyosarcoma positive cases. The intensity of FAK immunostaining was mild, moderate and intense in 65%, 30% and in 5% of neuroblastoma, in 80%, 10% and 10% of nephroblastoma and in 14,3%, 71,4% and 14,3% of rhabdomyosarcoma positive cases, respectively. FAK positivity, overexpression and intensity of immunostaining were not correlated with patients' clinical stage and tumor cells' proliferative capacity. Conclusion: FAK expression was prominent in the most common pediatric solid tumors, nevertheless no correlation was found with clinicopathological parameters important for patients’ outcome, suggesting an uncertain involvement of FAK in either diagnosis or management of patients with neuroblastoma, nephroblastoma or rhabdomyosarcoma.

OP6-6

HTERT EXPRESSION IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS (MPNST) OF PEDIATRIC AGE: CORRELATION WITH TUMOR GRADE AND PROGNOSIS.

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Telomerase is a complex ribonucleoprotein enzyme that extends telomeric repeats on the ends of chromosomes, preventing the progressive shortening in each cell division that would lead to replicative senescence of cells. hTERT is the catalytic subunit

that provides the function to replicate the ends of chromosomes. In epithelial neoplasms hTERT is frequently increased, but in sarcomas it is less well characterized. We have investigated the expression of hTERT in a series of pediatric MPNST and its correlation with grading and prognosis Methods and results 19 MPNST enrolled in the Italian protocol for soft tissue tumors (RMS 88, RMS 96) were reviewed and graded according to the French FNCLCC system. Immunohistochemical staining for hTERT was performed, and the results were scored as follows: 0, negative; +1, weak positivity in rare cells; +2, strong and diffuse positivity. The age range of patients was 1-17 yrs; 7 suffered from NF1, 8 did not show any syndrome; for 4 patients the status was unknown. The mean follow-up was 4.8 yrs (range 1-9 yrs). 8 patients died (1 for toxicity), 1 had local relapse, 1 is alive with disease. hTERT expression was detected in 12 cases (63%), with +1 in 6 and +2 in other 6 tumors. NF1-associated MPNST were positive in 4/7, with +2 in only one case; conversely, 7/8 non NF1-associated MPNST were positive, with +2 in 4 cases; MPNST with unknown NF1 status were positive in 1/4 cases. The relationship of tumor grade and hTERT expression was the following: G1: hTERT 0 in 1/5, +1 in 3/5 and +2 in 1/5; G2: hTERT 0 in 2/4, +1 in 1/4 and +2 in 1/4; G3: hTERT 0 in 4/10, +1 in 2/10 and +2 in 4/10. Death occurred in 1/6 patients with hTERT 0, in 3/6 with hTERT +1 and in 4/6 with hTERT +2; follow-up was not available for 2 patients (1 hTERT 0 and 1 hTERT +2). Conclusions Pediatric MPNST express hTERT in 63% of the cases, with strong immunoreactivity in 31.5% of cases, and +2 positivity is more frequent in tumors not associated with NF1. In addition expression of hTERT seems to be related to an unfavorable prognosis.

OP6-7
ACTIVATION OF THE PI3K/AKT PATHWAY IN NEUROBLASTOMA

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Background: Neuroblastoma is a pediatric malignant neurorectodermal tumor with still a poor survival in children over 1 year of age. In cancer, the PI3K/Akt pathway contributes to tumor aggressiveness (proliferation, metastasis). It is a major target for novel anti-cancer therapies. The objective was to study the activation of the PI3K/Akt pathway in NB. Methods: 101 patients (median age 2.5 years, range: 0-13) with a resected neuroblastoma (16 stage I; 7, II ; 22 ,III; 48, IV and 8, IVS) were included. Tissue microarrays were constructed containing 101 primary tumors, 39 paired metastases and 56 paired normal tissues. Immunohistochemical staining was performed on sections using antibodies against PI3K, Akt and phosphoAkt (S-473). The expression of membrane receptors (EGFR, HER-2, VEGFR1, VEGFR2, PDGFRα, PDGFRβ, IGF1Rβ, TrkB), proteins directly interfering with AKT (PTEN, mTOR) and downstream proteins (phospho-p70S6 kinase, 4eBP1) was studied. The intensity was evaluated by a semi-quantitative score based on the percentage of positive cells. The Wilcoxon signed rank test was applied for the comparison of paired data and adjusted for multiple testing using the False Discovery Rate criterion. Spearman correlation values (rho) were used to compare the expression between genes in the primary tumors. Results: 60% of the tumors expressed PI3K, 80% AKT and 73% pAKT. In addition, phospho-p70S6 kinase and 4eBP1p were expressed in 95% and 85%. All these proteins had a higher expression in tumors as compared with paired normal tissues (fdr-adjusted $p<0.001$). Akt and pAkt showed a tendency for higher expression in metastases as compared to paired primary tumors (raw $p<0.05$ for both, but fdr-p=0.11 and fdr-p=0.17 respectively). While EGFR, IGF1Rβ, TrkB, PDGFRα and PDGFRα were significantly more expressed in primary tumors as compared to normal tissues (fdr-$p<0.05$), only IGF1Rβ seemed more expressed in metastases (fdr-p=0.09). A significant positive correlation was found between pAkt and the following: VEGFR1, VEGFR2 and IGF1Rβ (rho>0.34, $p<0.001$), phospho-p70S6 kinase and mTOR(rho>0.26, $p<0.01$). Conclusions: The PI3K/Akt pathway is activated in neuroblastoma and could be an attractive target for novel therapies. In addition, the data suggested that VEGFR and IGF1R are preferentially committed to this pathway activation.

OP6-8
MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHANGES OF PLACENTA OF WOMEN WITH MULTIFETAL PREGNANCIES REDUCED TO TWINS

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Introduction: Multifetal pregnancies are known to be associated with a higher risk in maternal, perinatal complications when compared to singletons or twins. Materials and methods: We made a retrospective study of placenta of 38 high multiple pregnancies following assisted reproduction that were reduced to twins (group 1). They were compared with nonreduced twins pregnancies (group 2) and control group (natural pregnancy). The study of structural and functional features of the placenta included morphological, morphometrical and immunohistochemical methods (expression of the antigen tumor necrosis factor (TNF) and proliferative cellular nuclear antigen (PCNA) by streptavidin-peroxidase method). The Results of the immunohistochemical detection were assessed by traditional approaches using the score to reflect the degree of the expression of detected antigens and the apoptosic index (AI). Results: Chronic placenta insufficiency was detected in the group 1 in 71.5% compare to 28.5% in the group 2 according to the results of histological examination. Destructive changes and chronic placenta insufficiency mild (28.58%) were detected together with sever (71.42%) compensatory changes including angiomatosis of the vessels, increased number of syncytial inserts and sinusoidal transformation of capillary. Described compensatory changes were further supported by the detection of the regeneration markers such as PCNA expressed in the nuclei of the cells. Increased expression of PCNA (relevant to the score of 3 to 4) in the cyto- and syncytiotrophoblast cells of the women from IVF and embryo reduction group compared to group 2 was detected by immunohistochemical methods. The Expression was lower in stroma fibroblasts (mostly score 2, in some cells 3), compared to group2. The level of apoptosis (AI) in placenta barrier was increased in the patients with embryo reduction almost by 2 fold in the villies sincitii (12.0+-0.42% and 7+-0.21 % in control group). AI in stroma cell was lower than in the group 2. Significant number of TNF-positive cells with mild and high TNF expression (score 2-3) was detected in placentas of patients from the group 2 (patients who had IVF) compared to the control group. Conclusions: Chronic placenta insufficiency, increased expression of TNF (especially in epithelial cells), inreased AI in epithelium, stroma and decidual cells and elevated PCNA in epithelial cell of the villies were characteristic findings in the placentas of the women after IVF and embryo reduction.

OP6-9
ESTIMATION OF APOPTOSIS IN PLACENTAS WITH INTRAUTERINE GROWTH RETARDATION

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Background: Intrauterine growth retardation (IUGR) and preterm delivery remain the most important risk factors of perinatal

morbidity and mortality. However, the underlying pathophysiological mechanism of IUGR still remains the subject of extended studies. This immunohistochemical study aims to analyze the incidence of apoptosis in placenta tissue with IUGR due to uteroplacental insufficiency, to compare its incidence to uncomplicated gestation and to interpret its possible role in case of IUGR. Method: Placental tissue samples were obtained from 15 normal pregnancies (control group) and 20 pregnancies complicated with IUGR (study group). All the samples were examined with fluorescence microscopy using fluorescence TUNEL staining and the number of apoptotic nuclei was measured using an image analysis system. Results In general, few apoptotic cells were seen in term placental tissue of uncomplicated pregnancies. By contrast, a significant increase in the incidence of apoptosis was identified in placental tissue of IUGR pregnancies at term, with the highest apoptotic index in the syncytiotrophoblast layer ($p<0.0001$). Conclusion: Whether the observed increase in the incidence of apoptosis is a result of IUGR or is a part of the pathophysiological process leading to these complications is yet not clearly identified. Placenta may respond to hypoxic stress by enhancing the number of apoptotic cells. This could be interpreted as an attempt from the placenta to compensate for this relative ischemia and so increase the nutritional transport and gas exchange.

OP7-1

E-CADHERIN/CATENIN COMPLEX AND THE RECENTLY IDENTIFIED DYSADHERIN ARE DIFFERENTIALLY EXPRESSED IN PRIMARY LIVER TUMOURS

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Background: The aim of the present study is to examine the expression and localization of the E-cadherin cell-cell adhesion molecule and its associated undercoated catenins (α-, β-, and γ-catenin), as well as the recently identified by members of our research team, anti-adhesive protein termed dysadherin, in primary endo-hepatic carcinomas. Method: Fifty-six (56) formalin-fixed, paraffin-embedded tissue specimens of primary liver tumours (39 hepatocellular carcinomas and 17 cholangiocarcinomas) were examined immunohistochemically. The EnVision system (DAKO, Netherlands) and the monoclonal antibodies CM170B (Biocare Medical, Menarini Hellas) against E-cadherin, anti- α-, β-, and γ-catenin (Menarini, Hellas), as well as NCC-M53 against dysadherin, were used. The staining was evaluated by a semiquantitative method. Results: In the vast majority of hepatocellular carcinomas there was decrease in the membranous immunostaining for E-cadherin and increase in immunostaining for α-, β-, and γ-catenin. The expression of E-cadherin was inversely correlated with the grade of hepatocellular carcinomas, while that of α-, β-, and γ-catenin was directly correlated. Furthermore, in high grade hepatocellular carcinomas, nuclear localization of β-catenin was noted. Dysadherin was not expressed in non-neoplastic hepatocytes. Membranous dysadherin immunostaining was noted only in 3 (7.6%) hepatocellular carcinomas, all of which exhibited decrease in E-cadherin expression. In non-neoplastic bile ducts there was membranous immunostaining for E-cadherin and α-, β, and γ-catenin, but not for dysadherin. In cholangiocarcinomas reduction in the expression of E-cadherin, α-, β-, and γ-catenin, and positive membranous staining for dysadherin (10/17, 59%) were noted. There was no statistically significant correlation between dysadherin and E-cadherin expression. Conclusion: Hepatocytes and intrahepatic bile duct cells, although situated in the same area, differ in the signal transduction pathways that are implicated in carcinogenesis, at least regarding the role of molecules involved in cell adhesion and dysadhesion processes.

OP7-2

HEPATOCELLULAR CARCINOMA IN ALPHA-1-ANTITRYPSIN DEFICIENCY: A GENOTYPE STUDY IN SINGLE NEOPLASTIC AND NON-NEOPLASTIC HEPATOCYTES.

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Background. Alpha-1-antitrypsin (AAT) deficiency is associated with mutation in the SERPINA 1 gene and represents the most common cause of metabolic liver disease in children. Around 10-15% of adults develop hepatocellular carcinoma (HCC). Hepatocellular damage is due to toxicity of the mutant alpha-1-antitrypsin molecule retained within hepatocytes. The histological hallmark of the disease is the intracellular periodic acid-Schiff diastase (PASD) resistant positive globules in hepatocytes with heterogeneous distribution within liver tissue. Moreover, aging processes enhance the relative number of globule-devoid cells, which ultimately might result in neoplastic transformation. Methods. We attempted to correlate the AAT genotype with globule-devoid hepatocytes in single cells from two patients who had developed HCC. The first was a 56 year-old woman whose ultrasound examination had revealed a 3 cm nodule in the left hepatic lobe, while the second one, a 65 year-old man, was submitted to hepatectomy due to a 3.7 cm liver mass. Paraffin-embedded liver sections from both patients were evaluated on hematoxylin and eosin (HE) and PASD stains. Immunohistochemistry used polyclonal and monoclonal antibody anti-AAT. To assess the AAT genotype, DNA was extracted from paraffin-embedded liver sections and the 5 exons of the SERPINA 1 gene were PCR-amplified and directly sequenced. To test whether globule-devoid hepatocytes were the result of a self-induced correction of the genetic defect, laser-assisted microdissection on a HE-stained slide was used to collect separately cells from neoplastic liver as well as non-neoplastic hepatocytes. Results. The non-neoplastic liver cells were found to contain PASD globules, while the tumours hepatocytes were globule-devoid. The globules were positive for polyclonal AAT-antibody in both cases, whereas monoclonal AAT-antibody was positive in the first case and negative in the second one. DNA analysis revealed a PiSZ genotype in the first case and PiMM-Cagliari in the second patient. An identical genotype was observed in cells obtained with micro-dissection in both cases. Conclusions. This study presented the following considerations. First, not only Z but also M-Cagliari AAT deficiency might lead to HCC. Second, both conditions are associated with a heterogeneous morphological phenotype in liver tissue. Third, the presence of the same genotype in both cell population suggests that phenotype change is not a result of a retro-mutation during neoplastic transformation and that other mechanisms are responsible for the phenomenon.

OP7-3
CLUSTERIN EXPRESSION IN CHOLESTASIS AND LIVER FIBROSIS

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Background: Clusterin is a chaperone which shares functional properties with small heat shock proteins (sHSP), but its physiological function is restricted to the extracellular space. Cytoplasmic clusterin overexpression was found to be associated with poorer prognosis in hepatocellular carcinomas (HCCs). Furthermore, clusterin may play a role in HCC metastasis and the serum clusterin level was found to correlate with patient survival. Method: A tissue microarray was used to evaluate the expression of clusterin in 116 HCCs and 103 matched non-neoplastic livers (including fibrosis and cirrhosis) by immunhistochemistry (IHC). Additionally, in several cholestatic liver diseases, including primary sclerosing cholangitis (10 samples), primary biliary cirrhosis (18 samples), mechanical cholestasis (9 samples) and drug-induced cholestasis (12 samples) the presence and distribution of clusterin was explored. The distribution pattern of clusterin in portal tracts was correlated with staining of elastic fibers (Elastica-VanGieson stain). To examine the presence of clusterin in human bile western blotting was performed in three bile samples from autopsy cases. Results: Clusterin was found to be associated with fibrotic areas of the liver, with colocalization with elastic fibers. Furthermore, clusterin was detected immunohistochemically in bile plugs in all investigated cholestatic liver diseases. Additionally, 44 of 116 cases of HCC showed pseudoglandular features with clusterin staining of their content. Clusterin was also demonstrated in bile by western blotting. Conclusion: In liver fibrosis and cirrhosis clusterin was associated with elastic fibers but not with hepatocytes or bile duct epithelia. Moreover, clusterin was present in bile as revealed by western blotting and IHC. The biologic significance of the association of clusterin with elastic fibers is unclear. It may act as chaperone either protecting and stabilizing elastic tissue or shielding abnormal elastic material. In bile, clusterin may protect bile duct epithelium against aggressive bile components or inhibit precipitation of biliary proteins.

OP7-4
PROTEIN PROFILING OF CHOLANGIOCARCINOMA: ARE HILAR AND INTRAHEPATIC CHOLANGIOCARCINOMAS DIFFERENT MOLECULAR ENTITIES?

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Background: cholangiocarcinoma is a devastating malignancy diagnosed lately and associated with a high mortality. The tumour arises from the ductular epithelium of the biliary tree and two main forms are recognised: hilar and intrahepatic cholangiocarcinomas. In addition to different clinical patterns, they also differ in terms of etiopathogenesis and potential risk factors. However, up to now there are no data regarding their molecular signatures. The aim of the study was to investigate the protein profile of a large series of hilar and intrahepatic cholangiocarcinoma in order to better characterize their phenotype. Method: we reviewed the major morphological features including size, tumor differentiation, mucus secretion, perineural invasion of cholangiocarcinoma from 112 patients who underwent surgical resection at Beaujon hospital from 1996 to 2006. We compared the expression profiles between hilar (n=59) and intrahepatic cholangiocarcinomas (n=53) using a tissue microarray technology. Immunohistochemical staining of paraffin embedded tissue sections was performed for a total of 32 antibodies against a panel of keratins (CK), apomucins, cell cycle proteins, growth factors and cell signalization proteins. Two pathologists independently scored the protein expression. Results: Comparison of morphological features showed that hilar cholangiocarcinomas were smaller in size (mean: 2.9cm vs 7.8cm, $p<0.0001$), were more differentiated adenocarcinomas (62% vs 38% well_differentiated, $p<0.05$), secreted more mucus (91% vs 68%, $p<0.01$) and realized more perineural invasion (80% vs 45%, $p<0.001$) than intrahepatic cholangiocarcinomas. Regarding protein expression, 93% and 98% of cholangiocarcinomas were positive for CK7 and CK19, respectively. Among all proteins markers tested, a significantly different expression was observed only for three molecules. Hilar cholangiocarcinomas expressed more often MUC 5AC (60% vs 22%, $p<0.0001$) and Akt2 (53% vs 27% $p<0.001$). At the opposite, VEGF expression was more frequently encountered in intrahepatic cholangiocarcinomas (76% vs 53%, $p<0.05$). Conclusion: Our study shows that hilar and intrahepatic cholangiocarcinomas display a specific phenotype regarding their protein expression. These results suggest that different molecular pathways are involved in the carcinogenetic process of cholangiocarcinoma and that new therapeutic targets may be efficient.

OP7-5
TRANSCRIPTION FACTORS FBP-1 AND FBP-3 AND THEIR REPRESSOR FIR ARE PRO-TUMORIGENIC FACTORS IN HEPATO-CARCINOGENESIS

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Background: The single-stranded DNA binding transcription factors FBP-1 (far upstream element (FUSE)-binding protein) and FBP-3 as well as their transcriptional repressor FIR (FBP interacting repressor) bind to DNA by recognizing dynamic changes of DNA-conformation and influence the general transcription factor TFIIH. This system has been shown to suppress the intrinsic noise of transcription and to ensure a steady transcriptional output of genes like c-myc, which is necessary for cellular homeostasis. These data suggest a potential pro-tumorigenic function for these factors. So far, little is known about the functional relevance in carcinogenesis, especially in the case of hepatocellular carcinomas (HCC). Methods: Measuring transcription by real-time PCR, FBP-1 and FBP-3 as well as FIR are found to be over-expressed in human HCCs (70% for FBP-1 and FBP-3 [19/27] and 33% for FIR [9/27]), and their expression levels significantly correlate among each other. Determing protein levels by immunohistochemistry using tissue-micro arrays (215 tumors), a highly significant correlation is found between the nuclear expression of FBP-1, FBP-3 and the cytoplasmic enrichment of FIR with tumor progression (Spearman correlation: $r=0.355$ (FBP-1), $r=0.454$ (FBP-3), and $r=0.335$ (FIR); $p<10\text{-}6$). Furthermore, the expression of both FBPs and FIR significantly correlates among each other but not with the nuclear expression of c-myc in HCCs. However, this over-expression does not correlate with elevated c-myc transcript levels. Using RNAinterference, the transient inhibition of FBP-1, FBP-3 and FIR in different HCC cell lines does not influence the c-myc expression but significantly reduces tumor cell viability due to diminished proliferation and G0/G1-cell cycle arrest (for FIR) or moderate G2/M-cell cycle arrest (for FBP-1/-3). Interestingly, western-blot analyses shows that inhibition of FIR reduces the expression of FBP-1 but not the expression of the FBP-3 in different HCC cell lines. Results and Conclusions: The transcription factors FBP-1, FBP-3 and FIR are over-expressed

and co-regulated in the course of human hepatocarcinogensis. In this context, FIR seems to be an inducer of FBP-1, but not FBP-3 expression. Since in the case of HCC, FBP and FIR obviously do not influence the expression of c-myc, they probably exert their oncogenic properties through the activation of other tumorigenic factors, which we currently aim to identify.

OP7-6

SIGNIFICANCE OF THE E-CADHERIN REPRESSOR SNAIL IN PANCREATIC, AMPULLARY, AND SMALL BOWEL CARCINOMAS

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Pancreatic, ampullary and duodenal carcinomas are tumors originating in related anatomical structures that have the same embryogenetic origin. Alterations of the cell adhesion molecule E-cadherin have been commonly found and directly related to dedifferentiation, aggressive behavior, and lymph node or liver metastasis of pancreatic carcinomas. Recently, it was reported that the transcription factor Snail, a master regulator of epithelial-mesenchymal-transition (EMT) and E-cadherin inhibitor, plays a role in tumor-stroma interactions. The aim of our study was to examine Snail and E-cadherin immunoreactivities in pancreatic, ampullary and duodenal carcinomas in order to better understand their biological behaviour. A series of 104 cases, including 42 pancreatic, 45 ampulla, and 17 duodenal adenocarcinomas were analysed from the archive of the Institute of Pathology at the Technical University of Munich. One representative block from each case was selected and the intensity and localization (epithelial, stromal, mixed) of the Snail expression and the intensity and localization (membranous/cytoplasmic/mixed) of E-cadherin expression were evaluated. Chi square test was used to assess a possible correlation between expression levels for Snail and E-cadherin and the tumor localization. We found a statistically significant difference between tumor localization and desmoplasia: 42 (100%) of the pancreas cases have desmoplasia compared with 15 (88%) of the duodenum tumors ($p<0.0001$). Snail expression was observed in 38 (90%), 33 (73%), and 10 (58.8%) of the pancreas, ampulla, and duodenum cases, respectively ($p<0.012$). There was a significant relation between desmoplasia and Snail intensity ($p<0.003$). The most common localization of stromal Snail expression was in pancreas (n=31 or 73%), followed by 6 (55.3%) duodenal cases and 20 (47.6%) ampullary cases. There was a significant inverse correlation between E-cadherin and Snail immunoreactivities ($p<0.005$). In summary, we found a correlation between tumor localization, desmoplasia, intensity and localization of Snail immunoreactivity in the 3 types of cancer, most likely related to the stromal Snail expression in pancreatic adenocarcinoma. Our study demonstrates Snail-associated communication between tumor and stroma and suggests involvement of EMT in these tumors.

OP8-1

HUMAN SPERMATID CENTRIOLE ABNORMALITIES ARE CAUSATIVE FOR INTRACYTOPLASMIC SPERM INJECTION (ICSI) FERTILIZATION FAILURE.

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Background: A low birth rate for a number of European countries is predicted (Germany = 1.4 children per woman), the difference between the birth and death indices will rise over the next several decades, which would have an adverse social and economical impact. The infertility prevalence rate for all couples of reproductive age is estimated to be 15%, in 1/3 of them male infertility is causative and can be overcome by innovative therapeutic options. Fertilization failure in human after assisted reproduction technique with ICSI (Intracytoplasmic Sperm Injection) may be due to different dysfunctions affecting the spermatozoon. Because the mature oocyte is devoid of centrioles, an impaired capacity to promote the growth of microtubule (MT) arrays from the sperm centrosome may preclude pronuclear opposition required for syngamy resulting in developmental arrest of the embryo. Method: Native ejaculate with motile sperm evidence (50%) from an 40-years old healthy man with a history of 2 unsuccessful homologous ICSI cycles (woman= intact mature oocyte function evidenced by polar body ”spindle-view”; one embryo transfer with one irregular embryo) resulting in pronuclear stage arrest of the zygotes, was Karnovsky-fixed, processed for electron microscopy examination, and evaluated for potential ultrastructural abnormalities. Results: A significant number (80% of examined) of abnormal spermatozoa with an aberrant acrosomal complex (agenesia, dysgenesia, crater-deformation, vacuoles/membranous inclusions), head deformations (round-shaped, tapered-shaped, binuclear, uncompacted chromatin), and multiple centrosome lesions was observed. In eight spermatozoa out of 11 with optimally visible centrosomes (73%), the transversal centriole sections revealed a distinct loss of the MT-triplets or their reduction to a MT-doublet, and a coupled additional smaller centriole structure was evidenced. In the longitudinal centrosome sections overlong MT protruding laterally the spermatid neck were displayed. Conclusion: The spectrum and relatively high incidence of the observed centriolar aberrations suggest the causative sperm defect responsible for the early embryo development failure. At present no help could be offered to couples affected by totally impaired centrosome function, first heterologous trials to restore human sperm centrosomal function using cytoskeletal stabilizers were reported. In our experience, electron microscopic sperm examination could reveal crucial findings for decision making in male infertility therapy.

OP8-3

DIFFERENTIAL DIAGNOSIS OF CLINICALLY NONFUNCTIONING PITUITARY ADENOMAS WITH IMMUNOHISTOCHEMICAL AND ELECTRON-MICROSCOPY METHODS

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In the current WHO classification of pituitary adenomas immunohistochemical evaluation represents the “gold diagnostic standard”. However, immunohistochemical profiles may overlap, but ultrastructural features of these tumors are essential to reach a correct diagnosis of adenomas with specific biologic behavior, invasiveness, aggressive growth potential and recurrences. The aim of this study was evaluation of usefulness of immunohistochemical and ultrastructural methods in prognostic evaluations of pituitary adenomas. Material and methods: Among 1032 pituitary tumors diagnosed between 1998 and 2007 at the Department of Pathology of Cancer Center in Warsaw there were 889 pituitary adenomas. About of 35% of all cases were clinically nonfunctioning pituitary adenomas. All cases were studied by review of clinical features, light and electron microscopy and immunohistochemistry. Selected cases were examined using a postembedding immunogold technique. The proliferative index was determined by MIB-1 immunohistochemistry. Results: Among 285 clinically nonfunctioning pituitary adenomas 18 cases of silent corticotroph adenomas (I, II and Crooke’s type), 6 of silent thyreotroph, 17 of silent lactotroph and 8 cases of silent somatotroph adenomas were revealed by electron microscopy.

All tumors were diagnosed as aggressive macroadenomas with invasion of the surrounding structures. The proliferative index had low values (0–3%). Conclusions: Immunohistochemical evaluation permits the diagnosis of silent adenomas associated with a poor prognosis. In the cases of overlapping immunohistochemical profiles ultrastructural analysis is valuable to provide useful information. Currently, there are no accepted means of predicting an adenoma's invasiveness and long-term aggressiveness, but morphologic separation of some subtypes of pituitary adenomas by electron microscopy provided fundamental knowledge to classify and management. Proliferative markers, as MIB-1 may be helpful in diagnosis.

OP8-4

WHEN DOES THE CORNEA DIE?

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Background: The cornea is a unique tissue, being non-vascularized and dependent on diffusion of oxygen from the anterior chamber and the air. Even though the cornea is the body's most transplanted tissue, little is known about cell changes and cell survival post-mortem. Method: 28 corneas (14 pairs) were obtained during autopsies at Department of Pathology, Ullevaal University Hospital, Oslo, Norway. A corneoscleral disc with a diameter of 14 mm was removed with a trephine and curved scissors and placed in Hank's balanced salts solution for transport to the laboratory. The transport time did not exceed 20 minutes. The post-mortem time (interval between recorded death and fixation or cultivation) ranged from 14 to 163 hours and donor age from 24 to 91 years. The cause of death ranged from acute deaths to chronic disease. All donors died in the hospital and were on the same day as death brought to a cool storage room with a temperature of 4ºC. One cornea of a pair was examined for post-mortem changes and the second was placed in culture medium containing 8% serum for 3 or 6 days as a viability test. Examination was performed with scanning (SEM) and transmission electron microscopy (TEM), light microscopy, and immunohistochemistry with antibodies against n- and e-cadherin, PCNA, cytokeratin 3 and 12, vimentin and P63. Results: In the post-mortem epithelium, first the superficial cells detached in a sheet-like fashion, after 4 to 7 days the basal cells detached and the denuded basement membrane became visible in large areas. The endothelial cells lost the posterior membrane. When examined by TEM, cytoplasmic vacuolization was observed. Stromal keratocytes showed shrinkage. In specimens with very high post-mortem time (5 to 7 days) few epithelial cells remained in the peripheral cornea, and few endothelial cells remained structurally intact. Cultivation demonstrated rapid regeneration of the epithelium and endothelium. The endothelial cells became electron-dense with abundant organelles and the keratocytes increased in size and became electron dense when examined with TEM. Conclusion: With increasing post-mortem time, gradually more pronounced cellular changes and cell loss occurred in the human cornea. All three cell types (epithelial, endothelial and keratocytes) survive up to seven days post-mortem. Regeneration during cultivation restores the cell layers and the corneal architecture. Important factors for cell survival post-mortem are supposedly hypothermal donor storage and diffusion of oxygen to the externally situated and non-vascularized cornea

OP8-5

DETECTION OF SILICA PARTICLES IN LUNG TISSUE BY ENVIROMENTAL SCANNING ELECTRON MICROSCOPY

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Background: In lung pathology the role of silica is still debated at various levels of experimental, epidemiological, clinical and forensic evidence. To physicians and other health professionals it could be of relevance to know silica levels in lung tissue in order to better define cut-off limits of exposure. In this investigation, our objective was to apply Environmental Scanning Electron Microscopy (ESEM) in lung tissue microanalysis and to compare silica levels between subject with and without lung cancer. Methods: ESEM technology was employed to measure silica particles in tissues obtained from twenty-five patients with non-small cell lung cancer (Group A) and twenty patients without lung cancer deceased for extra-pulmonary diseases (Group B). Silica levels were quantified considering the number of spots (NS), (silica particles) and the number of positive zones (NPZ) in which there was at least one spot. Levels of NS and NPZ were assessed with Poisson-type regression models, and two patients with suspected workplace exposures were used to evaluate the performance of fitted models (Group C). Results. In Group A, 12 subjects were silica positive with a medium NS of 4.6 and a medium NPZ of 2.8. Three subjects were silica positive in Group B, with a medium NS of 1.3 and a medium NPZ of 0.8. In Group C, a medium NS of 210 and a medium NPZ of 27.5 were detected. Patients with lung cancer displayed significantly higher levels of silica relative to non-cancer controls, and smoking, age and gender had no significant effect on this relationship. While only evaluated in two patients, measured values of NS and NPZ for the workers in Group C were in agreement with model estimates. Conclusions: Higher silica levels were observed in lung cancer tissue in comparison non-cancer controls, and, if demonstrated effective in a larger number of patients, the fitted model between NS and NPZ might be useful in evaluating new observations and in the development of threshold limit values for silica in biological tissues. In this investigation, ESEM was a rapid, simple and valid tool for the determination of silica levels in lung tissues.

OP8-6

ULTRASTRUCTURAL AND STEREOMETRICAL PARAMETERS OF THE PLACENTAL BARRIER UNDER HYPOXIA CONDITIONS

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The electron microscopic investigation of placenta under hypoxia condition caused by maternal iron-deficiency anaemia and EPH-gestosis was carried. Electronograms from 30 placentas, including 10 cases of EPH-gestosis, 10-maternal anaemia and 10-uncomplicated pregnancy (controls) were studied stereometrically by point count method at magnification X 5600. Pathological ultrastructure's alterations of a placental barrier, including a damage of microvilli, thickening of basal membranes both of epithelium and endothelium, a stromal fibrosis were found. Compensatory proliferations of cytotrophoblast in cases of EPH-gestosis and in anaemia group; as well as hyperaemia of foetal vessels in EPH-gestosis group were seen too. The increase

of the syncytiotrophoblast volume fraction (VF) in anaemia group (35,71±0,66 vs. 32,19± 0,26 % in controls), as well as cytotrophoblast, epithelium and endothelium basal membranes VF's both in anaemia (A) and gestosis(G) groups had registered (correspondingly: A – 2,76±0,04; 3,52±0,02; 0,57±0,06; G – 1,05±0,03; 4,76±0,03; 0,74±0,04 vs. 0,76±0,04; 3,04±0,01; 0,38±0,12% in controls, P<0,05). The capillary lumen increased to 14,70±0,26% in gestosis group (13,5±0,21% VF in controls, P<0,05). Pathological changes of a placental barrier could be a structural basis of a placental insufficiency. It was suggested that the process of ultrastructural remodelling of the placental membrane may compensate for different pathologies at the terminal parts of the villous chorion, which is essential base of the morphological correction of a placental insufficiency.

OP9-2

DIFFERENT PROGNOSTIC ROLE OF MUTATIONS IN THE HELICAL AND KINASE DOMAINS OF THE PIK3CA GENE IN BREAST CARCINOMAS.

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In breast cancer, the PIK3CA gene is frequently mutated at "hot spots" in exons 9 and 20, corresponding to the helical and kinase domain respectively. We investigate the association of PIK3CA mutations with pathological features and clinical outcome in a large series of breast cancer patients. Experimental Design: frozen samples from 163 consecutive patients were analyzed for PIK3CA mutations using PCR-SSCP and sequence analysis. Results: we identified 46 missense mutations, 24 (53%) in exon 9 and 21 (47%) in exon 20. Twelve (50%) of the 24 mutations in exon 9 were of the E542K type and 11 (46%) were of the E545K type. Twenty (95%) of the 21 mutations in exon 20 were H1047R substitutions. Mutations in exon 9 were more frequent in lobular carcinomas (42% of cases) than in ductal carcinoma (11% of cases) (p=0.002). At univariate survival analysis PIK3CA exon 20 mutations were associated with prolonged overall (OS) and disease free survival (DFS) while mutations in exon 9 were associated with significantly worse prognosis. At multivariate analysis exon 9 PIK3CA mutations were the strongest independent factor to predict poor prognosis for DFS (P = 0.0003) and OS (P = 0.001). Conclusion: Our data show that exon 9 PIK3CA mutations are typical of infiltrating lobular carcinomas. In addition, they indicate that PIK3CA mutations in different exons are of different prognostic value: exon 9 mutations are independently associated with early recurrence and death, while exon 20 PIK3CA mutations are associated with optimal prognosis.

OP9-3

INTRATUMORAL T LYMPHOCYTES ARE A PREDICTIVE MARKER OF RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN BREAST CARCINOMA

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Background: The antitumor immune response plays a crucial role in development and spread of many malignancies. Cellular immunity represents an important factor in the antitumoral defense, however, certain immune reactions can have an adverse effect and support the growth of tumor, e.g. by induction of angiogenesis. Tumor infiltrating T lymphocytes (TIL) (CD8+ cytotoxic and TH1 subset of CD4+ T lymphocytes, NK cells) seem to play an essential role in antitumor response. Aims Investigation of the relationship between CD3+ TIL and the effect of neoadjuvant chemotherapy in breast carcinoma. Method: We have examined 73 cases of breast carcinoma treated by neoadjuvant chemotherapy with paclitaxel and doxorubicin during 1998-2003. In each patient, the core needle biopsy taken before the treatment and the resection specimen were available. In addition to routine histology, the tissue was examined immunohistochemically (CD3, ER, PR, Ki67, p53, HER2/neu). CD3+ intratumoral lymphocytes were quantified by counting in three high-power fields (400x) and recorded as average and maximal values. The values of TIL were correlated with results of examinations of hormonal receptors (ER, PR), proliferative activity (Ki67) and selected oncoproteins (p53, HER2/neu). The results in core biopsy and resection specimen were compared by Mann-Whitney and Wilcoxon paired tests. Results The age of patients was 22-75 years (median 56 years). There were 53 ductal, 13 lobular and 7 other type carcinomas; 3 cases were grade 1, 36 grade 2 and 34 grade 3, respectively. A complete pathologic response (pCR), i.e. absence of tumor in resection specimen - was observed in 10 patients (16%). There were statistically significant differences between maximal (14 ± 14 vs. 5 ± 7; p=0.004) and average (7 ± 8 vs. 2 ± 3; p=0.05) values of TIL in the groups with and without pCR. Maximal values of tumor infiltrating lymphocytes (7 ± 8 vs. 10 ± 16; p=0.05) and associated lymphocytes (220 ± 176 vs. 359 ± 370; p=0.004) significantly increased during chemotherapy. Conclusion: Our results indicate that besides the well known prognostic role of intratumoral lymphocytes, values of CD3+ TIL in core needle biopsy specimens can also serve as a predictive marker of response to neoadjuvant chemotherapy. The increase of maximal values of TIL after chemotherapy indicates that they play an important role in destruction and elimination of tumor cells during this treatment. Supported by a grant of Internal Grant Agency of the Ministry of Health of the Czech Republic NR 8392-3.

OP9-4

HER2 OVER-EXPRESSION QUANTITATIVELY ACT ON LEVEL OF PROLIFERATION IN BREAST CANCER

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Introduction: Over-expression of HER should lead to high level of proliferation in breast cancer via homo- and hetero dimmerization. However, we have some investigations which contradict to these terms and authors claim that over-expression of HER2 may lead to low level of proliferation. The aim: to analyze influence of HER2 over-expression on proliferation of breast cancer's cells and pathways via which this influence is exist. Material and methods: We studied 414 breast cancer cases in which level of proliferation (Ki-67), expressions of HER2, progesterone and estrogen receptors were analyzed via immunohistochemistry and 23 samples of breast cancer with known positive immunohistochemical reaction of HER2 ("++", "+++") where analysis of HER2 gene amplification (FISH or CISH), morphometric comparative immunohistochemical analysis of Ki-67, P21/waf1, topoisomerase-II-a, E-cadherin, CD44v6 were performed. Exact calculation of percent of cancer cells expressed markers (among 2000 cancer cells by station LeicaCTR5000 and program LeicaQwinPlus) in each tumor with further their comparison allows us to make conclusions about activity of p21/waf1, level of expression of topoisomerase-II-a, allocation of cancer cells in the cell cycle. Results: Difference between proliferations of HER2 positive and negative groups was

statistically inauthentic (p>0,05). Thus HER2 positive cases don't have higher proliferated level than HER2 negative ones. Analysis of HER2 gene amplification has shown that over-expression of HER2 generally caused by gene amplification (87% of cases), but in 13% investigated samples the cause of over-expression was polysomia of 17 chromosome. Analysis of p21/waf1 protein showed that over-expression of HER2 is accompanied by active condition of p21/waf1 (17% of cases), inactive (13% of samples), mixed (43% of cases in which some cancers cells with active p21, others – with inactive), loss of p21/waf1 expression (27% of samples). Analysis of topoisomerase-II-a showed that normal and over-expression of this marker observe in 39% of cases. Hypo-expression was found in 61% of samples. Analysis of adhesion molecules (E-cadherin, CD44v6) also showed much differed conditions from low level of expression to high one with invert correlation of model expression of these two molecules. Conclusion: Over-expression of HER2 accompanies opposite conditions of pathways which quantitatively act on level of proliferation.

OP9-5

MAY THE QUANTIFICATION OF ESTROGEN RECEPTOR MRNA SERVE AS THE BASIS FOR MOLECULAR PROFILING OF BREAST CARCINOMAS?

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Background: A cDNA microarray technology has allowed to make molecular profiling of breast carcinomas by monitoring expression levels of many genes simultaneously. Clustering of microarray data indicates that expression of estrogen receptor (ER) plays a fundamental role in such molecular division. Cancers with high expression of ER are numbered among luminal phenotypes characterized by better clinical outcome. Tumors with low expression of ER form basal-like and HER2 subtypes associated with the shortest survival times. The aim of the present study was to compare ER-mRNA level and ER status assessed by immunohistochemistry. We also tried to validate which method of assessing ER status may be more reliable for molecular subtyping in relation with basal keratins (5 and 17) and HER2 genes expression. Patients and Methods: This retrospective study was conducted on 97 patients with operable invasive ductal breast carcinomas not otherwise specified. A median median follow-up period was 60 months. mRNA levels for ER, HER2, keratin 5 (KRT5), and keratin 17 (KRT17) genes were measured by real-time RT-PCR in fresh tumor specimens frozen immediately after excision at -800C. ER status was also assessed by routine immunohistochemistry. Results: In 45 cases (46.4%) identified immunohistochemically as being ER-negative, mean ER-mRNA level was 8.51±15.71, whereas in 52 (53.6%) cases classified by immunostaining as being ER-positive it was 17.35±31.66 (p=0.013). However, a significant discordance between real-time RT-PCR and immunohistochemistry in assessing ER status has been found. In a group of 27 tumors with low level of ER-mRNA (<1.00), there were 8 ER-positive cases as assessed by immunostaining, and among 70 cases with high level of ER-mRNA (≥1.00), 26 were ER-negative by immunohistochemistry (p=0.003). Regardless of the method of dichotomization of real-time RT-PCR data, lack of prognostic relevance of ER-mRNA level has been demonstrated, whereas ER-positive patients as assessed by immunostaining had a relative risk of cancer-related death lower by 65% when comparing with ER-negative ones (p=0.020). ER-mRNA level did not correlate significantly with HER2-mRNA (τ=0.14, p=0.061), KTR5-mRNA (τ=0.01, p=0.873) or KRT17-mRNA (τ=-0.13, p=0.071). Conclusions: Our observations may question the rationale for the assessment of ER-mRNA in a molecular division of breast tumors and indicate that results of cDNA microarray experiments should be interpreted cautiously.

OP9-6

PSEUDOANGIOMATOUS STROMAL HYPERPLASIA (PASH) TUMORS ON CORE NEEDLE BIOPSY (CNB): IS EXCISION NECESSARY?

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Background: Tumor formed by PASH (PASH-TU) is rare, with only a few reported case series in literature. Clinical management of PASH-TU diagnosed by CNB is still controversial. The aims of our study were: 1) to evaluate clinical, radiologic, and histologic features of PASH-TU; 2) to correlate CNB findings of PASH-TU with subsequent excision and/or clinical follow-up. Methods: PASH-TU cases were retrieved from the Department of Pathology database at UT M. D. Anderson Cancer Center and retrospectively studied. PASH was histologically classified as simple/classic or fascicular/proliferative. Associated pathologic findings, including epithelial and stromal atypia, location of the PASH [intralobular (IL), perilobular (PL)], and the presence of hamartomatous changes were correlated with patients´ clinical data, hormonal use, imaging study results, and follow-up data. Statistical analysis was performed using Fisher's exact test. Results: Twenty-six cases were found. Patient ages ranged from 19 to 70 years (mean, 47±13 years), 62% of patients were premenopausal, and 13 had a long-standing history of oral contraceptive use or hormone replacement therapy (6). Nine patients (35%) presented with a palpable mass; in the others, tumor was detected by mammography mostly as a hyperdense mass with irregular margins and/or by sonography as a hypoechoic mass. Lesion size ranged from 0.8 cm to 11 cm (mean, 4.2±4.3 cm). PASH was simple/classic in 18 patients (69%) and fascicular/proliferative in 8. PL involvement was noted in 5 patients, and PL and IL involvements, in 21. The associated pathologic findings were invasive ductal carcinoma in 1 patient (4%), gynecomastia-like change (GLC) in 17 (65%), and other benign findings in 8 patients (31%). In 5 patients, GLC was originally read as columnar cell change. The presence of GLC was significantly associated with IL PASH (p=0.00085). Hamartomatous change was noted in 54% of patients. Among 15 patients with clinical follow-up ≤27±17 months after surgical excision (7) or radiologic surveillance (8), none demonstrated additional pathology or significant change in lesion characteristics on imaging. Conclusion: PASH-TUs occur more frequently in premenopausal women and are associated with hormonal therapy use in the majority of patients. The GLC frequently occurs in PASH-TUs and its presence is strongly associated with IL involvement by PASH. CNB-diagnosed PASH-TUs not subsequently excised remained clinically and radiologically stable, thus offering the option of close clinical surveillance instead of surgery in those patients.

OP9-7

NEAR TILING MICROARRAY-BASED CGH IDENTIFIES CCNE1 AMPLIFICATION IN BASAL-LIKE BREAST CANCER

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Background: Basal-like breast cancers (BLBC) constitute a heterogeneous group of aggressive neoplasms, accounting for around 15% of invasive breast carcinomas. BLBCs are characterised at the molecular level by lack of oestrogen receptor

(ER) and HER2 expression, and expression of genes consistently expressed in normal basal/ myoepithelial cells. Current tailored therapies are not amenable for the management of these tumours, given that they lack hormone receptors and HER2 expression. The aims of this study were to characterise the molecular genetic profiles of BLBCs using a high resolution microarray-based CGH (aCGH) platform and to novel amplifications in BLBCs. Methods: A series of 20 fresh frozen grade 3 BLBCs were subjected to aCGH analysis using whole genome 16K near tiling bacterial artificial chromosome (BAC) arrays. Specific amplifications were confirmed by means of chromogenic in situ hybridisation (CISH) analysis using in-house generated probes and immunohistochemistry was used to investigate the expression of oncogene candidates. Furthermore, we investigated the prevalence of CCNE1 amplifications with CISH on a tissue microarray containing 97 grade III tumours. Results: 20%, 50% and 30% of cases exhibited the recently described "simplex", 'sawtooth' and "firestorm" patterns of genomic alterations, respectively. The most common regions of gain (>25% of cases), included 1q, 6p, 8q, 10p and 17q, and the most common regions of loss (>25% of cases), encompassed 3p, 6q, 8p, 13q and 17p. Common amplifications observed in greater than 10% of cases included 1p12-q25.3, 7q11.21, 8q24.21, 11q13.3, 12p13.33-p13.31, and a novel amplification on 19q12. Further delineation of this novel amplicon identified CCNE1 (cyclin E1) as a possible amplicon driver. CCNE1 amplification was confirmed by means of CISH in all samples identified as CCNE1 amplified by aCGH. In the TMA, CCNE1 gene amplification was found in 7% of cases and was significantly associated with basal-like phenotype (p=0.0082, Fisher's exact test), with CCNE1 protein expression as determined by immunohistochemistry (p=0.008, Fisher's exact test) and lack of CCND1 amplification (p<0.001, Fishers exact test) as defined by CISH. Conclusion: These results demonstrate that CCNE1 amplification may be one of the mechanisms driving cyclin E1 protein expression in basal-like breast cancers. These results also suggest that CCNE1 and CCND1 amplification act in a mutually exclusive manner.

OP9-8

BASAL-LIKE PHENOTYPE IS FREQUENT IN INVASIVE BREAST CARCINOMA FROM UGANDAN AFRICANS AND IS STRONGLY ASSOCIATED WITH A HIGH EXPRESSION OF EGFR/C-KIT AND FEATURES OF AGGRESSIVE TUMORS

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Breast cancer is the most common neoplasm-affecting women and a leading cause of death from cancer in women worldwide. Therapeutic targets are needed to improve clinical outcomes, which are still poor in Africa, although incidence is increasing rapidly. Basal-like breast carcinoma has been recognized as a subtype with poor prognostic features and specific therapeutic implications. It frequently expresses epidermal growth factor receptor (EGFR) and c-Kit. The prognostic significance of c-Kit in breast cancer is still controversial. EGFR has emerged as an important prognostic parameter and a promising strategy in tumor specific therapies. There is a paucity of reports about basal-like breast carcinoma and expression of EGFR/c-Kit in Africa. The aim of this study was to explore invasive breast carcinoma from Ugandan African women in association with basal-like phenotype, EGFR/c-Kit expression and other prognostic factor. We studied 65 archival paraffin blocks of invasive breast carcinoma from female patients in the archives of the Pathology Department, Makerere University. Five μm-thick sections were stained with antibodies against Cytokeratin 5/6, P-cadherin EGFR, c-Kit, oestrogen receptor (ER), progesterone receptor (PR), HER-2 and Ki-67. Sections were scored according to recommended criteria. Tumors that expressed Cytokeratin 5/6 and/or P-cadherin were considered have a basal-like phenotype (BLP). Basal-like phenotype was expressed in 34% of cases and in 52% of ER negative tumors. Forty-five percent of basal-like tumors expressed EFGR, while 23% expressed c-Kit. Fifty-five percent of basal-like tumors expressed EGFR and/or c-Kit. BLP was expressed in 71% of the EGFR and/or c-Kit positive tumors (P = 0.000). The BLP positive tumors were more likely to express EGFR and/or c-Kit than non basal-like type (OR 9.1; 95% CI 2.6-32; P = 0.000). BLP was strongly associated with EFGR expression (P = 0.002), c-Kit positivity (P = 0.003), high histological grade (P = 0.001), high mitotic counts (P = 0.002), and marked nuclear pleomorphism (P = 0.002). All basal-like tumors were ER negative (P < 0.0001) and PR negative (P = 0.002). No association with HER2 expression (P = 0.410) and Ki-67 proliferative rate (P = 0.114) was found. Basal-like phenotype is frequent in invasive breast carcinoma from Uganda and is strongly associated with high expression of EGFR/c-Kit and features of aggressive tumors. Ugandan patients with BLP could benefit from EGFR-targeted therapies. More studies are required to validate this.

OP9-9

VASCULAR PROLIFERATION IS A STRONG AND INDEPENDENT PROGNOSTIC FACTOR IN BREAST CANCER

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Background: In 1971, Folkman et al. proposed that the growth of malignant tumors is dependent on angiogenesis, and can be inhibited by targeting its blood supply. Various measures of angiogenesis have been reported as prognostic factors, although no available marker can predict treatment response. Microvessel density (MVD) is a widely used indicator of angiogenesis, but clinical use is not currently recommended. Improved measures of angiogenesis are required, and we recently found that vascular proliferation might be superior to MVD. We have now studied vascular proliferation with regards to prognosis in a well characterized series of breast carcinomas. Methods: The study was restricted to 292 cases of first primary invasive breast cancer diagnosed in Ashkenazi Jewish women between 1980-95. Ki-67 and Factor VIII double immunostaining was performed on 239 cases with sufficient material, including 24 BRCA1 and 6 BRCA2 carriers. Microvessels in 10 consecutive 250x fields within vascular hot spots were registered, simultaneously counting microvessels with Ki-67 positive endothelial cells. Results: Vascular Proliferation Index (VPI): Median 1.3%.There were significant associations with higher histologic grade (p=0.05), higher mitotic rate (p= 0.020), positive expression of CK 5/6 (p=0.017), P-cadherin (p<0.0005), EGFR (p=0.004), p53 (p=0.031), negative ER-expression (p= 0.001), and the presence of glomeruloid microvascular proliferation (GMP) (p<0.0005). Survival analysis: Univariate survival analysis showed no significant effect of MVD. VPI was highly significant, with 10 year survival 78.6% in negatives versus 57.4% in positives (Log Rank test: p<0.0005; HR 2.47). By multivariate analysis, tumor size (p=0.001, HR 2.839), GMP (p= 0.043, HR 1.918) and VPI (p=0.007; HR 2.247) were significant and independent prognostic factors. Conclusions: Vascular Proliferation Index (VPI) is a novel prognostic factor in breast carcinoma, and is superior to microvessel density (MVD). We suggest that VPI should be evaluated further in studies of prognosis and response to adjuvant treatment.

OP10-1

TUMORS OF UNCERTAIN MALIGNANT POTENTIAL OF THE THYROID : MYTH OR REALITY ? AN HISTO-MOLECULAR ANALYSIS OF 24 CONSECUTIVE CASES FROM A SINGLE INSTITUTION

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The WHO classification of thyroid tumors has recently described and introduced borderline lesions called « well –differentiated tumor of uncertain malignant potential » (WDT-UMP) and « follicular tumor of uncertain malignant potential » (FT-UMP). Although histologic definition of these lesions is readily available, application of the diagnosis criteria and personal experience may lead to disagreement among pathologists. FT-UMP designate tumors that show either questionable capsular invasion without vascular invasion or minor nuclear changes of papillary carcinoma. WDT-UMP designate tumors having questionable papillary thyroid carcinoma-type nuclear changes, and if capsular invasion is questionable or absent. Although as rarely as possible, the term « uncertain malignant potential tumor of the thyroid » (UMPTT) must be employed. However, the different categories of lesions described under this terminology should not be an easy option to avoid the intellectual commitment involved in making a clear diagnosis of malignancy or benignity. The aims of this study were i) to investigate interobserver variation in assessment of different UMPTT diagnosed from 2004 to 2007 in a single institution (CHU of Nice, France); ii) to see for UMPTT if ancillary molecular techniques assist one in defining more precisely benign from malignant tumors. For each case, the entire capsule was examined by 5 pathologists. Twenty four UMPTT (14 FT-UMP and 10 WDT-UMP) were diagnosed independently by each pathologist from a unique institution. All these diagnosis were reviewed by all the pathologists from the 2 different institutions. Surprisingly, in more than 95% of the cases, there was complete agreement for the diagnosis of FT-UMP and WDT-UMP. None of the 24 UMPTT were BRAF positive. Detection of PAX8-PPAR gamma rearrangements was performed in all cases and these rearrangements were found in most of the FT-UMP. RET/PTC (RET/PTC1 and RET/PTC2) alteration was found in some WDT-UMP. RAS alteration was detected in both some WDT-UMP and FT-UMP. In conclusion this work showed first that diagnosis of UMPTT (both WDT-UMP and FT-UMP) can be reproductive among pathologists, but the observer variation is considerably reduced when the entire lesion, and particularly the entire capsule, is examined. Secondarly, it seems that further study performed in a larger series must be done to see if gene analysis can contribute to the prognosis of these lesions, but to our opinion it is doubtful whether even this tool will provide reliable separation of the clinical behaviour of the UMPTT.

OP10-2

IMMUNOHISTOCHEMICAL STUDY OF PROTHYMOSIN-α , Ki67 AND p53 IN BENIGN AND MALIGNANT THYROID DISEASE

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BACKGROUND: The distinction of benign from malignant follicular thyroid lesions is difficult. There is controversial evidence for the significance of Ki67 and p53 and very few studies refer to the expression of prothymosin-a (Pro-Ta) with evidence of gradual increase from normal to hyperplastic, benign neoplastic and malignant thyroid epithelium. The fact that Pro-Ta, an immunoregulator, plays a role in cell proliferation, made us study its expression in normal and diseased thyroid, and its probable relationship to Ki67 and p53. METHODS: 105 sections were selected from 51 thyroidectomy specimens: normal follicles (23), hyperplasia nodular (NH,) 30, diffuse (DH) 2, adenomatous (AH) 4, adenoma, follicular (FA) 4, oncocytic (OA) 1, 3 cases of follicular tumour of uncertain malignant potential (FTUMP) and 31 carcinomas. Papillary (PTC) 27: classical 10, follicular 13, tall cell 4 , oncocytic carcinoma(OC) 1, anaplastic 1 and myeloid 2. Pro-Ta, Ki67 and p53 were immunohistochemically studied. The percentage of positive nuclei was measured using digital morphometry and the p53 intensity was arbitrarily assessed as +,++,+++.For statistical analysis of Pro-Ta the SPSS v13.0 was used.RESULTS: Pro-Ta nuclear positivity in 4 categories(1,2,3,4), 0-20,21-40,41-60 and >60% respectively. Normal follicles showed <20% positivity (19/23) and none exceeded 40%. Only the microfollicles of NH and DH were in categories 1 and 2 (17/25) but follicles of Hashimoto thyroiditis , AH and adenomas reached category 3. 1/3 FTUMP showed >60% positivity. 12/27 PTC and the 2 myeloid increased to category 3 while 12/27 reached category 4. The anaplastic Ca showed 100% positive and totally negative areas. The oncocytic Ca was <20%. Pearson's correlation index showed statistically significant increase of Pro-Ta in malignancy compared to normal and benign follicular cells (p0.086). Ki67 % categories 1,2,3,4. <0.5, 0.6-2.9, 3-6 and 6.1-30 accordingly. Normal thyroid follicles were almost negative (22/23). Hyperplastic, neoplastic benign and malignant epithelial cells in category 2, with exception of 8/27 PTC in category 3 and only the myeloid and anaplastic Ca showed >6% positivity. p53 ranged between 0-6% with negative normal follicles, weak focal positivity in all hyperplasias and FTUMP (categories 1-2) and only the microfollicles of Hashimoto in category 3.There was marked increase in intensity and grade for 15/27 PTCs (+++) and 22/27 (category 3).CONCLUSIONS: Increased Pro-Ta and intense albeit low p53 characterise thyroid malignancy. Pro-Ta overexpression needs further investigation.

OP10-3

CELL-MATRIX INTERACTIONS IN ADRENOCORTICAL CARCINOMA: PATHOGENETIC ISSUES AND DIAGNOSTIC IMPLICATIONS

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Background: The molecular mechanisms leading to adrenocortical carcinoma (ACC) development, as well as clear-cut diagnostic parameters in the differential diagnosis with adrenocortical adenoma (ACA), are currently poorly defined. Reports exist on the de-regulation of cellular to extracellular matrix interactions in ACC, with the identification of putative novel diagnostic markers. Methods: PCR-based array profiling of 86 molecules involved in cell to cell and cell to matrix interactions was performed in 6 frozen ACC and 4 control ACA samples. Statistical significance in transcript expression fold changes of target genes was evaluated by Student's T test. A large series of 55 ACC and 25 ACA paraffin embedded control cases was also available for histochemical and immunohistochemical analysis. Complete pathological and

clinical information, including follow up, were available for all cases. Results. By array analysis, most genes were generally down-regulated in ACC as compared to ACA samples. Four of them had a significantly ($p<0.05$) altered expression in ACC, including laminin alpha 3 and hyaluronan synthase 1 (both 4-fold up-regulation), osteopontin (OPN) (20-fold up-regulation), and laminin alpha 2 which was down-regulated. Based on such findings, OPN protein expression was evaluated in archival paraffin specimens and found to be absent in all 25 ACA and specifically expressed with a typical paranuclear dot-like pattern in 22/55ACC ($p<0.0001$). However, OPN protein expression did not correlate with any of the clinico-pathological parameters considered (presence of necrosis, mitotic index, presence of capsular/vascular invasion, overall Weiss score, clinical course). With regard to laminins, since the alpha 2 type is a major basal membrane component of human adrenal cortex (as opposed to the alpha 3 isoform), we speculated that its down-regulation could be associated to a disruption of the reticular network. By means of reticulin histochemical stain, all ACC cases had a disruption of the reticulin framework, as opposed to ACA that maintained a fine reticulin network, thus providing a simple and useful additional morphological parameter in the differential diagnosis between ACA and ACC. Conclusions: Some molecules involved in cell to matrix interactions have specifically altered expression in ACC: by selective cDNA array screening, we provide novel data on OPN expression as well as constitutive alterations of the reticular framework in ACC, which may be useful to better understand its pathogenesis and also support ACC morphological diagnosis.

OP10-4
DUODENAL AND PANCREATIC SOMATOSTATIN-PRODUCING NEUROENDOCRINE TUMORS: INCIDENCE, TYPES, BIOLOGICAL BEHAVIOR, ASSOCIATION WITH INHERITED SYNDROMES, AND FUNCTIONAL ACTIVITY

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Background: The somatostatin-producing neuroendocrine tumors (SOM-NETs) of the duodenum and pancreas are very rare and a heterogeneous group of neoplasms. Therefore, we determined their clinicopathological profiles. Methods: Clinical and pathological data of a series of 82 duodenal and 541 pancreatic NETs were screened. In addition, the clinical records of 821 patients with duodenal or pancreatic NETs were reviewed for evidence of a somatostatinoma syndrome. Results: Predominant or exclusive expression of somatostatin was found in 27% (n=22) of duodenal and 4% (n=22) of pancreatic NETs. They were classified as sporadic (n=31) or neurofibromatosis type 1 (NF1)-associated duodenal NETs (n=3), gangliocytic paragangliomas (GCPGs) (n=7), poorly differentiated neuroendocrine carcinomas (pdNECs; n=2), or mixed acinar endocrine carcinoma (MAEC; n=1). In addition, 5 duodenal and 4 pancreatic SOM-NETs were found in 5 patients with multiple endocrine neoplasia type 1 (MEN1). Metastases occured in 42% of the sporadic and NF1-associated SOM-NETs, but in none of the duodenal MEN1-associated SOM-NETs or GCPGs. Sporadic advanced (stage IV) SOM-NETs were more commonly detected in the pancreas than in the duodenum. None of the patients (including the 808 patients for whom only the clinical records were reviewed) fulfilled the criteria for a somatostatinoma syndrome. Conclusion: Somatostatin expression is not only seen in sporadic NETs, but may also occur in GCPGs, pdNECs and hereditary NETs. Surgical treatment is effective in most duodenal and many pancreatic SOM-NETs. Benign behavior is seen in MEN1-associated SOM-NETs and GCPGs, whereas highly malignant somatostatin-producing pdNECs/MAECs have a poor prognosis. The so-called somatostatinoma syndrome seems to be an extreme rarity.

OP10-5
HUMAN ASH1 TRANSCRIPTION FACTOR EXPRESSION IN PROSTATE CANCER WITH NEUROENDOCRINE DIFFERENTIATION

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Background: Prostate cancer (PC) is a leading malignancy in the aging male population, with a heterogeneous clinical course. The occurrence of neuroendocrine (NE) phenotype in PC correlates with overall prognosis and prostate cancer progression after androgen-deprivation therapy. The mechanisms leading to NE differentiation in PC are currently poorly understood. A role of Notch pathway has been reported in determining NE phenotype of normal and neoplastic tissues. Aim of this study is to analyse whether this pathway might affect NE differentiation in prostate cancer. Methods: Human achaete-scute homolog 1 (hASH1), a pivotal member of the Notch signalling pathway, was investigated in 80 PC selected and grouped according to chromogranin A (CgA) immunohistochemistry, as follows: PC without NE differentiation, untreated (group PCNE-, 25 cases); PC with NE differentiation, untreated (group PCNE+, 40 cases); PC with previous androgen deprivation therapy, all having NE differentiation (group PCNE+Ther, 15 cases). Three cases of prostatic pure large cell NE carcinomas were also analyzed. hASH1 protein was analyzed by immunohistochemistry, whereas the presence of hASH1 mRNA transcripts was investigated on representative microdissected tumor areas from serial paraffin sections by Real Time PCR. Results. By immunohistochemistry, hASH1 was co-localized with CgA in NE cells of the normal prostate. It was strongly expressed in pure NE prostate cancers, absent in all but one PCNE- cases, whereas it was positive in 25% of PCNE+ cases, with a strong correlation with the extent of NE differentiation ($p=0.02$). Moreover, comparing PCNE+ and PCNE+Ther cases, a strong positive association with androgen deprivation therapy was observed ($p=0.01$). In PC with NE differentiation, RNA analysis confirmed the association of higher transcript levels in androgen deprivation-treated (PCNE+Ther) versus untreated (PCNE+) patients ($p=0.01$). In addition, hASH1 mRNA analysis in microdissected CgA positive and negative areas within the same tumor demonstrated heterogeneous upregulation of hASH1 mRNA expression, with a 2 to 7 fold increase in the CgA positive tumor cell population. Conlusions: hASH1 mRNA and protein expression correlates with the presence and extent of NE phenotype in PC, especially in cases undergoing preoperative androgen deprivation therapy, and our data identify hASH1 as a likely critical factor in PC to drive NE differentiation, which represents an independent poor prognostic indicator and is a major responsible for hormonal refractory disease.

OP10-6
CYTOKERATINS 8 AND 18 IN SILENT PITUITARY ADENOMAS: FROM IMMUNOHISTOCHEMISTRY TO PROGNOSIS

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Background: Cytokeratin 8/18 expression is recognized in some subtypes of clinically functioning and nonfunctioning pituitary adenomas. In nonfunctioning lesions, when pituitary hormones and cytokeratins are identified with immunohistochemical

reaction, the profile of expression resembles that of respective functioning adenomas. Cytokeratin 8/18 immunoreactivity characterizes different pituitary adenomas with difficult clinical management: sparsely granulated somatotroph and acidophil stem cell adenomas, characterized by fibrous bodies, which respond poorly to therapy and show invasive growth, and silent corticotroph adenomas particularly prone to invasion and recurrences. In the present work, we studied 36 clinically nonfunctioning adenomas exclusively immunoreactive for gonadotroph and thyrotroph hormones (silent gonadotroph and thyrotroph adenomas), normally negative for cytokeratin 8/18 and representing the largest group of silent adenomas in several series. Immunohistochemical expression of CAM5.2 was investigated and related to biological behaviour. Methods: All 36 specimens were immunostained for the full panel of pituitary hormones and for MIB1/ki67 and CAM 5.2 antibodies. The patterns of CAM 5.2 immunoreactivity were classified in 3 subtypes according to cellular expression, intensity and distribution: cytoplasmic/intense/diffuse (CID), dot-like/intense/patchy(DIP) and dot-like/mild/patchy(DMP). Age, sex, endocrine function, tumour size and disease status were obtained from patient charts. The mean follow-up was 57 months (range 18-96). Results: 13 (36%) cases were variably immunoreactive for CAM5.2; 23 (64%) were completely negative. 7 (54%) CAM5.2+ patients recurred, while only 2 (9%) of CAM5.2- patients had recurrent disease. According to CAM5.2 expression patterns: 4 CID cases were identified; all were females with aggressive behaviour (recurrences, invasive growth and 1 exitus for disease). In DIP subgroup (7 patients, 5 males) we observed 4 recurrences, 1 death for other disease and 2 negative follow-ups. Finally, the 2 DSP adenomas did not recur. Conclusion: CID and, to a lesser extent, DIP pattern of immunoreactivity for CAM5.2 in nonfunctioning gonadotroph and thyrotroph adenomas may help to identify a recurrent/aggressive phenotype. The control group confirmed that CAM5.2- cases rarely show an aggressive behaviour. These results suggest that cytokeratin 8/18 expression is associated with aggressive behaviour of pituitary adenomas independently of tumor functional status. The biological bases of this phenomenon need to be further clarified.

OP10-7

DNA COPY NUMBER STATUS IS A POWERFUL PREDICTOR OF POOR SURVIVAL IN ENDOCRINE PANCREATIC TUMOR PATIENTS

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Background: The clinical behavior of endocrine pancreatic tumors (EPTs) is difficult to predict in the absence of metastases or invasion to adjacent organs. Several markers have been indicated as potential predictors of metastatic disease, such as tumor size ≥2 cm, Ki67 proliferative index ≥2%, cytokeratin (CK) 19 status, and recently in insulinomas, chromosomal instability (CIN). The goal of this study was to evaluate the value of these markers, and in particular of CIN, to predict tumor relapse and / or tumor-specific death, using a series of insulinomas and non-insulinoma EPTs. Method: From seventy-one EPT cases, including 47 insulinomas, a genomic profile could be generated and follow-up data could be obtained. The proliferative index could be determined in 68 tumors and a CK19 expression pattern in 50 tumors. Results were statistically analyzed using Kaplan-Meier plots and the log-rank statistic. Results General CIN, as well as specific chromosomal alterations such as 3p and 6q loss and 12q gain, turned out to be the most powerful indicators for poor tumor-free survival ($p \leq 0.0004$) and tumor-specific death ($p \leq 0.0113$) in insulinomas. CIN, chromosome 7q gain, and a proliferative index ≥2% were reliable in predicting a poor tumor-free survival in non-insulinoma EPTs ($p \leq 0.0181$), whereas CK19 expression was the most optimal predictor of tumor-specific death in these tumors. Conclusion: DNA copy number status is the most sensitive and efficient marker of adverse clinical outcome in insulinomas and of potential interest in non-insulinoma EPTs. As a consequence, this marker should be implemented as a prognosticator to improve clinical diagnosis. This study was supported by Netherlands Foundations Vanderes and Sacha Swarttouw-Hijmans, and the Association for International Cancer Research (AICR), UK.

OP10-8

THE COEXISTENCE OF PRIMARY HYPERPARATHYROIDISM AND PAPILLARY THYROID CARCINOMA

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Background: The coexistence of primary hyperparathyroidism and nonmedullary thyroid carcinoma has been reported by several authors. The incidence was reported between %2.6 and %17.4. Some authors believe that this is fortitious on the contrary some of them believe this is more than fortitious and accepting an underlying aetiology. Methods: The records of Cerrahpasa Medical School Department of General Surgery reviewed between January 2002 and December 2006 for the patients who underwent surgery for primary hyperparathyroidism. Results: There were 147 patients underwent to surgery due to primary hyperparathyroidism and 46 patients had coexistent thyroid pathology. 13 patients had total thyroidectomy , 11 patients had near totoal thyroidectomy and 15 patients had subtotal thyroidectomy. We found 8 patients of parathyroid adenoma coexistent with papillary thyroid carcinoma . The mean age of these patients is 58.2(29-64) and male:female ratio is 1:7. Conclusion: The prevalence of minimal thyroid carcinoma in Turkish Community was reported 1.3%. The incidence of parathyroid adenoma and papillary thyroid carcinoma in our patients is 5.4 %.We believe that this is more than coincidental. Three factors considered to be responsible in the aetiology. These are external irradiation to the head and neck, goitrogenic effect of Ca and genetics.Our patients had no history of external head and neck irradiation . We are performing further studies to find an underlying aetiology. While performing a parathyroid surgery especially mininal access parathyroid surgery, surgeons should be careful in detecting the patients preoperatively. We recommend US-FNAB if any thyroid nodule is found with parathyroid adenoma before parathyroid surgery.

OP10-9
PAPILLARY THYROID CARCINOMA GENE EXPRESSION PROFILE: SEARCHING FOR GENES RESPONSIBLE FOR ITS SPECIFIC HISTOMORPHOLOGICAL FEATURES.

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BACKGROUND: Papillary thyroid carcinoma (PTC) is the most common type of endocrine malignancy. PTC is diagnosed by defined morphological features, which include optically clear nuclei, irregular nuclear profile, pseudoinclusions and grooves. These features probably are driven by specific molecular mechanisms in PTC cells. In this study we analyze the relationship between gene expression profile and the microscopic futures of PTC. MATERIAL AND METHODS: Thyroid post-operative samples were studied. 41 cases with histologically verified diagnosis were analyzed. Light microscopy with morphometry was performed with the help of a semiautomatic computer image analyzer. One hundred thyroid nuclei were assessed in each sample. RESULTS Karyometric, densytometric and textural parameters were assessed for PTC and normal thyroid (NT) tissue. Obtained information was related to gene expression profiles obtained from DNA microarrays. Difference between normal thyroid tissue (NT) and PTC tissue was noted using complex of morphometrical parameters: circular rate (NT – 0.68; PTC – 0.57), diameter equivalent circle (NT – 51.0; PTC – 63.09), coefficient of variation of nuclear area (NT – 30.65; PTC – 19.36) and others. In the next step we assessed the relationship between morphometrical parameters and gene expression profile of papillary thyroid tumors, looking for genes with expression correlated with analyzed features. Genes, highly correlated with morphometrical parameters were discovered and discussed. CONCLUSIONS: Histomorphometry of PTC is reflected in its gene expression profile.

OP11-1
HER2/ TOP2A AMPLICON IN BREAST: A MICROARRAY-BASED AND CHROMOGENIC IN SITU HYBRIDISATION ANALYSIS

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Background: Although the HER2 amplicon has been subjected to extensive molecular genetic analysis, the characteristics of the 17q12 amplicon in cases harbouring HER2/ TOP2A amplification are not well known. The aims of this study were 1) to provide a detailed molecular genetic analysis of the 17q12 amplicon in breast cancers harbouring HER2/ TOP2A co-amplification and 2) to describe additional recurrent coamplifications in cases with HER2/ TOP2A amplification. Methods: A tissue microarray containing replicate cores of 245 breast carcinomas were subjected to chromogenic in situ hybridisation analysis (CISH) using Zymed SpotLight probes for HER2, TOP2A and chromosome 17 (CEP17) centromere. Amplification was defined according to the manufacturer's guidelines. All cases harbouring HER2/ TOP2A co-amplification and 5 cases with HER2 but not TOP2A amplification by CISH were microdissected and subjected to microarray-based CGH (aCGH) using the Breakthrough Breast Cancer 16K bacterial artificial chromosome (BAC) array platform. Results: HER2 and TOP2A amplification was found in 37 and 20 samples, respectively. All cases harbouring TOP2A amplification were also HER2 amplified. Out of 25 microdissected cases, 13 rendered optimal results with microarray-based CGH analysis – 9 HER2/TOP2A amplified and 4 HER2 amplified. Cases displaying HER2/ TOP2A high level amplification by CISH were confirmed by aCGH analysis. The smallest region of amplification on 17q12 in the whole series extended from 34719.9 to 35454.8Kbs, and encompassed HER2 but not TOP2A. In TOP2A/ HER2 co-amplified samples, the smallest region of overlap was extended from 34719 to 36537kb, comprising a region of 1817kb. This region comprised several other genes that are reported to be overexpressed when amplified, such as GRB7, THRA, CASC3, RARA, CDC6, SMARCE1 and KRT10. HER2 amplified cases displayed recurrent co-amplifications mapping to 8p12, 13q34, 17q23, 18q21.1 and 20q13. Conclusion: HER2/ TOP2A amplified breast cancers characteristically display the so-called 'firestorm' molecular genetic pattern, being characterised by multiple, focal, high level amplifications. The 17q12 amplicon is complex and harbours multiple genes that may be associated with breast cancer development and progression.

OP11-2
ANALYSIS OF BREAST NORMAL AND TUMOR TISSUES BY USING MICROIMAGING FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY

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BACKGROUND AND OBJECTIVE : Fourier Transform Infrared Spectroscopy (FT-IR) has received great attention from various fields because it can provide information about the molecular structure and the chemical composition of biological materials. Recently, infrared spectroscopy has been considered as an early diagnostic tool for the detection of tumours in synergy with traditional methods such as histopathological analysis, ultrasound, X-rays, etc. Micro imaging FT-IR is a powerful technique applied in tissue analysis, because it allows to detect biochemical changes that can be extremely subtle and located in a small area of tissue . Recently, micro-FT-IR imaging of normal and tumor breast cells has been carried out on breast tissues and cell lines showing certain spectral features, which could distinguish different breast neoplastic lesions and normal tissue. MATERIALS AND METHODS: In this study we have investigated 19 breast tumors (4 invasive lobular carcinomas with LCIS component, 13 invasive ductal carcinomas with DCIS component, 1 mixed type carcinoma and 1 osteosarcoma). The carcinomas were of variant histologic grade of malignancy. Thin paraffin sections of 5μm were obtained and spectral analysis of their solid state spectra was performed by using a Perkin-Elmer Spectrum One FT-IR equipped with a Perkin-Elmer Auto-image microscope. RESULTS AND CONCLUSIONS: By analysing these spectra, we have found characteristic bands in the infrared region, where the main components of these signature bands are located. For example, characteristic DNA band near 1680-1660

cm-1, Amide I, II, III protein bands and phospholipid band near 1140-1170 cm-1. These characteristic bands have been monitored as a function of the degree of cancer progression, and displayed spectral differences between normal breast tissue and different types of breast tumors. The results have been obtained with chemometric methods, such as cluster analysis, principal component analysis and custom analysis.

OP11-3
ANALYSIS OF THE EFFECTS OF A HIGH CORN OIL AND A HIGH OLIVE OIL DIET ON THE HISTOPATHOLOGIC CHARACTERISTICS AND ON P21RAS EXPRESSION IN DMBA-INDUCED RAT MAMMARY ADENOCARCINOMAS

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Background: Breast cancer is associated with lifestyle and environmental factors. Animal studies have shown that dietary lipids influence mammary carcinogenesis, mainly at promotion stage. c-Ha-ras is involved in proliferation pathways in normal and tumoral cells and can be activated by point mutations and overexpression mechanisms. Methods: We investigated the effects of a high corn oil and a high olive oil diet on the histopathologic characteristics and on p21ras expression in rat dimethylbenz(a)anthracene (DMBA)-induced mammary adenocarcinomas in comparison with those of a control low-fat diet. We analyzed nine histologic parameters and we applied a grading method adapted to rat tumors, described previously by us, on 227 carcinomas from three dietary groups of an experimental series: C (n=69), fed a normolipidic diet; M (n=91), fed a high corn oil diet and O (n=67), fed a high olive oil diet after the DMBA induction. An immunohistochemical study with an antip21ras monoclonal antibody (Ab-1 Oncogene Research) was performed on 209 carcinomas (65 C, 83 M and 61 O) and we evaluated the results using the image analysis software VISILOG 5.1 (NOESIS). Results: More than 50% of carcinomas from M exhibited architectural or nuclear grade 2 or 3 and > 3 mitosis/10HPF compared to the prevailing grade 1 and a lower mitotic count on control and high olive oil groups. An unequivocal stromal desmoplasia and tumoral necrosis were more prominent than tumors from O and a prevailing cribriform pattern was observed. High olive oil diet adenocarcinomas exhibited a predominantly low histologic grade, similar to the control, few necrotic and invasive areas and the highest percentage of papillary areas. Lymphoplasmacytic and mast cell infiltration were also influenced by diet. The different degrees of morphological malignancy, evident when analyzing the isolated parameters, was highlighted when we applied the adapted grading method: 72% and 64% of carcinomas from C and O, respectively, were well differentiated whereas only 50% of the tumors from M displayed a grade I. Regarding the expression of p21ras, we found no significant differences on the expression between the three groups: mean positivity of 49,66% in C; 56,43% in M and 54,26% in O. Conclusions: Our study demonstrates that dietary lipids affect the degree of morphological aggressiveness of the experimental mammary tumors according to their clinical behavior, which provides more arguments in favor of their relationship with breast cancer, and an unlikely role of p21ras overexpression in rat mammary carcinogenesis.

OP11-4
NESTIN POSITIVE MYOEPITHELIAL / PROGENITOR CELLS IN THE BREAST

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Nestin (neuroepithelial stem cell protein) is an intermediate filament (IF) protein considered to be a marker of neural stem cells. Nestin expression has been confirmed in stem/progenitor cells of the dermis, hair follicles, intestine, pancreas, bone marrow as well as in neural, muscle and other tissues. Nestin expression has also been detected in the endothelium and we have noted the activation of nestin expression in newly formed human blood vessels. The myoepithelial cells of the breast form an outer layer of the terminal duct lobular unit. Several markers have been reported for the immunohistochemical detection of breast myoepithelial cells, however, the specificity and sensitivity of these markers vary widely. We analysed the expression of nestin by an indirect immunohistochemical method in 300 archival formalin-fixed and paraffin-embedded tissue samples of breast cancer and surrounding mammary tissue as well as in tissue microarrays consisting of 109 cases. Nestin expression of various intensities was found in the cytoplasm of myoepithelial cells of almost all normal ducts and lobular acines found in the vicinity of tumours. The intensity of expression varied from strong /marked (32 cases, ~10%), to moderate/clearly visible (91 cases, ~29%) to low/visible (113 cases, ~38%) and to very low/visible in less than 50% cells only (64 cases, ~21%). Pathological lesions like adenosis with atypical hyperplasia and in situ carcinoma exhibited very strong positivity and the detection of nestin could be also used for the diagnosis of pseudoinvasion in some controversial cases. In invasive carcinomas we found nestin positivity in a portion of epithelial cells in a minority of cases. The positivity in the epithelial component was confirmed by simultaneous AE1AE3 cytokeratin immunostaining and was significantly higher in hormone receptor and c-erbB-2/HER2/neu negative cases which likely corresponded to basal cell phenotype cancer ($p \leq 0.003$). Beside the epithelial component, nestin positivity in carcinomatous samples was also confirmed in some stromal elements and endothelium of the capillary network (confirmed by simultaneous CD34 immunostaining). In summary, we have described the potential utility of nestin immunostaining in breast pathology for the detection of myoepithelial/progenitor cells and suggest its potential involvement in the processes of tumour development and neoangiogenesis. Acknowledgement: This work was supported in part by grants MSM 6198959216 and 0021620820.

OP11-5
TPL2/COT AS A NEW PROGNOSTIC AND PREDICTIVE FACTOR IN BREAST CANCER?

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Inhibition of protooncogene Tpl2/Cot (serin/threonin kinase) lead to its inability to activate signalling pathway ERK-MAPK, which, among others, regulates synthesis of cytokines TNF-alpha and IL-1 and expression of cyclo-oxygenase 2 (COX-2). Moreover there is a change in signalling pathway mediated by NF-kappaB. These molecules are also affected by the STAT protein family. STAT mediated signalling pathways participate not only in the modulation of immune response but also in the development of several types of malignant umours. Therefore, we can hypothesise that there is a relation between expression f STAT and Tpl2/Cot which has not been studied to date. The latest studies show Tpl2/Cot over-expression in about 40% of breast carcinomas. Experimental increase in expression of Tpl2 in breast carcinoma cell lines leads to activation of COX-2 expression. Over-expression of COX-2 detected in approximately 40% of breast carcinomas seems to play an important role especially in regulating angiogenesis, cell proliferation, cell differentiation and apoptosis. The aim of this pilot study was immunohistochemical detection of expression of TPL-2, COX-2 and STAT1,3,5 proteins in tissue microarrays (TMA) of 32 cases of invasive ductal carcinomas and analysis of its expression in relation to hormonal status and c-erbB-2 status. The results showed significant increase in Tpl2/Cot expression in c-erbB-2 negative carcinomas (p=0,001). This increase correlated with enhanced nuclear expression of STAT3 in the same group of tumours (p=0,014). In contrast to these findings, multivariate analysis showed significantly lower values of STAT1 expression in tumours characterized by ER+/PR+/cerb2- immunophenotype in comparison to other tumour types (p=0,001). COX-2 expression was increased in all studied types of tumour. No significant correlation was observed for other proteins. These results indicate that increased Tpl2/Cot and STAT3 expression together with decreased STAT1 expression in c-erB-2 negative breast cancer tumours could by an other important prognostic and predictive factor. Supported by grants VZ MSM 6198959216 and 6FP LSHC-CT-2006-037344.

OP11-6

MORPHOLOGICAL AND MOLECULAR EVOLUTIONARY PATHWAYS OF LOW AND HIGH GRADE BREAST CANCERS AND THEIR PUTATIVE PRECURSOR LESIONS

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BACKGROUND: In this study, further support to our proposed histogenetic pathways concerning low and high grade breast cancer (LGBC and HGBC) and their putative precursors was provided. MATERIALS & METHODS: 495 successive cases of invasive tubular (TC), cribriform (ICC), lobular (ILC), tubulolobular (TLC), low and high grade ductal carcinomas (IDC) and tubular/classic lobular mixed tumours were reviewed for the frequency of pre-invasive lesions. Tissue microarrays containing 790 representative areas of the putative precursors, co-existing terminal lobular units and carcinomas were immunoprofiled for putative tumour suppressor genes, cell cycle regulators, and proliferation and differentiation markers. RESULTS: More than 85% low grade IDC, ICC, TC, TLC, and ILC had associated CCLs, predominantly flat epithelial atypia (FEA). FEA, ADH, LN, and low grade DCIS was rare in high grade IDC; instead, high grade DCIS were the common precursor lesions (43%). The epithelial cells in FEA, ADH, LN, DCIS lesions and their co-existing LGBC/ILC were negative for basal and myoepithelial markers, but positive for CK19/18/8, ER-α, Bcl-2, and cyclin D1. The ER-α/ER-β expression ratio increased during carcinogenesis, as did cyclin D1 expression. Bcl-2 was expressed in almost all epithelial cells lining CCLs, ADH, LN, low grade DCIS and LGBC but was reduced, or absent, in high grade IDC. FHIT expression was reduced in hyperplastic CCLs, ADH/DCIS, LN and the associated invasive lesions. In LGBC/ILC, p53 immunopositivity was very rare (3%) while ATM expression was reduced in only 22% of LGBC cases. Positive expression of p53 was detected in 35% of high grade IDC. CONCLUSION: Our findings support the concept that FEA is the committed precursor cell lesions of LGBC/ILC. These may represent a family of precursor, in situ and invasive neoplastic lesions belonging to the luminal 'A' subclass of breast cancer. Our results suggest that the committed progenitor cells (PCs) for low grade breast neoplasia are CK19/18/8 positive and exhibit ER-α mediated CCND1 and BCL2 gene expression. Alternatively, breast cancer (PCs) regardless of their original phenotype may acquire stochastic genetic and epigenetic hits that lead to the activation of separate histogenetic pathways for LGBC and HGBC, and that these events determine the phenotype of the pre-invasive and invasive lesions. Furthermore, these hits may be an early event in the progression of 'luminal A' tumours and once committed to this 'molecular pathway', progression to a 'high grade' (basal-like or HER2+) phenotype would be unlikely.

OP12-1

IS BACTERIAL TRANSLOCATION IN CROHN'S DISEASE DEPENDENT ON CARD15 GENOTYPE?

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Background: The intestinal bacterial flora is involved in the pathogenesis of Crohn's disease (CD). Bacterial translocation may be required for the development of chronic transmural inflammation. Three single nucleotide polymorphisms (SNPs) of the CARD15 gene are associated with an increased susceptibility to develop CD and a decreased expression of alpha-defensins in ileal Paneth cells. We questioned whether the CARD15 gene status influences the type and ultimate distribution in the bowel wall of translocating bacteria in CD patients. Methods: 4 CD patients were selected on the basis of their status for the Arg702Trp, Gly908Arg, and Leu1007fsinsC SNPs of the CARD15 gene (1 wild type; 1 heterozygous and 1 homozygous for Arg702Trp; 1 compound heterozygous for Arg702Trp and Gly908Arg). Normal and pathological mucosa, myenteric plexus and mesenterial lymph node tissue were microdissected from snap-frozen ileal biopsies taken from the surgical bowel specimens. Extracted DNA was used as template in a 2-round PCR using universal primers for the 16S rDNA gene. Purified PCR products were subcloned, sequenced and subjected to a BLAST-search against the GenBank-, EMBL- and RDPII-databases. Results: PCR and cloning of 16S rDNA was successful in all samples. Sequence analysis revealed the presence of typical bowel bacteria (Enterobacteriaceae; Clostridia; Bacteroidetes) at all levels of the bowel wall, but there was no relation between the CARD15 gene status and the type of translocating bacteria. There was also no difference between the types of bacteria detected at the mucosal level and deeper in the bowel wall. Interestingly, 2 samples of the myenteric plexus in macroscopically involved bowel segments contained DNA derived from Legionella species. Conclusions: The majority of translocating bacteria in CD belong to the typical bowel microflora. The status of the CARD15 gene for the Arg702Trp, Gly908Arg, and Leu1007fsinsC SNPs does not influence the type and ultimate distribution in the bowel wall of these bacteria. Interestingly, this is the first report that demonstrates the presence of Legionella species deep in the bowel wall in CD patients. Further exploration of additional biopsy samples will be needed to confirm this finding.

OP12-2
HEDGEHOG PATHWAY IN COLONIC LESIONS
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Hedgehog signaling is a main regulation cascade in embryonic differentiation of the whole gastrointestinal tract and mutations of some components have been associated with birth defects like gut malrotation. Unusual expression of Hedgehog components has been associated with malignancies of the GI-tract. Methods Immunohistochemistry was performed on FFPE non-pathological (n=10) and pathological (n=45) tissue from human colon. The benign colonic lesions (n=23) comprise tubular/villous adenomas and hyperplastic polyps, malignant lesions (n=22) include adenocarcinomas and neuroendocrine carcinomas. Additionally Tissue array with 274 samples of colon cancer and normal tissue sections were used. Antibodies for Gli1, Gli2, Gli3, Shh, Ihh, Dhh, Ptch, Smo, β-Catenin and monoclonal antibodies for CK20 and Ki67 were used. For real time PCR tissue specimens were obtained from coloscopy. Colon cancer cell lines, including HCT 15, HT 29, DLD-1, C 201, C 205 and C 320 were investigated by real time PCR and BrdU assay. Functional assays were performed using cyclopamine as a pathway inhibitor and retroviral Gli1 and Gli2 constructs as promoters of Hedgehog signaling. Results Hedgehog signaling components are expressed in normal tissue samples as well as in benign and malignant lesions of the colon . In normal colon tissue the ligands Sonic Hedgehog (Shh) and Desert Hedgehog (Dhh) are expressed exclusively in the lining epithelium. Strong and focally distributed Shh and Dhh expression was detected in all malignant cases, although the expression of Hedgehog ligands is reduced in adenocarcinomas. Shh/Dhh expression was inversely correlated with proliferation using Ki67. Possible regulation of differentiation was proven with CK20. Corresponding tissue samples were analyzed by real time PCR. Cell culture experiments were performed to verify correlation of active Hedgehog signaling and proliferation or differentiation, respectively. The colon cancer cell lines investigated did not show significant changes neither on treatment with pathway inhibitor cyclopamine, nor by ectopic Hedgehog expression. BrdU incorporation assay also confirmed immunohistochemical findings. Conclusion The Hedgehog signaling pathway seems to play an essential role rather in maintaining normal colonic tissue than promoting cancer formation or even accelerating cancer growth. Relating to cancer dedifferentiation and proliferation loss of Hedgehog function may be associated not with regulation of well differentiated cancer cells but with cancer stem cells.

OP12-3
HEDGEHOG SIGNALING IN THE PANCREATIC NEUROENDOCRINE SYSTEM AND MALIGNANT TUMORS
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The Hedgehog (Hh) pathway is one of the most fundamental signal transduction pathways in pancreatic embryonic differentiation. The presence in mature tissue is still to be investigated. Three mammalian Hh ligands Sonic (Shh), Indian (Ihh) and Desert Hedgehog (Dhh) are all able to bind to the receptor Patched (Ptch) on target cells at the same extent. Binding of the ligand at Patched receptor looses its inhibitory effect on the protein Smoothened (Smo), activating an intracellular signaling cascade which leads to the release of the transcription factors Gli1, Gli2 and Gli3 and therefore expression of downstream target genes. Hh signaling in developing pancreas determines boundaries, as inhibition by cyclopamine results in expansion of pancreatic tissue and expression of pancreatic hormones. Indian Hedgehog and Patched seem to promote correct development of the pancreas as targeted mutation of one of them results in pancreatic defects. Patched especially affects organization of endocrine cells, as seen by a reduction of islet size and pancreas specific gene expression and impaired glucose homeostasis in the absence of the receptor. The expression of Hh pathway members (Gli 1, 2, 3, Shh, Dhh, Ihh, Ptch, Smo, Hip) in normal human and murine pancreatic tissue and neuroendocrine tumors (n=30), including e.g. insulinoma, glucagonoma and gastrinoma was analyzed. For immunohistochemistry a standard S-ABC detection system (Dako) and a double immunofluorescence technique on FFPE tissue was performed. Interaction of Hh activity with Wnt and Notch signaling has been analyzed. For mRNA detection and quantification real time PCR was performed on a Corbett RotorGene 6000. The insulinoma cell lines TGP52 and Beta TC-6 were used for in vitro experiments. Therefore the pathway has been inhibited by cyclopamine to show changes in gene expression and correlation with hormone secretion. By adding glucose the impact of Hh signaling on hormone secretion and release was investigated. Immunohistochemical results showed an active Hh pathway in the β- and δ-cells of normal mature pancreas. We detected a specific expression pattern of the Hh components and members of other pathways like Notch and β-Catenin. Activity of the Hh signaling cascade is also demonstrated in neuroendocrine tumors. These findings are confirmed by real time PCR. Our results indicate an involvement of Hh signaling in formation of pancreatic neuroendocrine tumors. Hh pathway may be associated with neuroendocrine cell differentiation and stem cell determination in pancreatic Langerhans islets.

OP12-4
QUANTITATIVE MONITORING OF THE MRNA LEVELS OF CYCLIN D1 AND PROLIFERATION MARKERS (Ki-67, TOPOISOMERASE IIα, AND TPX2) ASSOCIATED WITH UNFAVORABLE PROGNOSIS IN PATIENTS WITH MANTLE CELL LYMPHOMA
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Background: Mantle cell lymphoma (MCL) is an aggressive neoplasm with a poor long-term outcome. The median overall survival is usually 3–4 years. However, the clinical behavior may be variable. Some patients suffer from an aggressive disease with survival less than a year, while other patients have a relatively slow clinical evolution and survive more than 10 years. It is therefore of a great importance to recognize MCL at initial diagnosis and to establish routinely applicable prognostic markers. Cyclin D1 is described as a molecular hallmark of MCLs. Expression of proliferation markers (Ki-67, topoisomerase IIα and/or TPX2) is shown to be the most effective prognostic factor for the clinical outcome. The aim of the study was to analyze the cyclin D1 expression and to design a methodology allowing a precise quantitative assessment of the proliferation markers. Method: We developed a real-time quantitative (RT-RQ-PCR) measurement of the cyclin D1, Ki-67, topoisomerase IIα, and TPX2 transcripts. We studied 113 fresh frozen or formalin fixed, paraffin embedded biopsy specimens of non-Hodgkin's lymphomas, of which 77 were diagnosed as MCL. Results: We detected the cyclin D1 overexpression in all MCL specimens including bone marrow aspirates infiltrated by MCL. We designed and approved a novel quantitative assessment of the proliferation markers. We demonstrated RT-RQ-PCR as a highly reliable, specific, objective and routinely applicable technique to examine the proliferation activity. The proliferation activity varied from one tumor to another reflecting the heterogeneity between individual patients, which is described to be of a clinical relevance. Generally, we observed the highest proliferation activity in Burkitt lymphomas and the lowest in reactive lymph

nodes. The proliferation activity assessed by the quantitative measurement of topoisomerase IIα and TPX2 correlated with the cyclin D1 level indicating the quantitative regulation of the cell cycle rather than an on/off switch control. Conclusion: RT-RQ-PCR is a reliable method for molecular monitoring of patients with MCL. A quantitative detection of cyclin D1 mRNA is a useful tool to recognize MCL and also a tool for monitoring the bone marrow during the disease course. The quantitative assessment of the proliferation markers provides a potent tool to predict the survival of MCL patients. This work was supported by the Internal grant of Faculty Hospital in Motol No. 9756, Grant of Charles University GAUK 200055, 46/2006/C/2.LF, and the Research project of the Ministry of Health No. 00064203/6704

OP12-5

MINIMAL DISSEMINATED DISEASE IN ALVEOLAR RHABDOMYOSARCOMA USING PAX3/FKHR AND PAX7/FKHR FUSION GENES.

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Background: Rhabdomyosarcoma (RMS) has 2 major histological subtypes: alveolar (ARMS) and embryonal (ERMS). ARMS is more aggressive and prone to distant dissemination, whereas ERMS tends to expand and recur locally. The aim of the study was to monitor minimal disseminated disease (MDD) using real-time quantitative reverse-transcription PCR (RQ-RT-PCR) of the PAX3-FKHR or PAX7-FKHR fusion gene. Design: We prepared an assay using RQ-RT-PCR for the quantitative assessment of MDD in ARMS by using hydrolysis probe for quantification of PAX3-FKHR or PAX7-FKHR genes and beta-2-microglobulin housekeeping gene, which was used as an internal cDNA quality and quantity control. We constructed plasmid standards with either PAX3/7-FKHR fusion genes or beta-2-microglobulin gene. Normalized expression of PAX3/7-FKHR was determined as a ratio between PAX3-FKHR or PAX7-FKHR and beta-2-microglobulin levels assessed by RQ-RT-PCR. We determined the sensitivity and specificity of PAX3/7-FKHR transcripts as markers for detection of MDD. Primary tumor samples (18), local recurrent samples (16), bone marrow samples (91), peripheral blood progenitor cell samples (24), peripheral blood samples (16) or bone marrow grafts (3) in 27 patients with ARMS were examined by the RT-PCR and RQ-RT-PCR techniques. Results: PAX3/7-FKHR transcripts are specific and sensitive tool for detection of MDD in ARMS. We were able to identify 13/25 patients with BM involvement using RQ-RT-PCR and 15/25 using nested RT-PCR method. Nine of eighteen BM samples were positive for the fusion transcript at the time of diagnosis. We analyzed PAX3-FKHR or PAX7-FKHR expression during the course of the disease in 133 sequential BM, PB or PBPC (93, 24, 16) samples. The RQ-RT-PCR results correlated well with nested RT-PCR results ($p<0.0001$). Conclusion: The presence of metastases is the most adverse prognostic factor in RMS, and bone marrow is a frequent site of the tumor dissemination in ARMS. Our results detecting the fusion transcripts typical of ARMS in the BM or peripheral blood suggest that patients with positive findings are at a high risk of the tumor progression. Of the 9 patients in whom we were able to identify bone marrow involvement by the tumor cells at the time of presentation, four died of progressive disease and two live with the tumor progression. In three patients the follow-up is too short to make a conclusion. The project was supported by grant GACR 304/07/0532, by research project FNM MZO 00064203 and by MSM 0021620813.

OP13-1

CD44 (H-CAM) EXPRESSION IN BREAST CARCINOMA. AN EXPERIENCE OF 314 CONSECUTIVE CASES IN VERONA

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Background: CD44 is a family of plasma membrane glycoproteins encoded by a single gene in chromosome 11. This adhesion molecule has been recently reported in breast carcinoma, but the role and the prognostic impact is not clear. CD44v7-v8 expression is associated with poor long term prognosis. CD44s expression is associated with increased diseased free survival in node negative patients. Our aim is to study CD44 (H-cam) expression in breast carcinoma and the correlation with other molecular markers. Methods: We studied CD44 (H-CAM) immunoexpression in 314 consecutive breast carcinomas consisted of 310 primary neoplasm and four metastasis. All cases were also submitted to routinely immunohistochemical evaluation for Estrogen Receptor (ER), Progesteron Receptor (PgR), E-caderin, Ki-67 and Hercep-test (HcT). To evaluate CD44 expression, the cases have been stratified into three different categories according to the pattern of cell positivity. High-positive: diffuse membrane expression in all neoplastic cells; low-positive: low rate of neoplastic cells positive in a context of highly heterogeneous expression; negative: lack of staining in neoplastic cells. Results: 141 (45%) cases were CD44 high-positive and consisted of 71 ductal invasive carcinoma, 26 ductal carcinoma in situ, 2 microinvasive carcinoma,19 lobular invasive carcinoma, 9 mixed carcinoma ductal and lobular, 3 colloid carcinoma, 2 papillary carcinoma, 2 metaplastic carcinoma, 1 lobular carcinoma in situ, 1 tubular carcinoma, 1 inflammatory carcinoma, 4 metastasis of breast carcinoma (liver, pleura, nodal and bone), 51 (16%) cases were low-positive, 122 (39%) cases were negative . In our analysis, CD44 was not correlated to histotype, ER (P=0,50), PgR (P=0,88), ki67 (P=0,9) E-caderin (P=0,47), HcT (P=0,78) and nodal metastasis (P=0,88). Conclusion: In preliminar data CD44 is not correlated to histotype and other immunohistochemical markers. This work was supported by: Fondazione Cassa di Risparmio di Verona Vicenza Belluno e Ancona. “Carcinoma della mammella: marcatori fenotipici e molecolari indicatori di prognosi e risposta terapeutica. Bando di ricerca scientifica 2004, indirizzo biomedico. Resp Scientifico: Prof Franco Bonetti

OP13-2

MOLECULAR GENETICS AND IMMUNOPHENOTYPICAL CHARACTERIZATION OF MICROPAPILLARY CARCINOMAS OF THE BREAST

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Background: Micropapillary carcinoma (MPC) is a special type of breast cancer with a distinctive histological growth pattern, high prevalence of lymph-node metastasis and aggressive clinical behaviour. Methods: We studied 28 MPCs, 14 pure (PMPCs) and 14 mixed carcinomas (MMPCs). A tissue microarray was constructed and immunohistochemistry for ER, PR, HER2, p53, basal markers (CK5/6, CK14, CK17, CAV-1, EGFR) and adhesion proteins (E-cadherin, beta-catenin) was performed. Eleven primary PMPCs and 2 matched lymph-node metastases

were microdissected and studied by microarray-based comparative genomic hybridisation (aCGH) using the Breakthrough Breast Cancer Research Centre aCGH platform containing 16K bacterial artificial chromosomes clones arranged at ~100Kb intervals throughout the genome. Validation of genetic alterations was performed with chromogenic in situ hybridization (CISH). Results: The vast majority of MPCs showed luminal (93% of PMPCs, 71% of MMPCs) or HER2+ (7% of PMPCs, 29% of MMPCs) immunophenotype. Neither positivity for any of the basal markers studied nor EGFR amplification was found. E-cadherin expression was seen in both PMPCs and MMPCs, beta-catenin showed only membranous and cytoplasmic staining in all cases but one pure carcinoma which also displayed nuclear positivity. p53 was positive in 21% of PMPCs and in 42% of MMPCs. aCGH analysis revealed recurrent (> 30% of cases) copy number gains on 1q, 6p, 7p, 8p, 16p, 17p, 17q, 20q, 20q; losses at 3q, 6q, 6q, 8p, 9p, 13q, 13q, 16q, 16q, 17p, 18p, 18q, 18q and 22q. Recurrent amplifications mapping to 1q23.3, 1q31.3-q32.1, 8p12-p11.21 (FGFR1), 8q21.12-q24.3 (MYC), 11q13.3 (CCND1), 16p13.3, and 17q21 (HER2) were also found. CISH analysis of the whole series revealed HER2 amplification in 7% of PMPCs and 29% of MMPCs. TOP2A amplification was found in 2 HER2 amplified MMPCs. MYC amplification was found in 21% of PMPCs and 28% of MMPCs. FGFR1 amplification was found in 14% of both PMPCs and MMPCs. CCND1 amplification was only found in 7% and 14% of PMPCs and MMPCs respectively. 7% of PMPCs harboured co-amplification of HER2 and TOPO2A and of MYC and CCND1. The 14 MMPCs showed coamplification of HER2 and TOPO2A in 2 cases, coamplification of HER2, TOPO2A and CCND1 in 1 case, and HER2, MYC, FGFR1 in 1 case. Conclusions: MPCs do not display a basal-like immunophenotype and are predominantly of luminal phenotyope. At the molecular genetic level they show varying patterns of genomic aberrations and frequent coamplification of key oncogenes that may account for their aggressive clinical behavior.

OP13-3
MAMMAGLOBIN, A USEFUL ADDITION TO THE CURRENTLY AVAILABLE IMMUNOHISTOCHEMICAL MARKERS FOR THE DETECTION OF METASTATIC BREAST CARCINOMA

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Background: Gross cystic disease fluid protein (GCDFP-15) and estrogen receptor alpha (ER α) are immunohistochemical markers routinely used in antibody panels for the diagnosis of metastatic breast cancer. These markers are valuable diagnostic tools, but there is a need to improve the sensitivity and specificity of the existing panel of breast markers. Mammaglobin is a member of the secretoglobin-uteroglobin family and its expression is largely restricted to normal and neoplastic breast. Mammaglobin protein expression has been demonstrated in the majority of well, moderately, and poorly differentiated invasive ductal carcinomas and in metastatic breast cancers by immunohistochemistry (IHC). In this study, we compared the expression of monoclonal antibodies to Mammaglobin, GCDFP-15 and ER α on metastatic breast cancers to evaluate whether mammaglobin improves the sensitivity of the antibody panel in detecting metastatic breast carcinoma. Method Formalin-fixed, paraffin-embedded (FFPE) tissue specimens from metastases of breast cancer, renal cell carcinoma, melanoma, and large cell carcinoma were obtained. Immunostaining was performed using mouse monoclonal antibodies to human mammaglobin (clone 304-1A5), GCDFP-15 (clone 23A3) and ER α (clone 1D5) on tissue pretreated with Tris/EDTA target retrieval solution. Bound antibody was visualized with the Dako EnVision+ Dual Link Detection System. Results: Mammaglobin, GCDFP-15 and ER α were found to be expressed in the majority of metastatic breast carcinomas tested. The highest positivity rate was found with anti-mammaglobin, followed by anti-ER α and finally anti-GCDFP-15. While expression of all three markers was observed in the majority of cases tested, the percentage of positively stained tumor cells varied significantly. Both GCDFP-15 and mammaglobin exhibited focal positivity in a subset of tumors, however their expression was complementary in that cases that were only very focally positive for GCDFP-15 exhibited significant mammaglobin expression and vice versa. Additionally, cases with significant mammaglobin and GCDFP-15 expression frequently labeled different cell populations within a tumor, further demonstrating the complementarity of the two markers. Conclusions: In this study we have demonstrated that mammaglobin is a sensitive marker for the identification of metastatic breast cancer. Furthermore, our findings suggest that the addition of mammaglobin to the panel of currently available breast markers improves the sensitivity when establishing breast as the site of origin.

OP13-4
IMMUNOHISTOCHEMICAL ANALYSIS OF TOPOISOMERASEIIα EXPRESSION IN SPORADIC INVASIVE BREAST ADENOCARCINOMAS

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BACKGROUND-OBJECTIVE: TopoisomeraseIIα gene is located at chromosome band 17q12-q21 and encodes a 170 kDa protein which alters the topological state of DNA through DNA strand cleavage, strand passage and religation, actions vital to cell division. Anti-cancer drugs called topoIIα inhibitors target this protein and induce tumor cell death via stabilization of DNA-TopoIIα complexes and double-stranded DNA breaks. The oncogene HER2 is located close to TopoIIα chromosomal locus and is overexpressed or amplified in 14-34% of breast carcinomas. Patients who respond to targeted therapy with monoclonal antibodies against HER2 often benefit from the parallel administration of TopoIIa inhibitors. In vitro studies with cell lines have demonstrated that TopoIIa gene amplification leads to TopoIIa protein overexpression and increased sensitivity to TopoIIa inhibitors. On the other hand loss of TopoIIa gene or decreased protein expression leads to chemoresistance against those drugs. Therefore, information on the expression levels of TopoIIa could be a significant predictive factor for therapeutic response in breast cancer treatment. In this context we investigated the expression of TopoIIα in sporadic invasive breast carcinomas and correlated its expression with tumor type, histological grade, lymph nodes status and HER2 receptor's expression. MATERIALS AND METHODS:Retrospective study of 368 cases of sporadic invasive breast carcinoma, from total and partial breast resections. Immunohistochemical detection of TopoIIa and HER2 protein expression using monoclonal antibodies. Semiquantitative protein expression assessement according to the accepted guidelines. RESULTS AND CONCLUSIONS: A. Overall and independently of tumor type 54.07% of breast carcinomas showed intense nuclear expression of TopoIIα B. Correlation of TopoIIa expression with tumor type, revealed high levels in 61.92% of ductal type, 41.82% of lobular, 25% of mixed and 33.3% of other types. C. Correlation of TopoIIa expression with histological grade showed high expression in Ductal: Grade I 23.8%, II 51.8%, III 69.7%, Lobular: Grade I 50%, II 38.46%, III 33.4%, Mixed: Grade I 20%, II 20.4%, III 30%.D. There was not statistically significant association between TopoIIα expression levels and lymph node status.E. Correlation of TopoIIα expression status with HER2 expression levels revealed : 72.3% of HER2 (3+) and 81.4% of HER2 (2+) carcinomas had high TopoIIα expression levels, while 39.6% of HER2 negative tumors showed increased TopoIIα expression.

OP13-5

ANALYSIS OF HER-2/NEU EXPRESSION AND GENE AMPLIFICATION IN 1020 INFILTRATING BREAST CARCINOMAS BY IHC AND CISH.CORRELATION WITH TUMOR TYPE, HISTOLOGICAL GRADE, LYMPH NODE STATUS AND ERα, PGR, P53 AND Ki-67 EXPRESSION.THE HELLENIC MULTICENTER STUDY

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OBJECTIVES: To evaluate the expression of HER2 in infiltrating breast carcinomas by IHC, to compare the reactivity against HER2 of three different antibodies, to correlate HER2 expression levels with its gene amplification by CISH, tumor type, grade, lymph node status and ERα, PgR, p53 and ki-67 expression levels.MATERIALS AND METHODS: Prospective analysis of 1020 specimens of infiltrating breast carcinomas (680 ductal, 117 lobular, 69 mixed, 154 others) by IHC, using AO485, CB11, TAB-250 antibodies against HER2. Immunostaining was evaluated according to accepted guidelines.Cases scored 1+ and 2+ were further analysed by CISH. Gene amplification was graded as negative (1-2 gene copies/nucleus), putative Chr 17 polysomy (3-5 gene copies/nucleus), low amplification (6-10 gene copies/nucleus) and high amplification (>10 gene copies/nucleus). The same specimens were assessed IHC for ERα, PgR, p53 and Ki-67 expression status.RESULTS AND CONCLUSIONS: 1. AO485 IHC showed 24.80% of the tumors had 3+, 16.57% 2+, 26.47% 1+ and 32.16 % negative HER2 reactivity 2. There was concordance in evaluating HER2 expression levels among the antibodies used (AO485 vs CB11 92.8%, $p < 0.0001$ and AO485 vs TAB250 95.1%, $p<0.0001$) especially in 3+ cases. 3. Concerning CISH, among 1+ IHC analysed cases (249/270), 47.8% had no gene amplification, 45.8% showed putative chromosome 17 polysomy (not evaluated by Chr. 17 centromeric probe), 4.4% displayed low gene amplification and 2.0% high gene amplification. Cases scored 2+ (160/169) showed low gene amplification in 13.8%, high gene amplification in 8.8%, putative chromosome 17 polysomy 33.8% and no amplification in 43.8%. In conclusion CISH is a highly accurate method in evaluating HER2 gene copy status, more sensitive than IHC and should be used not only in 2+ IHC cases but also in 1+ IHC ones 4. HER2 expression levels were correlated with tumor type showing lower levels in lobular carcinomas ($p=0.0006$) and with tumor grade displaying higher expression in higher grade ($p<0.0001$). There was not statistically significant association between HER2 expression levels and lymph node status ($p=0.2744$) 5. Correlation of HER2 expression status with ER/PgR expression revealed higher HER2 levels in negative hormone receptors tumors and lower levels in positive ones ($p<0.0001$) 6. Higher HER2 levels have found in tumors with high p53 and Ki-67 expression ($p<0.0001$) 7. Stratified analysis revealed that tumor grade is more important than type in determining the expression status of HER2 (tumor grade $p<0.0001$ tumor type $p=0.7827$)

OP13-6

ANALYSIS OF THE CONCORDANCE BETWEEN THE BI-RADS CLASSIFICATION WITH USNCB AND BETWEEN USNCB WITH DEFINITIVE HISTOLOGICAL DIAGNOSIS IN 595 PATIENTS (601 CONSECUTIVE CASES)

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Introduction: Preoperative ultra-sound guided needle core biopsy (USNCB) in breast cancer is becoming the standard of care and is used as the initial sampling method for non palpable, breast lesions detected by radiology. Objective: The aim of this study is to analyze the correlation between the breast imaging reporting and data system (BI-RADS) and the histological classification B categories for NCB of the European Working Group on Breast Pathology (EWGBP). We further analysed the pre-operatory histological diagnosis of the NCB with the definitive histological diagnosis after the definitive surgery was performed. Material and Methods: 601 consecutive USNCB from 595 patients with suspicious breast lesions (23% =0-9mm; 57%=10-19mm) were carried out from March 2003 to June 2006. Biopsies were performed using US equipment with a 7.0 -12 MHz multi frequency transducer; a minimum of three cores from each lesion were obtained using disposable automatic needles 14-G. The biopsies were formalin-fixed, paraffin-embedded, three-micro-thick sectioned and haematoxylin and eosin stained. Results: The data revealed 99% agreement of the 5 category BI-RADS with B5 category of the EWGBP classification, 71% of the category 4 BI-RADS with B5 category of the EWGBP classification. In case categories 5 and 4 BI-RADS are considered together the agreement is 80% with B5 category of the EWGBP classification. Furthermore, one found 57% agreement of 3 category BI-RADS with B3 category of the EWGBP classification (33% were B1; 4% were B5 and 5% were B3).The analysis of the concordance of the pre-operative histological diagnostic of the NCB with the definitive histological diagnosis after the definitive surgery was performed revealed 100% agreement for B5 category of the EWGBP classification with the definitive histological diagnosis (95% invasive carcinoma; 5% in-situ). For B3 category of the EWGBP classification we found 59% benign lesions, 22% borderline lesions (phyllodes tumour), 7.4% in-situ malignant lesions (intra- papillary carcinomas) and 11% invasive carcinomas (invasive papillary carcinomas). Discussion and conclusions: Ultra-sound guided needle core biopsy (USNCB) is an effective diagnostic method for a pre-operatory histological diagnosis; when definitive surgery was performed the definitive diagnosis with the histological diagnosis in NCB was 100% of the agreement for B5 categories which allowed that women to be submitted to one surgery only.

OP13-7

BASAL DIFFERENTIATION WITH CK5/6 IN INVASIVE DUCTAL CARCINOMAS OF BREAST AND PROGNOSTIC FACTORS

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Aim: Currently human breast carcinomas are classified according to 2002-WHO histological classification. This system doesn't take into account prognostic factors. Recent studies on breast tumors have identified distinct subtypes of breast carcinomas that are associated with different outcomes. These subtypes are: ER+/ luminal, normal breast-like, HER-2 over expressing and basal-like groups. The latter two are associated with poor prognosis, and basal-like tumor group express basal cytokeratins like CK5/6. Our study aimed to asses basal phenotype in invasive ductal carcinomas of breast by the expression of CK5/6 and examine the relationship between their expression and other pathological and immunohistological factors that have clinical significancy.

Material- methods: Expression of CK5/6 (basal marker), SMA (myoepithelial marker) and CK8/18 (luminal marker) was analyzed by immunohistochemistry in 93 invasive ductal carcinoma (NOS) of breast diagnosed between December 2004-November 2006 in Ankara Oncology Hospital, pathology department. CK5/6 positive and negative groups are identified and these groups are compared according to known prognostic and immunohistological factors which are; patient age, tumor size, histological grade, mitosis count, lymphovascular tumor invasion, necrosis, lymph node status (metastatic node number, extracapsular tumor extension, conglomeration of metastatic nodes) and ER, PR status, cerbB2 expression. Results: Of 93 tumor samples 16 expressed basal marker CK5/6 (%17.2). All of the cases expressed CK8/18 but just one case was SMA positive. Between CK5/6 positive and negative groups 4 parameters showed statistically significant difference; histological tumor grade, conglomerated metastatic lymph nodes, ER and PR status. Most of the CK5/6 positive basal group tumors was grade 3 (%75), %54.5 had conglomerated metastatic lymph nodes. Twelve of 16 CK5/6 positive cases were ER negative (%75), eight of them were PR negative (%50) whereas in CK5/6 negative group results were respectively %9,1 and %5.2. Conclusion:Our results are correlated with the literature. Immunohistochemically basal like phenotype of breast carcinomas are identified and CK5/6 positive tumors are associated with poor prognostic factors; conglomerated metastatic lymph nodes, higher histological grade and negative steroid hormone receptors.

OP13-8
SELECTIVE FROZEN SECTION IN SENTINEL NODE BIOPSY: A RELIABLE APPROACH

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Background: Independently of existing guidelines the role of frozen section (FS) as obligate constituent of intraoperative pathological examination in axillary sentinel biopsy (SLNB) is still under debate. However, frozen sectioning can lead to significant tissue loss with the risk of missing metastases at all, or failing to determine their largest diameter. To avoid this, our multidisciplinary protocol includes exclusively selective FS. That means that FS examination is done only when standardized pathological gross exploration provides findings suspicious for metastasis. Aim of this study was to estimate reliability of selected FS in SLNB for breast cancer. Methods: In 145 consecutive patients with unifocal invasive breast cancers of two to 24 mm in diameter (median 14 mm) SLNB procedure detected 272 lymph nodes (LN). Pathology protocol: Slicing of LNs parallel to the length axis in 2 mm slices; macroscopic inspection and palpation of any slice; FS in case of suspicious finding only; embedding of all slices; step sectioning (500 µm levels) throughout the LN; in all H&E-negative cases cytokeratin immunohistochemistry from each level. Interpretation of microscopic findings was accomplished according to recommendations of the European Working Group on Breast Screening Pathology; the findings were documented according to the UICC-TNM-classification. We determined the rates of positive LNs with macrometastases (MaMet), micrometastases (MiMet), as the rate of LNs with isolated tumor cells (ITC), the false-classification-rate (FCR), and the rate of secondary axillary dissection in a separate operation (SAD). We analysed sensitivity and specificity of the standardised macroscopical examination of SLNB referred to final complete work-up results, separately per LN and per patient. Results: 29/145 patients showed one ore more positive LN (20 MaMet, 7 MiMet, 2 ITC). The FNR was 0.13% per LN and 0.17% per patient. The rate of SADs accounted to 6/145 (4.1 %). Sensitivity and specificity measured solely for standardised macroscopical examination was by 87% and 98%, respectively per LN, and by 82% and 98%, respectively per patient. (Two macroscopically suspicious LN proved to be negative by FS which enhanced the specificity of the SLNB procedure up to 100%.) The negative predictive value of the SLNB procedure including selective FS examination was 96%, the FCR was 0.04%. Conclusion: A selective FS only in case of suspicious finding after macroscopical examination of LNs by the pathologist is a reliable approach to intraoperative decision making for axillary dissection.

OP13-9
PROGNOSTIC SIGNIFICANCE OF SKI PROTEIN IN BREAST CANCER PATIENTS

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Introduction: Ski protein has recently been identified as a co-repressor of TGF beta signaling pathway through interaction with Smad family proteins. The activation of the signaling cascade begins by activation of TGF-beta receptor II (TbRII) after the ligand's binding. The purpose of the present study was to investigate the potential role of TbRII and Ski proteins in breast carcinogenesis and their correlation with several clinicopathological parameters. Materials and methods: TbRII and Ski expression was studied by immunohistochemical methods in 154 cases of breast lesions (8 cases of atypical hyperplasias, 22 cases of in situ carcinomas and 124 cases of invasive breast carcinomas). There was a median follow up time of 70 months (4-150) during which 47 developed blood borne metastases and 31 finally died of metastatic cancer. The statistical analysis of the results was performed using SPSS 13 for Windows. The statistical significance was set at 0.05. Results: Ski immunostaining exhibited a granular and mainly cytoplasmic pattern, though both nuclear and cytoplasmic immunostaining was observed in a few cases. TbRII showed a solely cytoplasmic expression. Ski protein was detected in 3 cases (37,5%) of atypical hyperplasias, 10 cases (45%) of in situ and 86 cases (69,4%) of invasive carcinomas. TbRII was positive in 2 cases (25%) of hyperplasias, 6 cases (27%) of in situ and 30 cases (24,1%) of invasive carcinomas. Kaplan Meier survival analysis showed that Ski protein's expression indicated a worse outcome (Spearman's Rho=0,290, p=0.001). Ski protein's expression was associated with TbRII expression ($p<0.001$), while a similar association existed between Ski proteins' expression and the presence of invasive carcinomas (p=0.006). No association was found between TbRII expression and patients' survival or the presence of invasive component. Conclusions: Ski protein may play a pivotal role in several steps of breast tumorigenesis and is probably correlated with a more aggressive phenotype.

OP13-10
HER2 DIAGNOSIS PROGRAMME IN ARGENTINA: MAIN RESULTS

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Background: Early HER2 status determination becomes essential for primary and metastatic disease management. Identifying HER2 positive patients enables offering specific therapy leading

to a significant change in treatment and prognosis. Technique standardization is a key to success in identifying HER 2 over-expression in an accurate, precise and truthful way. Objectives: Implement a program enabling access to replicable and standardized HER2 diagnosis country-wide. Develop a team of pathologists trained in IHC to meet demands, for fast and truthful detection of HER2 over-expression. Material and methods: In August 2003, 13 pathologists formed a cooperative group and created a national framework to train and set HER2 diagnostic centres in each country region. A coordinator responsible for coaching and evaluating centres and two technical consultants in charge of quality control and technique standardization were designated. In February 2004, the program was launched. Tumors were received from 82 oncologists and breast surgeons, using a private courier created "ad hoc". HER2 over-expression was analyzed by Inmuno-Histo-Chemical (IHC) test between February 2004 and March 2006. HER-2 analysis was performed using policlonal antibody anti Her 2 (DAKO), microwave antigenic recovery, detection system EnVision (Dako) and developed with diaminobenzidine. Results were interpreted as hercepTest® guideline's. Results: 7011 HER 2 tests were completed; HER 2+ prevalence in our sample is 16.4%. Internal quality control, executed by the Advisory Board,as well as independent and external quality control, carried out by the American College of Pathologists have assured accurate results Conclusions: HER2 National Program enabled country-wide HER2 testing. A clear and defined strategy within the regulated and controlled framework, with a standardized and replicable technique, renders excellent results. HER2+ prevalence in our sample (16.4%) is in line with previously published ones. We presented guides of performance, interpretation, and technical issues for HER2 testing based on our experience endorsed by Argentinean Society of Pathology

OP13-11
HER2 IN INVASIVE BREAST CARCINOMA: ASSOCIATION WITH PROGNOSTIC AND PREDICTIVE FACTORS

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Background: Since its discovery HER2 has been linked with poor prognosis in node positive and negative primary invasive breast carcinoma (IBC). Association between HER2 status and various prognostic and predictive factors has been analyzed in numerous studies; however, multivariate analysis has only rarely been used and HER2 status was rarely assessed by both IHC and FISH. Methods: HER2 status was assessed by IHC (HercepTest™) and FISH (PathVysion™) in 1519 consecutive primary IBC. Strong protein expression (3+) and/or gene amplification indicated positive HER2 status (HER2+). Correlations between HER2 status and patient age, tumor size, histologic type and grade, lymph node involvement, number of involved lymph nodes, lymphovascular invasion, estrogen (ER) and progesterone receptor (PR) were analyzed univariately by Chi-square test or Spearman's correlation and multivariately by logistic regression. Results: 14.5% of tumors were HER2+. In univariate analysis, HER2 positivity was strongly associated with younger age ($p<0.001$), higher histologic grade ($p<0.001$) as well as each of its components (gland formation, nuclear pleomorphism, mitotic count; all $p<0.001$), ductal type ($p<0.001$), presence of vascular invasion ($p<0.0001$), lymph node involvement ($p=0.003$) and number of involved lymph nodes ($p=0.004$). There was no significant association with tumor size. HER2 positivity was inversely associated with ER ($p<0.0001$) and PR ($p<0.0001$) expression. In multivariate analysis, ER status, histologic grade and lymphovascular invasion were the only independent predictors of HER2 status. Among ER+ tumors, HER2 positivity was independently associated with increasing grade and decreasing age, whereas among ER- tumors the only feature significantly related to HER2 positivity was lymphovascular invasion. Conclusions: The results of the present study are in line with findings from previous studies, which have shown that HER2 positive tumors usually exhibit other adverse features, such as ER/PR negativity and higher histologic grade. In addition, our study also reveals a strong and independent association between HER2 positivity and presence of lymphovascular invasion, which, to our knowledge has not been reported before.

OP14-1
MYXOID SOFT TISSUE TUMOURS HAVE A HETEROGENEOUS COMPOSITION OF THEIR EXTRACELLULAR MATRIX: A STUDY ON MYXOFIBROSARCOMA, INTRAMUSCULAR MYXOMA AND EXTRASKELETAL MYXOID CHONDROSARCOMA

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Intramuscular myxoma, myxofibrosarcoma and extraskeletal myxoid chondrosarcoma are soft tissue tumors characterized by their abundant, so-called myxoid extracellular matrix. We studied the biochemical composition of this extracellular matrix to unravel its constitution and functional consequences for tumor growth, which are still poorly understood. Using Alcian blue staining we showed that intramuscular myxoma and grade I myxofibrosarcoma contained heparan sulphate, hyaluronan, keratan sulphate, chondroitin-4 and –6 sulphate in comparable amounts; Myxofibrosarcoma showed a significant increase in heparan sulphate with increasing histological grade; In contrast, chondroitin-4 and -6 sulphates were the major glycosaminoglycans of the extracellular matrix of extraskeletal myxoid chondrosarcoma. Using Western Blot, we identified albumin as a major protein in tumor lysates of all three tumors. We showed its presence in the extracellular matrix of all tumors and in the cytoplasm of the majority of the tumor cells by immunohistochemistry. We confirmed that the tumor cells produced albumin using qPCR. However, we demonstrated the expression of albumin in the extracellular matrix and cytoplasm of many other tumors using a tissue microarray containing 28 unrelated mesenchymal tumors. This was therefore not specific for any tumor type. In conclusion, we showed that the composition of the extracellular matrix of myxoid soft tissue tumors is heterogeneous and dependent on the type and histological grade of the tumor. Based on these results we propose an active, interacting role of glycosaminoglycans and albumin in the morphogenesis of these tumors.

OP14-2
GENE EXPRESSION IN PERIPROSTHETIC MEMBRANES FROM ENDOPROSTHESIS LOOSENING - CHITINASE ACTIVITY FOR EARLY DIAGNOSIS?

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Aim: The identification of markers for the early diagnosis of hip and knee endoprosthesis loosening, for the differentiation between wear-particle induced and septic loosening and of new

insights into the pathogenesis of endoprosthesis loosening. Methods: Gene expression profiles were generated from 5 periprosthetic membranes of wear-particle induced and 5 of infectious (septic) type using Affymetrix HG U133A oligonucleotide microarrays. The results of selected differentially expressed genes were validated by RT-PCR (n=30 periprosthetic membranes). The enzyme activity and the genotype of chitinase-1 were assessed in serum samples from 313 consecutive patients hospitalized for endoprosthesis loosening (n=54) or for other reasons, serving as control subjects (n=259). Results: 824 genes were differentially expressed with a fold change greater than 2 (data sets on http://www.ncbi.nlm.nih.gov/geo/ GSE 7103). Among these were chitinase 1, CD52, calpain 3, apolipoprotein, CD18, lysyl oxidase, cathepsin D, E-cadherin, VE-cadherin, nidogen, angiopoietin 1 and thrombospondin 2. Their differential expression levels were validated by RT-PCR. The chitinase activity was significantly higher in the blood from patients with wear-particle induced prosthesis loosening (p=0.001). However, using chitinase activity as a marker for early diagnosis, it has a specificity of 83% and a sensitivity of 52%, due to a high variability both in the disease and in the control group. Discussion: This study shows that gene expression analyses from histopathologically defined tissue samples deliver valid and interesting results when applied to non-tumor tissue like periprosthetic membranes, but that only a portion of the differentially expressed genes is worth further clinical examination and that only a minor portion will be beneficial for the clinical routine work.

OP14-3
BIOLOGICAL AND PROGNOSTIC ROLE OF CCN3 PROTEIN IN EWING'S SARCOMA TUMORS

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Background: CCN3 belongs to the family of CCN proteins which participate in fundamental biological functions. Deregulation of their expression has been associated with tumorigenesis. Structurally, CCN proteins share a common multimodular organisation with 4 structural modules resembling Insulin-like growth factor binding proteins, Von Willebrand factor, thrombospondin 1, and a series of matricellular proteins and growth factors that contain a cystin-knot motif, (IGFBP, VWC, TSP1 and CT). Expression of the full-length CCN3 protein is associated with differentiation in rhabdomyosarcomas, neuroblastomas, Wilms? tumors and chondrosarcomas, as well as with the inhibition of the growth of cell lines derived from Ewing's sarcoma (ES). The aim of this study was to evaluate the biological and the prognostic significance of CCN3 isoforms using a panel of antibodies that recognize different modules of the protein. Method: Paraffin-embedded material from 226 ES subtyped: 66% classic, 10% large cell, 10% PNET, 3% clear cell, 3% atypical, 6% spindle cell and 1% hemangioendothelial. Seven tissue arrays (TA) of these cases were performed and immunohistochemical expression of CCN3 was evaluated with five different antibodies recognizing different parts of the protein: anti-NH2 (IGFBP), anti-NH3 (VWC), anti-NH4 (TSP1), anti-NH5 and K19m (CT). Results: Follow-up was available from 132 patients with a median of 49 months (range: 1-306 months). CCN3 expression was variable in all cases depending on the antibodies tested. We observed that 36% of cases expressed a full-length CCN3 protein (immunoreactive for the 5 antibodies analyzed) and no differences between histological ES subtypes were found. Interestingly, expression of full-length CCN3 was correlated with the progression of ES, its expression being higher in recurrence and in metastasis than in primary tumors (70-60% vs. 40%, p=0.033). Univariate analysis for progression-free (PFS) survival and overall (OS) showed a trend towards prognostic significance of the expression of different CCN3 isoforms, especially those isoforms detected with the NH3 (p=0.111 for PFS and p=0.118 for OS) and NH4 (p=0.039 for OS) antibodies. Conclusion: Full-length CCN3 protein is expressed in a third of ES cases being more frequent in recurrences and metastasis than in primary tumors. The isoforms detected with NH3 and NH4 have a prognostic value, the biological significance of which needs to be better understood. * Supported with grants FIS P1040822, Spain and PROTHETS Contract number: 503036 FP6 from the EC.

OP14-4
EXPRESSION OF ALPHA-B-CRYSTALLIN IN NEOPLASMS DERIVED FROM MESENCHYMAL PERIVASCULAR EPITHELIOID CELLS (PECOMAS)

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Background: Alpha-B-crystallin (ABCryst) is a major protein component of the eye lens with chaperone-like functions and is a member of the small heat-shock protein HSP20. ABCryst expression has been demonstrated to varying extent in epithelial neoplasms, but not in mesenchymal tumors. Abnormal expression of ABCryst in hamartomas of tuberous sclerosis has been reported. The family of tumors derived from mesenchymal perivascular epithelioid cells (PEComas) includes angiomyolipoma (AML), lymphangioleiomiomatosis (LAM), "sugar" tumor of the lung, and abdominopelvic tumor/sarcoma of perivascular epithelioid cells. The aim of the study is to examine ABCryst expression in PEComas and to assess the value of this protein as a new tumor marker. Methods: All tumors derived from mesenchymal perivascular epithelioid cells were retrieved from our files (n=43). We performed immunohistochemistry using the monoclonal antibody for ABCryst (clone G2JF, Novocastra) in formalin-fixed, paraffin-embedded tumors. Tissue microarray containing a wide variety of neoplasms and normal tissues was also stained with ABCryst antibody. ABCryst cytoplasmic immunoreactivity was scored based on intensity (0-3+) and distribution (% of positively tumor cells). Cases showing 2+ or 3+ in >75 % of tumor cells were considered as positive. Results: There were 32 renal AML, 1 hepatic AML, 1 lung LAM, 2 lymph node LAM, 1 sugar tumor of the lung, and 6 PEComas. Twelve patients were male (27.9%) and 31 female (72.1%), with an average age of 55.6 yr (range 30-83 yr). All cases studied showed granular immunostaining in the tumor cell cytoplasm with the anti-ABCryst Ab. Cytoplasmic reactivity was noted in the spindle cells, adipocytes, and perivascular epithelioid cells. All cases showed strong (3+) or moderate (2+) cytoplasmic labelling, in a diffuse pattern (75-100% cells). Endothelial cells of the blood vessels were negative in all cases. No correlation was found between ABCryst score and age, sex, tumor size, nuclear atypia, necrosis, and mitotic rate. In all cases, appropriate positive internal control (Schwann cells) was assessed. Other tumors that demonstrated diffuse (> 75 %) and intense immunoreactivity included alveolar soft part sarcomas, astrocytomas, schwannomas, and TFE3 renal cell carcinomas. Conclusions: We have demonstrated ABCryst positivity in all tumors derived from mesenchymal perivascular epithelioid cells. ABCryst may be a useful ancillary marker for the diagnosis of these neoplasms when used in a panel with other melanocytic markers and SMA.

OP14-5

PATTERN OF EXPRESSION OF CELL CYCLE/APOPTOSIS REGULATORS REFLECT LOCATION, MUTATION AND PROGRESSION FREE SURVIVAL (PFS) OF GASTROINTESTINAL STROMAL TUMOUR (GIST) TREATED WITH IMATINIB FROM RANDOMIZED PHASE III TRIAL 62005 EORTC/STBSG

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Background: A randomized phase III trial reported PFS in advanced GIST treated with imatinib to be dependent amongst others on drug-dose and mutational status. Impaired expression of key-regulators of cell cycle/apoptosis such as p53, p16, p21, chek2, ccnd1, bcl2, cdk4 and mdm2 is crucial for tumour progression, might reflect secondary genetic/epigenetic events and impair the response to therapy. We studied how pattern of expression of these molecules might correlate with: 1. clinico-pathological parameters at the start of therapy, 2. mutation status and 3. ultimately PFS upon treatment with imatinib. Method: A tissue array made up of 4 cores of tumour tissue per each of 353 GIST patients enrolled in the phase III trial was built. All were histologically validated. Immunohistochemistry was performed to assess expression of the above-mentioned molecules. Results were correlated with clinico-pathological data and mutation status. Expression patterns were analysed with hierarchical clustering. Results: The site of origin was: stomach,118 patients, other parts of the gastro-intestinal tract, 175 patients (94 small bowel, 27duodenum). High expression was found for bcl2, p53, cdk4, mdm2, and ccnd1 in 78%, 47%, 31%, 13% and 8% of the cases, respectively; while low expression of p21, chek2, and p16 was found in 73%, 50% and 43% of the cases, respectively. High bcl2 expression was found in GIST originating from small bowel and duodenum and low expression in gastric ones. GISTs of gastric origin are also associated with low expression of p21, p16 and ccnd1. 268 (76%) GISTs disclosed mutations in KIT: 198 (56%) harboured exon 11, 58 (16.4%) exon 9, six (1.7%) exon 13 and three exon 17. Eight (2.3%) tumors carried PDGFRA mutations, while 36 (10.2%) cases were wild type.High bcl2 expression was associtated witjh KIT-exon 9 mutation. Low bcl2 and high cdk4 expression was more frequent in KIT-exon 11 ones. High level of p53 and low level of p16 expression were associated with shorter PFS in a multivariate model including already identified prognostic factors ($p<0.05$). Hierarchical clustering showed 4 different clusters characterized by different PFS ($p<0.05$). Conclusions: Advanced GISTs show a heterogenous pattern of expression of the studied molecules which reflects the accumulation of distinctive secondary events in tumour progression. A distinct pattern of expression correlates with location and tumor genotype. Maintenance of cell cycles/apoptosis regulation in GISTs is relevant for the optimal response to imatinib.

OP14-6

THE IMPACT OF HISTOLOGICAL GRADING IN PREDICTING OUTCOME OF HIV-ASSOCIATED KAPOSI'S SARCOMA

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Background: Although the incidence of Kaposi's sarcoma has decreased in the era of HAART, clinical outcome varies and a considerable subgroup of patients experiences a bad outcome. Little is known, however, about the impact of histological grading of Kaposi's sarcoma in predicting outcome. Aim of the present study was to analyze histological grading parameters in respect to clinical outcome. Method: Index biopsies of 89 patients with Kaposi's sarcoma prospectively included in the Swiss HIV Cohort Study from January 1996 to December 2004 were analyzed using the grading parameters defined by the French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading system, including tumor differentiation, mitosis count per 10 high power fields and tumor necrosis. Based on the summing score, tumors were graded in G1 (2 or 3), G2 (4 or 5) and G3 (6, 7 or 8). Survival and outcome (use of chemotherapy and/or death) were correlated with the histological data. Results: Based on the summing score, G1, G2 and G3 tumors were observed in 73.0%, 22.5% and 4.5%, respectively. Of the total 34 (38.2%) of 89 patients with clinically bad outcome, 13 (38.2%) had Grade 2 and 3 tumor compared to 11 (20.0%) of 55 patients with favourable outcome ($p=0.085$) and Grade 2 and 3 tumors were significantly associated with a bad clinical outcome ($p<0.02$). Analyzing individual histological parameters revealed that the presence of necrosis and the cytomorphology of tumor cells were not significantly associated with outcome. In contrast, mitosis count of ≥ 3 was seen in 16 (47.1%) of patients with bad and in 13 (23.6%) of 55 patients with good outcome ($p<0.05$) and increased mitotic activity was significantly associated with a clinical inverse outcome (Hazard ratio 2.65) (95 % CI 1.35-5.23). Conclusion: The FNCLCC scoring system for soft tissue tumors can be applied to Kaposi's sarcoma. However, due to the low incidence of tumor necrosis and the often minor cytomorphological differences of the tumor cells, only the mitotic activity shows a significant correlation with prognosis. Mitosis count of ≥ 3 is strongly associated with bad clinical outcome and should therefore be included in the histopathological report of Kaposi's sarcoma.

OP14-7

PRIMARY MALIGNANT VASCULAR TUMOURS OF BONE: IMMUNOHISTOCHEMICAL INVESTIGATION ON ENDOTHELIAL PHENOTYPE AND HISTOPATHOLOGICAL CORRELATION.

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Background : Primary malignant vascular tumours of bone (PMVTB) are rare and represent less than 1% of the primary malignant bone tumours. Because of their rareness little is known. Clinically they are extremely aggressive. Due to the lack

of uniform terminology and accepted histological criteria, terminology and classification of PMVTB has been highly controversial. Today, angiosarcoma is the most accepted term for high-grade PMVTB, as recognized by the 2002 WHO Classification. However, the classification of intermediate and low-grade vascular tumours is still controversial and not well-defined. Method: We collected 66 cases of paraffin embedded PMVTB diagnosed between 1967 and 2006. All clinical, radiological, and pathological data were reviewed and different histological criteria were assessed. A tissue micro-array was constructed containing the 66 cases with the addition of 6 malignant vascular tumours of soft tissue, 3 benign vascular lesions of bone and, 2 haemangiopericytomas of bone as controls. To confirm the vascular origin of all lesions, and to investigate the diagnostic value of commonly used markers, immunochemistry was performed for CD31, CD34, Factor VIII, and keratin AE1/AE3. Staining was evaluated as positive or negative. Result: The PMVTB show positive staining for CD31 in 62/66 (94%), CD34 in 36/66 (55%), Factor VIII in 53/66 (80%), and keratin in 47/66 (71%). Of the 47 keratin positive angiosarcomas, only 18 tumours (27%) showed an epithelioid phenotype at classical morphology. All tumours with an epithelioid phenotype are keratin positive. Conclusion: CD31 and Factor VIII are the best diagnostic markers in PMVTB, which is comparable to its soft tissue counterpart. A combination of these markers should therefore be used to diagnose these lesions. While CD34 is valuable in vascular lesions of soft tissue, in PMVTB its diagnostic value seems limited. It is striking that keratin positivity is seen in the majority of PMVTB and is independent of epithelioid morphology. Because, in addition, PMVTB is often multifocal, one should be aware not to misinterpret this clinically, radiological, and histological as metastatic carcinoma.

OP14-8

CLINICOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF MALIGNANT VASCULAR TUMORS: GLUT1 AND FKBP12 EXPRESSION IN MALIGNANT VASCULAR TUMORS

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Introduction: Recently, many new immunohistochemical markers, for instance FKBP12 and GLUT1, have been introduced to exhibit vascular differentiation in assistance to CD34 and CD31. In this study, besides a detailed clinicopathologic assessment, we evaluated expression profile of FKBP12 and GLUT1 among low and high grade malignant vascular tumors. Methods: We collected 13 angiosarcoma and 16 hemangioendothelioma cases from the archives of Hacettepe University, Department of Pathology. Slides were reviewed by two pathologists. Clinicomorphological analysis and immunohistochemical panel including CD34, CD31, GLUT1, FKBP12, MDM2, p53, CD99 and c-kit were performed. Expression pattern of each antibody is assessed regarding location, intensity and extent. Results: Age at diagnosis ranged between 6-77 years (median, 49 yrs). Mass and pain were the two common presenting symptoms. The preoperative duration ranged from 1 month to 2 years. Extremities were the most common primary site, following spine and head&neck. Tumor size, varied 1 cm up to 20 cm in diameter (median, 6 cm), was >3 cm in 20 cases, 12 of which were high grade. Liver and lungs were the commonest metastatic sites. Follow up information were available for 19 of 29 patients with a duration ranging from 1 month to 120 years (median, 36 mos). Five patients, 4 of which had low grade tumor, were alive for at least 5 years whereas 9 cases, 6 of which with high grade tumor, were dead of disease. Lymphedema, vascular stent, radiation and chemical exposure (dye) are investigated as etiologic factors in five patients. Twenty-five cases were available for immunohistochemical study. Immune expression pattern did not varied significantly between low and high grade tumors. Ninety-two percent and %80 of cases expressed CD34 and CD31, respectively. Only 35% of cases expressed GLUT-1 and expression was stronger in epithelioid cells, while hobnail cells were GLUT-1 negative. All cases expressed FKBP12 strongly except one low grade hemangioendothelioma, in which expression was weak. In 34.6% of cases, either MDM2 or p53 was positive, suggesting that p53/MDM2 pathway may be included in pathogenesis. CD99 and c-kit were both negative in all cases. None of studied markers has correlated with survival. Conclusions: FKBP12 may take a role in the diagnostic panel of malignant vascular tumors. GLUT1, although expressed only in one third of cases, reveals the epithelioid character of tumor, which is likely to suggest worse prognosis. p53/MDM2 pathway, at least in some cases, may take place in the pathogenesis.

OP14-9

EGFR SIGNALING IN GIANT CELL TUMOR OF BONE (GCTB): AN IMMUNOMORPHOLOGICAL TISSUE MICROARRAY (TMA) STUDY USING DIGITAL SLIDES AND DATABASE MANAGEMENT

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Background. Giant cell tumor of bone (GCTB) is a relatively rare tumor, which is considered “an aggressive, potentially malignant lesion” by the WHO classification. Local recurrence may occur in 20-50% of cases, with 10% undergoing malignant transformation and 1-4% giving metastasis mainly to the lung. GCTB is characterized by osteoclast accumulation causing lytic bone lesions, surrounded by reactive monocytic-histiocytic cells and potentially malignant fibro-osteoclat-like stromal cells. The pathogenesis and the factors behing GCTB progression are still largely unknown. Method. In this work, EGFR receptor expression of 138 primary GCTB cases, 56 recurrent tumors, and 6 GCTB transformed into osteosarcoma were studied. 210 pieces of 2 mm tissue cores were collected into tissue microarrays (TMA) using Mirax TMA engeneer (3DHISTECH, Budapest, Hungary). For EGFR immunostaining, sections were treated with Ventana Confirm (Tucson, AZ, USA; clone: 3C6) mouse monoclonal antibody and Novolink detection kit (Vision-Biosystems, Newcastle Upon Tyne, UK) following heat induced epitope retrieval using Tris-EDTA. Immunostained slides were digitalized using Mirax Scan (Zeiss, Gottingen, Germany) analysed and linked to database using the Mirax TMA software. Results. 47% of studied GCTB cases were either moderately (2+), or strongly (3+) positive for EGFR, further 25% were weekly positive and only 28% were fully negative. Immunopositive cells were usually adjacent to osteoclasts, and most were elongated consistent with the potentially malignant stromal cells. Recurrence and tranformation into osteosarcoma were significantly more frequent in strongly EGFR positive cases. Conclusion. About half of the studied GCTB cases are possibly under the influence of EGFR signalling, which may facilitate tumor growth and recurrence, and/or osteosarcoma transformation. Further studies of mutation hot-spots in EGFR gene, and phosphorylated downstream signalling elements are under way to dissect the possible role of EGFR in GCTB. (Supported by EuroBoNet)

OP14-10
CANONICAL AND NON-CANONICAL PATHWAYS OF OSTEOCLAST FORMATION IN GIANT CELL TUMOUR OF BONE
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Background: Giant Cell Tumour of bone (GCTB) is tumour that contains numerous scattered osteoclast-like giant cells and a mononuclear cell component of fibroblast/osteoblast-like spindle-shaped cells and macrophages. The cellular mechanisms underlying the accumulation of osteoclast-like giant cells in these tumours is not clear. The canonical pathway of osteoclast formation involves differentiation of mononuclear (macrophage-like) precursors into osteoclasts in the presence of receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage-colony stimulating factor (M-CSF). Abundant RANKL and M-CSF-producing cells have been noted in GCTB. Other growth factors are known to be present in abundance in GCTB, notably vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF). In this study we determined whether these growth factors might play a role in osteoclast formation in GCTB. Method: The (CD14-positive) monocyte/macrophage-like fraction of peripheral blood mononuclear cells (PBMCs) was isolated and cultured in the presence of RANKL +/- M-CSF, VEGF or HGF. Osteoclast formation was assessed by noting the formation of multinucleated cells which expressed osteoclast-specific markers including tartrate-resistant acid phosphatase (TRAP), vitonectin receptor (VNR), F-actin rings and formation of resorption lacunae on dentine slices. Results: It was found that numerous TRAP+/VNR+/F-actin ring- forming multinucleated cells capable of lacunar resorption formed in PBMC cultures where HGF and VEGF was substituted for M-CSF. The presence of these growth factors and their receptors was noted immunohistochemically in GCTB. Conclusion: Our findings indicate that VEGF and HGF may play a role in the extensive osteoclast formation that occurs in GCTB and that these factors may provide a therapeutic target to inhibit the extensive osteoclast formation and osteolysis that is associated with these tumours .

OP15-1
DEFICIENCY OF COMPLEMENT FACTOR D RESULTS IN MESANGIAL IMMUNE COMPLEX GLOMERULONEPHRITIS
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Factor D, a serine protease essential for the activation of the complement alternative pathway, is expressed in adipocytes, macrophages and the kidney. Mice mutants with a targeted deletion of the Factor D gene were used to characterize the role of Factor D in complement and immune complex mediated glomerulonephritis. We show that mice with targeted deletion of the factor D gene (Cfd-/-) spontaneously developed mesangial immune complex glomerulonephritis. In Cfd-/- mice, mesangial deposits that stained predominantly for C3 and IgM were detectable in 8-month-old animals. The mesangial deposits results in mesangial expansion and cellularity, the mesangial hypercellularity is due to increase in mesangial cells and not infiltrating leukocytes. The Cfd-/- mice also showed impaired renal function evidenced by the development of albuminuria and reduced creatinine clearance. In contrast to the mesangial location of the immune deposits in the Cfd-/- animals, age-matched mice lacking the alternative pathway inhibitory protein factor H (Cfh-/) developed immune deposits along the capillary wall. These observations suggest that factor D or alternative pathway activation is required to prevent the spontaneous accumulation of C3 and IgM within the mesangium. Cfd-/- mice represent a novel model of spontaneous mesangial immune complex glomerulonephritis.

OP15-2
COMPARISON BETWEEN WHO AND ISN/RPS CLASSIFICATION OF LUPUS NEPHRITIS, IS ANYONE BETTER THAN THE OTHER?. ANALYSIS OF DISCORDANT CASES.
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Introduction: Lupus nephritis (LN) is one of the most common and serious complication of systemic lupus erythematosus (SLE). Given the diversity of morphologic alterations grouped and named as LN, it has been challenging to create a morphologic classification with good clinical correlation. Several attempts have been done, the latest one by the ISN and RPS. Objectives: to compare the previous WHO histological diagnosis and classification of lupus nephritis kidney biopsies with that established with the new ISN/RPS classification, to identify discordant histological cases between the two classifications,and to establish if clinical outcome differences were present in those histologically discordant cases. Methodology: Cases with adequate histological material, and complete clinical and laboratory data were included. Eighty three renal biopsies were reviewed and reclassified according to the ISN/RPS. Renal survival, considered as patients free of renal replacement therapy, was compared in discordant cases only. Kaplan-Meier survival curves were used. Results: Eighteen discordant cases (21%) were detected, all women, mean age 33.7 years.When Renal survival curves are shown, class III cases of both, the WHO and the ISN/RPS classifications are compared, it is noted that in the former, the renal survival rapidly drops and patiens show a worse prognosis than with the ISN/RPS. If class IV cases in the WHO classification are compared, with the ISN/RPS, they have a longer survival than with the latter classification. The class IV cases in the ISN/RPS classification has a worse prognosis than that with the WHO classification. Conclusion: Approximately one fifth of the initial cases with LN had discordant results. Reclassified cases with the ISN/RPS have a better clinical correlation, according to renal survival, displaying a worse outcome for class IV cases and improved outcome in class III cases.

OP15-3
PROGNOSTIC UTILITY OF CYCLIN D3, CYCLIN E, P27, MUC1 IN RENAL CELL CARCINOMA (RCC)
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Background: Renal epithelial neoplasms are a heterogeneous group of tumors with different prognosis. RCC has an unpredictable behavior and tendency to recur and metastasize, and there can be major differences in clinical outcome even within the same tumor stage. Clear cell type (CRCC) is the most common histologic variant of RCC. The prognosis of the patients with CRCC is significantly worse than that of the patients with other histologic subtypes. Although there are clinicopathological parameters have proven their prognostic significance in primary RCCs, there is an utmost need of biological predictors for individual tumor behavior with respect to response to treatment,

survival rate and progression to metastasis. If the potential for early metastatic spread of RCC or tumor aggressiveness can be predicted reliably, additional novel treatment alternatives could be studied. Design: Immunohistochemistry was performed on tissue microarray sections of 83 cases of RCC; 62 CRCC, 6 chromophobe RCC, 13 papillary RCC, 2 unclassified RCC, using antibodies against CyclinD3, CyclinE, p27, MUC1. Clear and non-clear variants were differentiated by CK7 expression. Results: CK7 expression patern was significantly different between clear and non-clear variants. Prognostic factors identified in this study were high nuclear grade, regional lymph node (RLN) involvement, distant metastasis (DM), advanced clinical stage (cT), histological tumor necrosis, renal capsule invasion, severe inflammation, lymphovascular invasion (LVI) and multifocality (M). cT and LVI were independent prognostic factors in RCCs. M of the tumor and high MUC1 expression were correlated and both were associated with DM. CyclinE overexpression was correlated with LVI and DM and so with poor prognosis. A significant correlation was found between high CyclinD3 protein expression and DM which leads to poor survival. p27 expression was significantly correlated with tumor size and RLN involvement. Conclusions: Negativity of CK7 helps to differentiate CRCCs from non-clear RCCs. cT and LVI are strongly related to increased metastatic potential and biological aggresiveness, and reduced survival. p27 expression is correlated with tumor size and RLN involvement. The association of MUC1 overexpression with DM indicates a potential therapeutic target in RCC. CyclinE overexpression indicates the risk of DM with regard to LVI potential. For the first time it is shown that CyclinD3 protein is an independent prognostic factor in RCC; patients with high CyclinD3 expression have worse clinical outcome and short survival.

OP15-4

BOSENTAN AND LOSARTAN AMELIORATE ACUTE RENAL FAILURE ASSOCIATED WITH MILD BUT NOT STRONG NITRIC OXIDE BLOCKADE INDUCED BY L-NAME

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Background. Acute renal failure (ARF) is a devastating illness, especially when it occurs in various conditions with impaired nitric oxide (NO) synthesis. We have directed our investigations to effects of both angiotensin II (AII) and endothelin (ET) receptor blockade associated with mild or strong NO deficiency on haemodynamic, biochemical and morphological parameters in experimental postischaemic ARF. Methods. In this study, we used bosentan (dual, ETA/ETB-receptor antagonist), losartan (nonpeptide, competitive antagonist of type I angiotensin II receptor), and NG-nitro-L-arginine methyl ester (L-NAME, inhibitor of NO synthesis). Experiments were performed in anesthetized, adult male Wistar rats. The right kidney was removed and the renal ischaemia was performed by clamping the left renal artery for 45 minutes. Experimental groups received receptor antagonists (bosentan or losartan) or vehicle (saline) in the femoral vein 20 minutes before, during, and 20 minutes after the period of ischemia. Investigation was designed in two separately performed setups, with high (10 mg/kg b.m.) and with low (3 mg/kg b.m.) dosage of L-NAME given as i.v. bolus before each antagonist infusion. Haemodynamic (mean arterial pressure, heart rate, cardiac output, total vascular resistance and renal vascular resistance) and biochemical (urinary and plasma creatinine, Na+, K+, protein concentrations and creatinine clearance) parameters were measured 24 hours after reperfusion and compared with histological findings. Results. Our results showed that strong NO blockade overcame effects of both ET and AII receptor blockade in experimental postischemic ARF. In addition, the AII receptor blockade had harmful effect in this condition, probably due to disturbed autoregulatory renal function. On the other hand, ET and AII receptor blockade in mild NO blockade associated with reperfusion injury, improves the most haemodynamic, biochemical and morphological parameters: tubular necrosis was less widespread and predominantly focal, proximal tubular dilatation was slightly prominent, and casts formation was very rarely present. The loss of brush border of proximal tubular epithelium was less evident. Conclusion. We concluded that experimental postishemic ARF is neither AII nor ET mediated in case of strong NO blockade, but, in more realistic conditions of mild NO deficiency, these peptides represent significant players whose receptor blockade expresses relevant therapeutic potential.

OP15-5

NONINVASIVE DIAGNOSIS OF POLYOMAVIRUS ALLOGRAFT NEPHROPATHY BY ELECTRON MICROSCOPY ON VOIDED URINE SAMPLES

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BACKGROUND: Polyomavirus Nephropathy (PVN) is a serious complication after renal transplantation. Noninvasive screening tools currently in use for identifying patients at increased risk for PVN include quantitation of decoy cells and quantitative urine and/or plasma PCR analyses for BK DNA load levels. These tests, however, lack specificity for accurately separating patients with PVN from asymptomatic viral activators. Negative staining electron microscopy (nsEM) is a well established but largely forgotten noninvasive tool that has been routinely used for decades to search for viruses in various body fluids. The aim of our retrospective analysis was to determine whether the detection of cast-like, three dimensional polyomavirus clusters (named “Haufen”) in voided urine samples by nsEM could serve as a noninvasive strategy to accurately diagnose PVN. DESIGN: 89 urines from 14 patients with biopsy proven PVN (2-15 samples/patient over 18 months followup; study group) and 48 urines from 25 control patients with signs of polyomavirus activation but no PVN (1-5 samples/patient over 14 months followup; control group) were analyzed (urine samples by nsEM and cytology for decoy cell sheding; plasma and urine samples by quantitative PCR for BK virus DNA). RESULTS: A) PVN Study Group: At time of initial biopsy diagnosis, 100% of patients showed shedding of Haufen in the urine by nsEM, decoy cells (median: 50, range = 10-150), and positive urine and plasma PCR readings for BK virus. During followup (median=9 months; range=3-18 months), 7 patients overcame disease over 3-30 weeks with clearance of Haufen and decoy cells along with negative or markedly decreased urine and plasma viral load levels; 1 patient had a repeat kidney biopsy negative for PVN. 4 patients had persistent PVN with continuous shedding of Haufen, urine decoy cells, and positive urine and plasma viral load levels. B) Control Group (no PVN): No urine samples (n=48) showed Haufen by nsEM. All urine samples showed decoy cells (median= 30; range =10-100). 19 serum samples from 5 control patients showed persistent positive PCR readings for BK virus. Renal biopsies were performed in these 5 patients and did not show histologic evidence of PVN. CONCLUSION: nsEM of voided urine samples to search for cast-like polyomavirus aggregates, i.e. “Haufen”, is a highly specific, noninvasive diagnostic tool for identifying PVN (positive and negative predictive values of “Haufen” for PVN: >95%). nsEM is a fast, easily performed, and cost effective method that can be used to optimize patient management.

OP15-7
INFECTION BY BK POLYOMA VIRUS IN RENAL TRANSPLANT PATIENTS: CONTRIBUTIONS OF MOLECULAR BIOLOGY, URINE CYTOLOGY AND ELECTRON MICROSCOPY

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BK virus can become activated and cause nephropathy after kidney transplantation. The clinical goal is to detect and treat patients early enough to limit the amount of damage to the transplanted kidney. The aim of our study was to compare the relative contribution of molecular and morphological approaches in the diagnosis of BK virus associated nephropathy. Thirty seven plasma specimens from 28 renal transplant patients were tested by PCR for the presence of a BK genomic sequence, a test which has the advantage of giving technically unambiguous results. The results were compared with 56 urine cytology specimens obtained from the same patients, searching for decoy cells. Ten urine specimens from 8 patients had a positive urine cytology and 1 of these 10 had a positive BK plasma test. Twenty eight out of the 56 urine cytology specimens (50%) were reported as indeterminate. The plasma of 7 patients was positive by PCR. The result of the PCR tests was confirmed by restriction enzyme digestion. One out 7 of these patients had a positive urine cytology; the other 6 were reported as having an indeterminate or negative cytology. Electron microscopy of patients who had a renal allograft biopsy showed typical viral particles but was less sensitive than PCR testing. We conclude that molecular testing of viremia for the documentation of BK virus replication in renal transplant patients is more specific than the demonstration of decoy cells in urine cytology specimens, or electron microscopy of renal allograft biopsies. Urine cytology for decoy cells leads to a large number of indeterminate and false positive results.

OP16-1
DERMATOFIBROSARCOMA PROTUBERANS (DFSP): CLINICAL, MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 83 CASES.

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Background: The DFSP is a sub- cutaneous tumor characterized by a strong tendency to recur,rarely metastasizant, which may present under several morphological aspects. These are named variants: sclerosing, schwanian, myxoïd, with giant cells, pigmented, atrophic, myofibroblastic, and fibrosarcomatous. Method: Our series is composed by 83 cases of DFSP observed during 7 years coming from Surgical, Plastician and Orthopaedics units. A clinical list is elaborated containing some elements: age-sex-evolution- trauma-anterior biopsy –number of recurrences. Multiple sections of the tumor and their margins are realized. The microscopic study with haematoxylin-eosin staining evaluates some parameters: classical storiform pattern-presence of variants-quantification of these variants-vascular component-inflammation- necrosis- mitotic index- cellular density-cellular atypia. Secondary, an immunohistochemical study using a panel of antibodies with CD34 is achieved. Results: They show that DFSP affects young adults and also children. Physical and clinical aspects are preserved during a long time even with very evoluated tumors.The classical storiform pattern is the most often found, diffusely and intensively CD34 positive. It's followed by sclerosing, neuroïd, myxoïd which express sometimes CD 34 but less diffusely and less intensively. Rarely (6 cases among the 83), a fibrosarcomatous form of poorer prognosis and CD34 negative is found in variable proportions near typical areas. Conclusion: Although it may grow very slowly during several years, DFSP is an authentic sarcoma but often without malignant cytological criteria. Its habitual microscopic pattern is that knowed by storiform cartwheel pattern. Some of the variants which may be associated and sometimes be predominant have no influence on the prognosis but the fibrosarcomatous form will be searched by the analysis of multiple specimens. The study of these 83 cases aims to show the clinical , morphological and immunohistochemical particularities in an Algerian population.

OP16-2
COLLAGEN V NASAL TOLERANCE IN EXPERIMENTAL MODEL OF SYSTEMIC SCLEROSIS

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Objective: Our aim was to study skin remodeling and autoantibody production in an experimental model of Scleroderma (Ssc), following nasal tolerance with human type V collagen (Col V). Methods: Female New Zealand rabbits (N=12) were immunized with two doses of 1mg/ml of Col V in complete Freund's adjuvant and additional two boosters in incomplete Freund's adjuvant to induce Ssc. After 150 days, half of from these immunized rabbits were submitted to type V collagen-induced tolerance receiving a daily nasal administration of 25μg of Col V. Control animals (N=6) were only submitted to type V collagen-induced tolerance. Serial skin biopsies were performed at 0, 150 and 210 days, and stained with H&E, Masson's trichrome and Picrosirius for morphological and morphometric analysis. Types I, III and V collagen, TGF-β and PDGF were identified by immunofluorescence. The animals serum samples were collected to determine anti types I, III, IV and V collagen and antinuclear antibodies (ANA). Results: Skin biopsies from immunized animals confirmed Ssc morphology as previously described, such as progressive decrease of papillary dermis, appendages atrophy, increased type I, III and V collagen deposition and augmented expression of TGF-β and PDGF. Rabbits with Col V-induced nasal tolerance showed reduction of skin involvement, with significant decrease of collagen amount and lower TGF-βand PDGF expressions. Humoral immune response did not change with nasal tolerance. Conclusions: Col V nasal tolerance promotes regression of skin remodeling process in an experimental model of Ssc. We suggest that nasal tolerance with type V collagen can be a promising therapeutic option to treat Scleroderma patients.Financial Support: FAPESP,CNPq.

OP16-3
HHV8 IN KAPOSI'S SARCOMA AND OTHER VASCULAR LESIONS: COMPARISON OF IMMUNOHISTOCHEMISTRY AND CHROMOGENIC IN-SITU HYBRIDIZATION METHODS

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BACKGROUND: Kaposi's sarcoma (KS) is an angioproliferative disease occurring in several clinical and epidemiological subsets: classical (Mediterranean), endemic (African), post-transplant or iatrogenic immunosuppression associated and AIDS-related. Kaposi sarcoma associated herpes virus or human herpes virus 8 (HHV8) discovered in 1994, has been implicated as the most important etiological agent and its presence had been demonstrated in all types and stages of lesions. The aim of this study was to assess by two different methods, immunohistochemistry and chromogenic in-situ hybridization, the presence of HHV8 in KS and a group of vascular lesions that can be included in the differential diagnosis. The main purpose was to compare the sensitivity and specificity of the two methods. METHOD: Paraffin embedded archival tissue of 45 KS patients were included in the working group. Four patients had two lesions in different stages. A total of 49 KS lesions, 4 patch, 13

plaque and 32 nodules were analyzed. Control group consisted of 42 various benign and malignant vascular lesions and 3 cases of non-involved skin of KS patients. Monoclonal anti-HHV8 LNA (clone 13B10) and polymer detection system was used in immunohistochemistry. In-situ hybridization was performed using an oligonucleotide probe directed to T1.1 mRNA transcript. Visualization was achieved using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate as chromogens. RESULTS: All cases of KS were positive for HHV8 LNA with immunolocalization in the nuclei of spindle cells and cells lining vascular channels, whereas all vascular lesions and non-involved skin of KS patients were negative. Sensitivity and specificity was 100%. Positive reaction was detected in only 29 KS cases by in-situ hybridization and percentage of labeled cells was very low, around 1%. No reactivity was detected on the control group, except one case. Sensitivity and specificity for this method were 64.4% and 97.8% respectively. CONCLUSION: We concluded that immunohistochemistry for HHV8 LNA is a highly sensitive, specific and simple technique compared to chromogenic in-situ hybridization and can be a reliable adjunct to conventional histopathology for the diagnosis of Kaposi's sarcoma.

OP16-4
P63 EXPRESSION AS A NEW PROGNOSTIC MARKER IN MERKEL CELL CARCINOMA.

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BACKGROUND: Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin tumor, mostly affecting elderly individuals. Histogenesis of this rare tumor as well as prognostic criteria are a matter of dispute. Several prognostic indicators have been proposed for MCC. Gender (male), tumour size >5 cm or > 2 cm, location in the buttock, thigh, trunk or head & neck, advanced clinical stage, small cell size, high mitotic index, diffuse pattern as well as mutation of TP53 gene family, have all been reported as indicators of poor prognosis. The p63 protein is a member of the p53 family, and it can be frequently amplified and/or overexpressed in human tumours including carcinoma of head & neck, lung, skin, esophagus, mammary glands, urothelia, cervix, prostate glands, oral squamous cell carcinomas and in odontogenic tumours. Although p63 per se is not general indicator of poor prognosis, nevertheless it is often associated to poorly differentiated basaloid carcinoma. Recently Fukushima et al. suggested that p63 expression is a disadvantageous factor for prognosis in a subgroups of B-cell lymphomas. Our study will show that the same statement can be made in MCC. METHODS: An immunohistochemical analysis of markers of proliferation (Ki67/MIB1), neuroendocrine differentiation (chromogranin A and synaptophisin) and basal cell differentiation (p63) was performed in a series of 47 cases of MCC. Significance of pathological data and of immunoreactivity with different markers was evaluated by chi-squared test. Survival curves were calculated by Kaplan-Meyer method. The difference of survival was estimated using Wilcoxon or Mantel-Cox test. RESULTS: Immunohistochemical evidence of neuroendocrine differentiation and immunopositivity for cytokeratin 20 were observed in all cases, while p63 positivity (≥10 of neoplastic cell) was detected in approximately half (25/47; 53.2%) of the cases. Cases positive for p63 showed a more aggressive clinical course than the negative ones (Z =2.93; P=.0003; HR=22.22). CONCLUSIONS: Our data indicate that p63 expression is associated with a worse prognosis in MCC and it represents a new independent marker of clinical evolution with a better predictive power than the other prognostic parameters.

OP16-5
MELANOCYTIC TUMOURS IN CHILDREN AND ADULTS ARE GENETICALLY DIFFERENT

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Background: Childhood melanomas, although very rare, do occur and even expert pathologists can not always arrive at an unequivocal diagnosis. The reason is its similarity to the, in this age group more common, Spitz nevus. The etiology of melanomas at childhood is unclear. In adults, the majority of cutaneous melanoma and common nevi harbour BRAF and NRAS mutations, whereas HRAS mutations occur in Spitz nevi. The purpose of this study is to evaluate the use of mutation analysis of BRAF, NRAS, and HRAS genes in the differential diagnosis of melanocytic tumours in children. Method: We reviewed histology of 67 cutaneous melanocytic lesions from children younger than 16 years. DNA was isolated from formalin-fixed paraffin-embedded blocks. We sequenced BRAF (exon 15), NRAS (exons 2 and 3), and HRAS (exon 3) genes to detect hotspot mutations. Results: Lesions were grouped into seven different diagnostic categories: common melanocytic nevus (15), Spitz nevus (13), Spitzoid tumour of uncertain malignant potential (STUMP) (22), Spitzoid melanoma (6), non-Spitzoid melanoma (7), melanoma metastasis (2), and combined nevus (common nevus with an atypical Spitzoid component) (2). We detected a BRAF or NRAS mutation in all common nevi. No HRAS mutations were found in common nevi. In the other diagnostic groups, only sporadic mutations were found. In the (Spitzoid and non-Spitzoid) melanomas and melanoma metastasis no mutations were found, except for one NRAS exon 3 mutation in a non-Spitzoid melanoma, which had an adjacent nevus component. No mutations were detected in Spitz nevi. Only one STUMP had an HRAS mutation. Furthermore, one of the two combined nevi harboured a BRAF mutation. --Conclusion-- In contrast to melanocytic lesions in adults, melanomas in childhood do not (or only sporadic) have BRAF mutations and Spitz nevi less frequently have HRAS mutations. Therefore, mutation analysis is not useful in the differential diagnosis of Spitz nevus and (Spitzoid) melanoma in childhood, in contrast to adults. Other, undiscovered, gene abnormalities must be responsible for the development of melanoma and Spitz nevus in children. Remarkably, the only BRAF and NRAS mutations detected, outside the common nevi, are in lesions adjacent to a nevus component. One hypothesis is that only childhood melanomas that develop from a congenital or common nevus have NRAS or BRAF mutations and that this is an infrequent event in children; we speculate that most childhood melanomas, different from adults, probably arise via a different pathway (de-novo).

OP16-6
MELK IS A NOVEL TUMOUR PROGRESSION MARKER IN MELANOMA

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Background: MELK (Maternal Embryonic Leucine Zipper Kinase) is a member of the snf1/AMPK family of serine-threonine kinases; its specific targets in the normal cell have not been identified yet, and its role is therefore still elusive. However, several studies report an increased expression of MELK in various tumours (colon, breast, paediatric brain tumours) compared to normal tissues. The role of MELK in melanoma has

not been explored yet; in a previous gene expression profiling study , we found a significant difference in MELK expression between patient with and patients without distant metastasis within four years after diagnosis (J Natl Cancer Inst 2006; 98: 472-482); moreover, besides its prognostic significance, MELK mRNA-levels increased with increasing thickness of the primary melanoma. The aim of the present study was to assess the expression of MELK by immunohistochemistry (IHC) comparing normal skin, nevi, primary melanomas and metastasis of melanomas. Method. Immunohistochemical studies were performed either on frozen sections from 124 cases using a goat polyclonal antibody or on paraffin embedded material using a mouse monoclonal antibody against Melk. The 31 benign cases included 7 congenital and 15 acquired common nevi, 2 acral nevi, 2 common blue nevi, 1 cellular blue nevus and 4 Spitz nevi. The 84 malignant lesions included 57 primary and 27 metastatic melanomas. Nine cases of normal skin were also studied. The results were confronted with clinical data and mRNA levels. Results: MELK was not expressed in normal melanocytes and was only focally expressed in common acquired nevi; on the other hand, 2 acral , 3 Spitz , 4 congenital and 1 cellular blue nevus showed immunoreactivity. 33/57 primary melanomas and 15/27 metastases were positive for MELK. The percentage of positive melanoma cells increased with tumor thickness and was highest in the metastases. Surprisingly, MELK was expressed by endothelial cells lining intact vessels in 16/29 melanomas and in 7/11 melanoma metastases. Conclusion: Our data showed that MELK is a tumour progression marker in melanoma. Whether it is of prognostic significance at the protein level needs further study. In line with other members of the AMPK family, MELK expression in endothelial cells may suggest a role in tumour angiogenesis .

OP17-1
GENE EXPRESSION PROFILING OF TFE/MITF RENAL CELL TRANSLOCATION CARCINOMAS

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Objective : In order to determine the gene expression profiling of TFE translocation renal cell carcinomas (RCC). Patients and methods : Frozen samples of 4 primary tumor specimens with a histological diagnosis of TFE-translocation RCC were obtained. Diagnosis was made according strong and specific nuclear immunohistohemical expression of TFE3 (3 cases) or TFEB (1 case). Institutional Review Board approval was obtained from each participating institution. 48 cases of renal carcinomas that were representative of their respective subtypes based on both histology and gene expression were selected to enable comparison of the gene expression of the TFE translocation RCC with other renal tumors. The reference set included 11 cases of clear cell, 7 cases of chromophobe, 11 cases of papillary type 1, 6 cases of papillary type 2, and 13 cases of urothelial RCC. In addition, we included 7 cases of Oncytoma and 13 samples of normal kidney. Total RNA extraction, RNA quality and oligonucleotide expression profiling were assessed as previously described. All statistical analyses were performed using the R statistical environment utilizing Bioconductor project version 1.9. Unsupervised clustering was performed based on the Euclidean distance between each case in terms of gene expression. Genes that were differentially expressed between the samples of TFE translocation RCC and our reference samples were identified using the Limma package from the Bioconductor project. Results: From our clustering analysis against 6 other subtypes of kidney tumors and normal kidney, we demonstrated that the three TFE3 translocation and the TFEB translocation cluster each closer to each other than to others renal tumors suggesting that this group of tumors could be further divided based on their molecular signatures. The main genes over expressed were TRIM63 (tripartite motif-containing 63, average fold change 107,54), Cytochrome p450 17A1, hyaluronan binding protein 2, integrin beta 1 binding protein 3, and synaptic vesicle glycoprotein 2B (over expressed). Zinc finger protein 204 cytochrome p450 2B6, MUC1 and CAXII (average fold change 0,04) were under expressed. Conclusion : Based on gene expression profiling, we confirmed that TFE3 and TFEB translocation RCC should be considered as one distinct group of RCC. The genes we identified as distinct from other subgroups of RCC represent potentially novel diagnostic makers, although further validation studies are warranted.

OP17-2
CELL SURFACE PEPTIDASES CD13 AND CD10 IN RENAL CELL NEOPLASMS.

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Background: the immunoexpression of cell surface peptidases, such as the metallopeptidase/CD10 in renal cell neoplasms have been found to be of diagnostic value among renal cell neoplasms. The aminopeptidase N/CD13 has been reported in some renal cell neoplasms, however little is know about its immunoexpression according to different histotypes of renal cell neoplasms. The current study attempts to evaluate and compare the CD13 and CD10 immunohistochemical staining in a large series of renal cell neoplasms. Method: we investigated 100 renal cell neoplasms, including 38 clear cell, 16 papillary, 17 chromophobe renal cell carcinomas (RCCs) and 14 renal onocytomas by CD13 (38C12, Novocastra) and CD10 (56C6, Novocastra) immunohistochemical expression. Results: a membranous staining pattern for CD13 was observed in 31/38 (81%) (19/38, 50% in >50% of neoplastic cells), and 11/16 (68%) (9/11, 81% in >50% of neoplastic cells) papillary RCCs. No staining was observed in all chromophobe RCCs and renal oncocytomas. CD10 was observed in 36/38 (94%) clear cell and 14/16 (87%) papillary RCCs; CD10 was also detected in 9/17 (52%) of chromophobe RCCs and in 7/14 (50%) (with patchy positive staining in 4/14, 28% and in 3/14, 21% with a microluminal and single cells stain) renal oncocytomas. Conclusion: we concluded that: 1) CD13 is a relatively sensitive and specific marker for clear cell and papillary RCCs; 2) CD13 is always absent in chromophobe RCCs and renal oncocytomas, whereas CD10 can be immunoexpressed in both chromophobe RCCs and renal oncoytomas; 3) CD13 should be included in a panel of antibodies to distinguish clear cell (most CD10+, CD13+) from chromophobe RCCs (most CD13-, CD10+/-).

OP17-3
MOLECULAR MORPHOLOGY IN RENAL ONCOCYTOMA: EVALUATION OF CYCLIN D1 IMMUNOEXPRESSION AND 11q13 INTERPHASE CYTOGENETIC REARRANGEMENTS.

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INTRODUCTION: cyclin D1 has been immunohistochemically demonstrated in approximately one-half of renal oncocytomas and CCND1 gene maps near to chromosome 11q13. Cytogenetically renal oncocytomas fall into three categories, namely characterized by normal karyotype, numerical or structural abnormalities on chromosome 1 and or 14, often with Y chromosome loss, and structural abnormalities of 11q13. We south to identify any correlation between cyclin D1 immunoexpression and different 11q13 structural rearrangements in renal oncocytoma. MATERIAL AND METHODS: we evaluated the cyclin D1 immunoexpression in 13 renal oncocytoma (11 with a solid-acinar and 2 with a tubulo-cystic pattern) and performed an interphase cytogenetic fluorescence in situ hybridization study, using a SpectrumGreen locus specific probe spanning to 11q13 on fomalin-fixed-paraffin embedded tissue. Fluorescence signal count was scored on 150 neoplastic nuclei. RESULTS: cyclin D1 was immunoexpressed in 10/13 (76%) renal oncocytomas (8/11 with solid/acinar and 2/2 with ubulo-cystic). Fluorescence in situ hybridization showed three cases (23%) with a 11q13 rearrangements, showing more than two signals for 11q13 probe (from three to eight signals) in most of neoplastic cells (>55%), and two of them immunoexpressed cyclin D1. Seven renal oncocytomas (70%) with cyclin D1 immunoexpression did not show any 11q13 genetic rearrangements. CONCLUSION: our study shows that 1) cyclin D1 is immunoexpressed in most renal oncocytomas without any difference between solid/microcystic patterns; 2) there is no correlation between cases with 11q13 genetic rearrangement with those with cyclin D1 immunoexpression; 3) different breakpoints near the CCND1 gene locus could explain different cyclin D1 immunoexpresion in renal oncocytoma.

OP17-4
PROSTATE ADENOCARCINOMA: IMMUNOHISTOCHEMICAL EVALUATION AFTER HIFU TREATMENT.

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Background: High Intensity Focused Ultrasound (HIFU) is a minimally invasive alternative treatment for prostate adenocarcinoma. High energy converging ultrasound waves induce a rise in temperature and subsequent destruction of target tissue. Necrosis and fibro-sclerosis are histological findings after HIFU treatment. Little is known about the evaluation of residual neoplasia in patients who received HIFU treatment. Residual prostate neoplasia is the most important point in the pathologist-report. We performed an immunohistochemical analysis to detect the presence of residual foci of prostate adenocarcinoma after HIFU treatement. Method: We tested 11 cases (7 from agobiopsies and 4 from chips obtained by trans-urethral resection) with morphologically residual neoplastic foci of prostate adenocarcinoma after HIFU treatment, using immunohistochemically the following antibodies: racemase, p63, cytokeratin 34βE12, PSAP, cromogranine, PSA and CD56. Cytokeratin 8-18 was performed to test material quality. Racemase expression was scored as "0" when negative, "+" when weakly or focally positive (in less than 10% of the cells), "++" when diffuse or strongly positive (in more than 10% of the cells). Results: Cytokeratin 8-18, PSAP and PSA where positive in almost all cases in both neoplastic foci and in normal tissue. p63 and cytokeratin 34βE12 marked the basal layer in normal prostatic glands tissue, while where absent in neoplastic glands. We found rare cells with staining for CD56 and cromogranine in four cases. Racemase was expressed in seven cases (in 3 "++" and in 4 "+") and negative in two cases. Two cases showed too small residual foci for a good quality evaluation. Conclusion: Immunohistochemistry is a useful tool to detect prostate neoplastic residual foci on biopsy or chips after HIFU treatment. A panel including neoplastic positive markers such as racemase and negative markers such as p63 and cytokeratin 34βE12 may confirm the presence of neoplastic glands.

OP17-5
LOSS OF EXPRESSION OF CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE-1 (CEACAM1) IN PROSTATE INTRAEPITHELIAL NEOPLASIA (PIN) AND INVASIVE PROSTATE CANCER INDUCES ANGIOGENESIS

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Background: Tumor growth and metastasis essentially depend on a sufficient neovascularization via angiogenesis, which is regulated by angiogenic activators and inhibitors. We previously showed that soluble human CEACAM1 exhibits angiogenic properties. It has been known, that mRNA of CEACAM1 is downregulated in several tumors, so a tumor-suppressive role of CEACAM1 has been postulated. Methods: prostates specimen were fixed in formalin or Bouin's solution (for electron microscopy) and embedded in paraffin. Human prostate cancer cell line DU-145 were cultured until 70% confluence. The cDNA encoding human full-length CEACAM1 was ligated into the plasmid pcDNA3.1/Hygro(-) (Clontech) and used for CEACAM1-overexpression studies. For CEACAM1 silencing, we used the siRNA technique. From the cell lysates, proteins were extracted and used in SDS-Page. From extracted RNA, qRT-PCR were performed on LightCycler Instrument (Roche) using the fluorescent SYBR-Green. Immunhistochemical stainings on prostate specimen were performed and on serial-sections, the CD34-positive and CEACAM1-positive blood vessels adjacent to the normal prostate gland and to tumor (PIN and invasive cancer) were counted separately. HDMEC's (human endothelial cells) treated with VEGF alone, with conditioned media of CEACAM1-verexpressing or CEACAM1-silenced DU 145 cells alone or the latter both together with VEGF were used to perform in vitro wound assay. Conditioned media of empty vector transfected and of luciferase-silenced DU-145 cells alone or in combination with VEGF were used as control. Results: We demonstrate here that epithelial CEACAM1 down-regulation in high-grade PIN and in invasive prostate cancer is inversely correlated with its up-regulation in adjacent blood vessels. CEACAM1 silencing in prostate cancer cell line DU-145 via siRNA increased but its overexpression suppressed the expression of angiogenic/lymphangiogenic factors such as VEGF-A, -C and –D, and the angiogenesis inhibitor collagen 18/endostatin. Furthermore, CEACAM1 overexpression in DU-145 cells increased but CEACAM1 silencing reduced angiopoietin-1 expression. Inverse relation was found for angiopoietin-2. Supernatant of CEACAM1-overexpressing DU-145 suppressed but that of CEACAM1-silenced increased the VEGF-induced endothelial tube formation. Electron microscopically, the majority of tumor-associated blood vessels were structurally destabilized, exhibiting endothelial fenestrations, trans- and inter-endothelial gaps. In some areas invasion of single tumor cells into the destabilized blood vessels were observed. Conclusions: These data show that disappearance of epithelial CEACAM1 in PIN and invasive prostate cancer is accompanied by its up-regulation in adjacent vasculature, which apparently correlates with vascular destabilization and increased vascularization of prostate cancer. Strategies to either conserve the epithelial CEACAM1 or to target endothelial CEACAM1 might be useful for anti-angiogenic therapy of prostate cancer.

OP18-1
MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITORS EXPRESSIONS IN INVASIVE CUTANEOUS MELANOMAS

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Background: Primary cutaneous melanomas are characterized by their tendency to metastasize. Essential steps in this process involve the degradation of basement membranes and remodelling of the extracellular matrix by proteolytic enzymes such as matrix metalloproteinases and their tissue inhibitors. In this study; we investigated the relationship between histopathological parameters and immunhistochemical expressions of the matrix metalloproteinases and their tissue inhibitors in primary invasive cutaneous melanomas. Materials and Methods: Seventy primary cutaneous invasive melanoma cases were re-evaluated for histopathological parameters including Breslow thickness, Clark level of invasion, ulceration, histological subtypes, mitotic rate, tumor-infiltrating lymphocytes (TIL), vertical and radial growth phases. Furthermore; immunohistochemical expressions of MMP-2, MMP-9, TIMP-1 and TIMP-2 have been evaluated by using staining intensity and percentage. Results: There was a statistically significant relationship between Breslow thickness, Clark level, mitotic rate, ulceration. However, there was no correlation between histologic subtypes and other histopathologic parameters. MMP-2 expression was correlated with Breslow thickness and tumor ulceration. There was also significant relation between MMP-9 expression and Breslow thickness. Although TIMP-1 was not correlated with any known histopathologic parameters, TIMP-2 was significantly correlated with mitotic rate and TIL. Although there was a statistically correlation between TIMP's, there had not been determined any statistical expressive correlation between MMP's and TIMP's. Conclusion: MMP-2 and MMP-9 expressions were correlated with Breslow thickness in primary invasive melanomas; which is support the relation with invasion.

OP18-2
OVER-EXPRESSION OF THE HEMIDESMOSOMAL ADHESION PROTEIN COLLAGEN XVII IN MALIGNANT MELANOMA

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Background: Collagen XVII (BP180, BPAGII) is a transmembrane protein anchoring proliferating basal keratinocytes to the underlying basal lamina in normal stratified epithelia. Collagen XVII is lost during normal keratinocyte maturation, however, it is still expressed in precancerous and cancerous epithelial lesions primarily of squamous cell origin as a sign of dedifferentiation. In this study we tested the potential neo-expression of collagen XVII in melanocytic tumors. Method: We produced mouse monoclonal antibody clones against the synthetic oligopeptide sequence aa542-564 of collagen XVII. Archived tissue blocks of 9 normal skins, 22 dysplastic- and 20 Spitz nevi, 83 primary melanomas (35 surface spreading, 33 nodular, 12 acrolentiginous and 3 lentigo maligna type) and 18 metastatic melanomas were tested for collagen XVII immunostaining using a non-biotin polymer detection EnVision+. Human melanoma mouse xenografts and melanoma cell lines in culture were also studied. Immunostains were analysed on digital slides scanned using Mirax Scan and the HistoQuant software(3DHITECH, Budapest & Zeiss, Gottingen, Germany). Results: Antibody clones 6D1 and 9G2 were selected out of 300 based on collagen XVII antigen specificity confirmed with ELISA and Western immunoblotting, and detectability of this antigen both in native frozen and routinely archived paraffin tissue sections following heat induced epitope retrieval. The presence of collagen mRNA in melanoma cell lines A2058, HT168M1, WM35 and WM983A was confirmed with RT-PCR technique. Substantial amount of collagen XVII protein was detected in paraffin sections of normal basal keratinocytes and primary and metastatic melanomas either in human tissue or in human melanoma mouse xenografts. When positive staining was defined by over 40% of tumor cell staining, the tested antibodies showed the following efficiency: 1) Collagen XVII 85% in melanomas; 6% in nevi; Melan A 87% in melanomas; 92% in nevi; S-100 94% in melanomas and 90% of nevi; HMB-45 72% in melanomas and 68% of nevi. In many instances collagen XVII staining was elevated on the tumor front in both primary and metastatic cases, and stained melanomas which were negative for HMB-45. Conclusion: This is the first study to show that immunodetection of collagen XVII protein, which is not associated to melanosomes or melanin production, 1) can be an efficient marker of malignant melanomas; 2) may be a useful adjunct in differentiating benign and malignant melanocytic lesions; and 3)may be positively linked to metastatic melanoma progression. Supported by OTKA K62758

OP18-3
THE ELASTIC TISSUE CHANGES IN GRANULOMA ANNULARE: A STUDY OF THIRTY CASES

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Background: Granuloma annulare (GA) is a common skin disorder, characterized by mainly two types of inflammation patterns which are interstitial and granulomatous. In the past; focal degeneration of dermal collagen fibers had been reported for this disease. However; recent studies also have been emphasized degeneration and fragmentation in elastic tissue, Objective: In this study we aimed to examine histopathologic characteristics and elastic tissue changes in GA. Material and methods: The hematoxylin and eosin-stained slides of the thirty patients which are diagnosed as GA between 1994-2007 were re-examined. The new serial sections of formalin fixed paraffin embedded blocks were stained with Verhoeff-van Gieson and Alcian Blue. All slides were examined by an experienced pathologist. Results: In the study population, 17 patients were female (70.8 %), 7 were male (29.2%). Their ages ranged from 4-72 years with a mean of 39.5. The biopsied lesions were localized on the leg (8/24), hand (6/24), arm (5/24), foot (1/24), shoulder (1/24), nape (1/24), trunk (1/24), arm and leg (1/24). Out of 30 patients, we were not able to reach 6 patients age, gender and biopsied lesion localizations. In hematoxylin and eosin slides, we observed 21 interstitial, 8 granulomatous and 1 both granulomatous and interstitial inflammation patterns. In 26 of 30 (86.7%) biopsy specimens, mucin was detected with Alcian-Blue staining. We observed elastophagocytosis in only 1 (3.3%, n=30) specimen, whereas giant cells were in 11 (36.7%, n=30). Elastic and collagen fiber fragmentation were seen in 28 (93.3) biopsy specimen. There was no remarkable changes in both elastic and collagen fibers in case 24 and 25. Additional histologic findings included vasculitis (8/30, 26.7 %) and necrosis (7/30, 23.3 %). Conclusion: Sunlight exposure has been considered as an etiologic factor in the pathogenesis of granuloma annulare. Furthermore, some authors concluded that degenerating elastic fibers may promote the granulomatous reaction seeing in GA. In

this study, on microscopy, elastic fiber degeneration was seen in almost all cases. All cases showed degenerated elastic fibers were surrounded by intact collagen fibers. These findings showed that the main alteration in GA is elastic fiber degeneration and strongly suggest that development of this disorder was injury to the elastic tissue.

OP18-4
THE NOTCH SIGNALING IN HUMAN SKIN CANCER

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BACKGROUND: The Notch pathway acts through the regulation of cell proliferation, migration, and apoptosis, with opposing outcomes that may result in oncosuppression or protumoral effects, depending on the cellular and tissue context. In the mouse skin, Notch-1 coordinates differentiation of squamous epithelia and deletion of Notch 1 results in hyper-proliferation of the basal epidermal layer, and development of skin tumors. However, little information is available on the level of expression of Notch-1 and its ligands, in precancerous lesions and invasive carcinomas in human skin. METHOD: The expression of Notch 1, Jagged 1, Jagged 2 and Delta-like 1 proteins was investigated by immunohistochemistry in a series of premalignant and invasive cutaneous carcinomas: 2 solar keratoses (SK); 4 Bowen's disease (BD), 4 squamous cell carcinomas (SCC) on sun-exposed skin, 6 SCC on sun-protected genital skin, and 14 basal cell carcinoma (BCC) of different histotypes (nodular, superficial type, sclerodermiform and baso-squamous). RESULTS: Expression of Notch 1 was decreased in solar keratoses and invasive SCC localized in sun-exposed sites. In contrast, marked Notch-1 staining was observed in extragenital Bowen disease as well as in genital HPV-related in situ and invasive SCC. Notch-1 was diffusely expressed in nodular and superficial BCC while sclerodermiform and baso-squamous BCC showed a low to absent Notch 1 expression. Jagged 1, Jagged 2 and Delta-like 1 proteins were expressed in all the tissues examined. CONCLUSION: Present findings suggest divergent expression of Notch-1 in cutaneous SCC depending on anatomical site: In UV-related squamous cell photocarcinogenesis Notch functions as tumor suppressor; in contrast, in sun-protected HPV-related SCC, Notch-1 is upregulated. Finally, Notch 1 expression is downregulated in BCC subtypes correlated with risk of recurrence (sclerodermiform and baso-squamous) in comparison with more common nodular and superficial-type subtypes.

OP18-5
THE RELIABILTY OF MULTIPLEX PCR AND HETERODUBLEX ANALYSIS USING BIOMED-2 PRIMERS ON MYCOSIS FUNGOIDES DIAGNOSIS

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Background: Mycosis Fungoides(MF) is the most frequently seen cutaneus T cell lymphoma. The morphological and clinical variability in the presentation of the disease can create diagnostic difficulties. The early phases of the disease can mimick inflammatory lesions both with their morphology and immunophenotype. Although demonstrating the clonal T cell proliferation does not always imply the neoplastic nature of the lesions, the previously performed molecular methods were less sensitive in detection of T cell clonality. Our aim in this study is to examine the efficiency of T cell clonality on differentiation of the inflammatory skin lesions from MF cases showing different morphological features and monitorisation of the therapy response. Method: We examined 15 MF (mean 53 years of age, 11 male, 4 female) cases showing characteristic morphological and immunophenotypic features, 8 suspicous MF (mean 61 years of age, 6 male, 2 female) cases. Post therapy control biopsies of 7 of 15 MF cases were also examined. Ten skin biopsies of licken planus lesions were included for polyclonal negative control. BIOMED 2 primers were used for detection of clonal TCR beta, gamma and delta rearrangements. Multiplex PCR with heterodublex (HD) analysis was performed for the molecular detection of T cell clonality. Results: Clonal TCR gene rearrangement were not demonstrated on licken planus. All of the MF lesions and 50% of the suspicious MF lesions showed clonal TCR gene rearrangements. 2 of 7 suspicious lesions were mimicking MF by their morphology but composed of predominantly CD8 positive lymphoid infiltration were within the clonal group. Conclusion: Multiplex PCR and HD analysis by using Biomed 2 primers are highly sensitive methods for detecting the monoclonal T cell proliferations in skin lymphoproliferative diseases. Molecular results were not enough for differential diagnosis of neoplastic and reactive lymphoid lesions. Combination of the molecular results with morphological, immunophenotypic and clinical features are essential in the interpretation of the behaviour of these lesions.

OP19-1
REGENERATIVE POTENTIAL OF CIRRHOTIC LIVER FOLLOWING VASCULAR LIGATION IN EXPERIMENTAL ANIMALS

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Preoperative occlusion of hepatic inflow vessels increased the volume and function of the contralateral lobe. We aimed to compare, biochemically and histopathologically, the effects of selective ligation of portal vein branch (PVL) alone or with hepatic artery branches (HAL) in cirrhotic liver on liver functions and regeneration. This study was carried out in the experimental unit of TBRI on 40 adult male dogs. Preoperative liver cirrhosis was induced in all animals.They were divided into 4 groups: Group1, in which laparotomy was done without any surgical manipulation; Group2, in which occlusion of portal vein branch only was done; and Group3, in which occlusion of portal vein and hepatic artery branches were done simultaneously (at the same time) & Group 4, in which occlusion of hepatic artery was done 48 hours after portal vein ligation. Blood samples for liver profile and wedge liver biopsies were taken from all animals . Hepatic regeneration and portal angiogenesis were determined. Also, tissue expression of TGF-β1 was established by immunohistochemistry. No significant biochemical differences could be detected between Groups 2,3 & 4 except for AST. By histology, significant regeneration was observed in group 4 ($p < 0.05$) which was more prominent than groups 2 & 3. There was significant portal angiogenesis in the non ligated lobes of all groups and significant decreased expression of TGF-β1 ($p < 0.001$) with no statistical difference between all groups. Extra-hepatic vascular exclusion with a little preference for PVL plus HAL in a heterchronous manner (Group 4) in induced cirrhotic liver is safe and efficacious. This improve survival rate and outcome of hepatic resection for liver diseases.

OP19-2
CLAUDIN 1, 3, 4, 5 AND 7 IN ESOPHAGEAL CANCER: LOSS OF CLAUDIN 3 AND 4 EXPRESSION IS ASSOCIATED WITH METASTATIC BEHAVIOR

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Background: Claudins are tight junctional proteins known to play a role in normal tissues and cancers.To elucidate their significance in esophageal cancer we analysed their expression in cases of surgically treated esophageal carcinoma. Methods: The expression of claudin 1, 3, 4, 5 and 7 was studied by immunohistochemistry. Tumor proliferation was assessed with Ki67 immunostaining and apoptosis by the TUNEL method and immunostaining of fragmented caspase 3. Results: Adenocarcinomas showed significantly more cases with moderate or strong claudin 3 ($p<0.001$) and claudin 5 positivity ($p=0.031$) than squamous cell carcinomas. Loss of claudin 3 expression was associated with the presence of distant metastases ($p=0.039$). Claudins 3, 4 and 7 had a significant association with either a high apoptotic index or a high number of caspase 3 positive cells, while claudin 5 was associated with increased proliferation. Conclusion: In esophageal carcinoma, claudin expression may vary along with the histology of the tumor. Claudin expression may also associate with apoptosis or proliferation, suggesting that they may contribute to tumor behavior and growth.

OP19-3
PROGNOSTIC IMPACT OF CLASS I HISTONE DEACETYLASES IN COLORECTAL CANCER: SPECIFIC ROLE OF HDAC2

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Post-translational acetylation of core histones by histone deacetylases (HDACs) play an important role in the regulation of activity and expression of a large number of genes intimately involved in the initiation and progression of cancer. Consequently, a variety of HDAC inhibitors have recently been validated as promising anticancer agents and are now entering clinical trials. However, the tumor specific function and expression of specific HDAC isoforms is still incompletely understood. In this study, we determined class I HDAC isoform expression in 140 colorectal carcinomas by immunohistochemistry and correlated our findings with clinicopathological parameters and patient survival. In addition, effects of unspecific HDAC inhibition by valproic acid (VPA) and suberoylanilide hydroxamic acid (SAHA) as well as specific HDAC isoform knockdown by short interfering RNA (RNAi) on cell number and cell cycle were investigated in the colon adenocarcinoma cell line CX-2. We found Class I HDAC isoforms to be highly expressed in a subset of colorectal carcinomas (HDAC1:36.4%, HDAC2:57.9%, HDAC3:72.9%). High expression of HDAC isoforms was pronounced in strongly proliferating dedifferentiated tumors and implicated reduced patient survival, with HDAC2 being an independent survival prognosticator. Unselective inhibition of HDACs by both, VPA and SAHA as well as selective inhibition of HDAC2 and to a lesser extend HDAC1 but not HDAC3 depleted growth of CX-2 tumor cells in vitro, partly by initiating specific cell cycle alterations. In conclusion, we showed that long known in vivo effects of HDACs on differentiation and proliferation could be confirmed in human cancer tissue. Furthermore, we proved that class I HDAC isoforms are differentially expressed and highly prognostic in colorectal cancer. This may influence response to HDI treatment, which should be taken into account when clinical trials are being planned and carried out.

OP19-4
GASTROINTESTINAL STROMAL TUMORS: RECENT RESULTS OF A NATIONWIDE DATABASE INCLUDING 1008 CASES WITH HISTOPATHOLOGICAL AND IMMUNOPHENOTYPICAL FEATURES

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Background:Gastrointestinal stromal tumors (GIST) which are the most common mesenchymal tumors arising from the gastrointestinal tract, omentum, and retroperitoneum, are known for their wide variability in biologic behavior and for the difficulty in determining their malignant condition. In this multicenter study, 1008 Turkish patients with GIST, were reviewed in order to determine their histopathological features and immunohistochemical profiles. Design: Working Group of GIST in Turkey, developed a nationwide database, including the standardized gross and microscopic features as well as the results of immune antibody panel including CD117, CD34, Desmin, Smooth Muscle Actin (SMA), S-100 protein, and Ki67. The risk of aggressive behavior of the tumors was calculated according to the NIH consensus statement of 2001. Statistical analysis was performed in 1008 cases after the elimination of cases with incomplete data. Pearson correlation analysis was used for describing the linear association between each pathologic features and immunohistochemical expressions. Kruskal Wallis, Mann Whitney U and Spearman tests were performed for nonparametric values. Results:1014 GIST cases were registered into the database by 29 centers from Turkey. In 1008 cases, male to female ratio was 1.24, mean age was 56.45 and 79.14% of them were more than the age of 50 years. The most common location was stomach (42.4%) followed by small intestine, omentum-peritoneum, large intestine, and esophagus (31.1%, 13.1%, 9.5%, 1%, respectively). The risk groups consisted of 5.9% very low, 20.5% low, 18.9% intermediate and 52.2% high risk cases. There were significant relationships ($p=0.01$) between each of the mean size, number of mitoses and Ki67 index. Many other histopathologic features like location, cell type, cellularity, cytologic atypia, infiltrative growth pattern, necrosis, hemorrhage, ulcer and distant metastasis were positively correlated with increasing risk ($p<0.001$). CD117 was positive in 94.7% of GISTs but no significant relation was found between CD117 expression and tumour location and risk groups. CD34, SMA and Ki67 expressions significantly varied in different locations ($p<0.001$). A similar difference was observed in CD34, SMA, Desmin, Ki67 expressions in relation to risk groups: SMA and Ki67 expressions increased in parallel with the risk while CD34 and Desmin decreased. Conclusion: The results of this study demonstrated that features other than tumor size and mitosis, and other immune markers besides Ki67 also seem to be useful predicting factors.

OP19-5
THE SIGNIFICANCE OF APC/BETA-CATENIN PATHWAY AND TGF-BETA/SMAD PATHWAY IN ASSOCIATION WITH MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITORS IN THE NEOPLASTIC PROGRESSION OF THE COLORECTAL MUCOSA

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Background: The classic genetic pathway for the pathogenesis of colorectal cancer (CRC) is based upon the concept of an adenoma-carcinoma sequence. However, in recent years, a "serrated pathway" involving hyperplastic polyp-serrated adenoma-carcinoma sequence has been proposed as an alternative. In the present study, the neoplastic continuum of colorectal mucosa was evaluated in terms of molecules responsible for the initiating events (APC/Beta-catenin pathway) as well as molecules involved in the progression to invasive carcinoma (TGF-beta/Smad pathway together with matrix metalloproteinases and their tissue inhibitors) in a large series of cases represented by tissue array. Method: Core tissue biopsies of 4mm in diameter were prepared from paraffin-embedded tissue blocks of 252 cases, including 11 hyperplasic polyps, 8 serrated adenomas, 98 colorectal adenomas (24 tubular; 24 tubulovillous, 26 villous adenomas, and 24 FAP polyps), 22 adenomas with intramucosal carcinoma, 48 conventional CRC, 24 mucinous CRC, 17 FAP-associated CRC and 24 normal colon mucosae. Sixty two percent of adenomas showed low grade while 38% presented with high grade dysplasia. Immunohistochemistry was performed using monoclonal antibodies to beta-catenin, MMP-2, MMP-7, TIMP-2, TGF-beta1 and Smad4. Distribution of positivity was assessed using percentage expression while an arbitrary grading scale was used for staining intensity. Results: Our results showed that, 24,4% of adenomas and 30% of adenocarcinomas had nuclear beta-catenin expression which was significantly highly expressed in cases with high grade dysplasia ($p<0,001$). MMP-7 expression was significantly higher ($p<0,05$) in adenomas than in normal mucosae and carcinomas while carcinomas at later stages showed stronger MMP-7 expression compared to early stage tumours. MMP-2 expression demonstrated a significant increase ($p<0,01$) in parallel with neoplastic progression. TIMP-2 expression, on the other hand, decreased significantly ($p<0,001$) from normal mucosae to carcinomas in a similar manner as Smad4 expression which also showed a significant decrease through the sequence. Adenomas and carcinomas showed increased TGF-beta1 expression compared to normal mucosae. Expressions of TGF-beta1 and MMP-2 were significantly ($p<0.001$) correlated with the neoplastic sequence. Conclusion: Our results suggest that, MMP-2, MMP-7 and TGF-beta1 seem to take place in early stages of carcinogenesis while beta-catenin and Smad-4 seem to have a role at both early and later stages of the neoplastic sequence of the colorectum.

OP19-6

HER-2 OVEREXPRESSION/AMPLIFICATION IN ESOPHAGEAL DYSPLASIA PREDICTS EARLY TRANSITION TO ADENOCARCINOMA: A CLINICO-PATHOLOGIC STUDY.

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Background: Barrett's esophagus (BE) is the primary preneoplastic lesion to esophageal adenocarcinoma (ADC). The natural history of metaplasia-dysplasia-carcinoma sequence remains largely not known. The HER2/neu oncogene results to be overexpressed/amplified in preneoplastic lesions and in ADC of the esophagus and it has been associated with poor prognosis. Our aim is to evaluate the value of HER2 overexpression / amplification and other clinical variables in predicting the conversion from preneoplastic lesion to ADC. Methods: We retrospectively analysed clinical records and histological specimens of 14 patients with a confirmed diagnosis of BE or esophageal dysplasia. All patients performed surveillance endoscopic controls at regular intervals or when clinically indicated. Clinical variables included age (≤65/>65), gender, alcohol and smoking intake, presence of symptoms (pirosis, disphagia) and endoscopic features (length). The HER2 status was studied by immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH), on paraffin-embedded tissue. Numerical alterations of chromosome 17 (CEP17) were also evaluated by FISH. The endpoint was the time-to-progression (TTP) from the initial histologic lesion to the worst histologic diagnosis. Univariate analysis of single variables was performed using the Log Rank test and a level of significance of $p<0.05$ was established. Results: Median age at diagnosis was 60 years, 10 patients were male and 4 female. BE median length was 5.2 cm. HER2 was overexpressed/amplified in 8/14 (57%) patients. Median time-to-progression was 39 months. At univariate analysis, age older than 65 years and HER2 overexpression/amplification were the only variables associated with a shorter TTP although, due to the small size of this series, a statistically significant association was not reached. Conclusion: This study provides evidence for a pathogenetic role of HER2 in the transition from low grade to high grade dysplasia and to ADC of the esophagus. HER2-positive cases showed a trend toward an early progression of their initial lesion to a higher grade lesion. This result should be confirmed in a larger study and could help in identification of patients at high risk of malignant transformation.

OP19-7

INTRAHEPATIC MACROPHAGE MIGRATION INHIBITORY FACTOR EXPRESSION AND MIF GENE-173G/C POLYMORPHISM IN NON ALCOHOLIC FATTY LIVER DISEASE

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Background: Macrophage migration inhibitory factor (MIF) is a cytokine which plays an important role in the innate immunity. Increased expression of MIF at liver tissue and serum levels had been reported in alcoholic liver disease and chronic hepatitis B infection. In-vitro studies revealed that anti-MIF monoclonal antibodies decreased MIF expression and prevent liver damage by reducing inflammatory response. Aim: to investigate role of the MIF expression and 173G/C polymorphism of the MIF gene in non alcoholic fatty liver disease (NAFLD). Method: 91 patients with diagnosis of NAFLD were included in the study. After informed consent, blood was collected and DNA was obtained from patients and 104 healthy controls. MIF 173 C/G polymorphism was detected using the PCR-RFLP based method. NAFLD were stratified as non alcoholic steatohepatitis (NASH), probable NASH and steatosis, respectively group 1, group 2 and group 3, according to NAFLD study group definitions by a blinded pathologist. Intrahepatic expression of MIF was detected by immunhistochemistry staining. Results: Mean age of patients with NAFLD was 50.1±9.6 (19-69) years, and 54 were male. There were 37/44/10 patients in group 1/group 2/group 3, respectively and demographic features (sex, age, BMI) were similar in all three groups. ALT and AST were found as 50/83, 42/63 and 31/32, respectively in group 1, 2 and 3, ($p<0.05$). MIF expression of hepatocytes were similar in all groups, on the other hand, MIF expression of mononuclear cells was significantly more prominent in both group 1 and group 2 than group 3. There was not any significant correlation between MIF expression of hepatocytes and fibrosis stage. On the other hand, MIF expression of mononuclear cells significantly increased according

to fibrosis stage ($p<0.05$, R:0.2). There was not any significant correlation between MIF genotype and MIF expression in the liver ($p>0.05$). MIF-173 C allele frequency and genotype were similar in all three groups and control group ($p>0.05$). Conclusion: MIF-173 C allele and genotype of MIF gene have no effect on NAFLD subgroups and on expression of MIF in liver tissue in patients with NAFLD. Our results suggest that MIF expression is significantly increased especially by mononuclear cells in liver tissue of patients with NASH secondary to inflammation. Thus, it should be considered as a consequence not a causal factor.

OP19-8
HEPATOCYTE DIFFERENTIATION IN CHOLANGIOCARCINOMA, SUGGESTING HEPATIC PROGENITOR CELL ORIGIN

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BACKGROUND/AIMS: Cholangiocarcinoma (CC) is a primary malignant liver tumour which is known to have a poor prognosis and the incidence and mortality is increasing worldwide. Recent studies showed that hepatic progenitor cells (HPCs) play a role in the carcinogenesis of hepatocellular carcinoma. The relationship between cholangiocarcinoma (CC) and HPCs have not yet been reported. Since HPCs can differentiate into both hepatocytes and cholangiocytes, we evaluated the hepatocyte differentiation and presence of HPCs in CC. MATERIAL AND METHODS: We studied 93 cases of CC by immunohistochemistry for hepatocellular differentiation markers (Hepar, canalicular polyclonal carcinoembryonic antigen (pCEA), CD10), biliary/progenitor cell markers (cytokeratin (CK) 7, CK19 and neural cell adhesion molecule (NCAM)) and the ATP-binding cassette (ABC) superfamily of membrane transporters (MDR1, MRP1, MRP3 and BCRP). RESULTS: Thirty-seven (39.8%) cases of CC showed hepatocyte differentiation. The morphological features of hepatocyte differentiation was classified into two patterns: (i) a trabecular pattern which show thick trabecular with little stroma consisting of tumour cells with abundant eosinophilic cytoplasm and mild atypia; and (ii) a cord pattern which show atrophic trabecular and/or cord structure of small uniform cells. A trabecular pattern was observed in 17 /37 cases (45.9%), a cord pattern in 16/37 (43.3%) cases, and both patterns in 4/37 cases (10.8%). In these areas, CK7 positive intermediate-sized cells which showed a submembranous staining pattern was observed in all cases, a canalicular staining pattern of pCEA in 30/32 cases (93.8%), CD10 in 21/29 cases (72.4%) and Hepar in 5/24 cases (20.8%). In addition, cytoplasmic CK7 and/or CK19 positive small round oval cells resembling HPCs were present in all cases and also positive to NCAM in 19/24 (79.2%) cases. These immunoshitochemical staining pattern were same in both pattern. BCRP, MRP3 and MRP1 staining showed a basolateral staining pattern and MDR1 showed canalicular staining pattern in both pattern, but the expression was down-regulated in comparison with surrounding normal hepatocyte. CONCLUSION: In our study, about 40% cases of CC showed hepatocyte differentiated features. According to both morphological and immunohistochemical features, these findings strongly suggest a possible HPCs origin of a subset of CC and the fact that MRP1, MRP3 and BCRP are up-regulated, can be responsible for chemoresistance of CC.

OP20-1
MICROMETASTASES IN PELVIC LYMPH NODES IN SURGICALLY TREATED PATIENTS WITH CARCINOMA OF THE CERVIX UTERI ARE OF PROGNOSTIC VALUE

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Background: Althought, metastatic disease in pelvic lymph nodes is the strongest prognostic factor in carcinomas of the uterine cervix (CX), limited information exist about the prognostic impact of micrometastatic disease (i.e. metastatic deposits < 0.2cm = pN1mic). Methods: Pelvic nodes of 894 surgically treated CX FIGO-stage IB to IIB were histologically re-examined regarding metastatic disease. In cases with lymph node involvement, the metastatic deposits were measured using ocular micrometer. Pelvic lymph node status (pN0 vs. pN1mic vs. pN1 = metastasis > 0.2cm) was correlated to recurrence free and overall survival. Results: 29.7% (266/894) patients represented with pelvic lymph node involvement. 22.1% of these patients showed micrometastases (pN1mic). Patients with macrometasases (pN1) and those with micrometastatic disease (pN1mic) represented significant reduced recurrence free survival (RFS) time (112.6+6.8 and 122.5+9.6 months), when compared to patients without metastatic disease (pN0: 190.9+3.0 months; $p<0.0001$). The 5-year-overall survival (OS) was decreased in patients with metastatic disease (pN0: 86.6%, pN1mic: 63.8%, pN1: 48.2%; $p<0.0001$). Conclusions: The results suggest that micrometastases in pelvic lymph nodes represent an important risk factor for reduced RFS and OS in cervical cancer patients, and all lymph nodes, especially resulting from sentinel lymph node procedure, should be examined very careful histologically.

OP20-2
GENE EXPRESSION IN ENDOMETRIAL CANCER CELLS (ISHIKAWA) AFTER SHORT TIME HIGH DOSE EXPOSURE TO PROGESTINS

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Background: The potent antiproliferative effect of progesterone and the mimic progestin has been utilized in clinical regimens in the treatment of endometrial disorders. The treatment of endometrial hyperplasia with progestin infiltrated intrauterine device yields in a hundred-fold increase of local progestin concentration in the endometrium compared to per oral treatment. The genetic basis for the complex effects of high dose progestins and the details of the different signalling pathways regulated by these genes has never been accurately surveyed. Therefore, the aim of the present study was to reveal gene expression in highly differentiated endometrial cancer cells (Ishikawa) after short time high dose exposure to progestins. Method: In eight independent experiments cells were treated with progestin (30 mg/ml) for four hours. Cells not treated with progestin served as control groups. Analysis of progestin-treated cells with microarrays representing 24,650 genes identified 939 genes with changed gene expression levels ($p<0.01$). The False Discovery Rate (FDR) was estimated

to be 25%. Genes present on [GTEQ] seven microarrays and with a fold changed expression level threshold set to 1.3 were used for further analysis. Results: Analysis revealed 247 differentially expressed genes of which 126 were up- regulated and 121 were down- regulated. Of these, 135 genes are known to be involved in biological processes like cell cycle, cell proliferation and differentiation, developmental processes, immune responses, intracellular protein traffic and transport. Our study shows that microarray analysis can detect relevant gene expression changes in endometrial cells treated with progestin, and that several alternative transcriptional factors and signalling pathways are involved. In addition, many genes are identified that were not previously known to be affected by progestin or that have unknown biological functions. Conclusion: Characterization of the roles of the differentially expressed genes may give new insights into molecular responses to treatment with high dose of progestins. Alternative signalling pathways for progestin different from the classical steroid receptors are suggested.

OP20-3

EXTRANUCLEAR STEROID-INITIATED SIGNALING BY PROGESTIN AND MIFEPRISTONE: CONCENTRATION DEPENDENT INHIBITION OF ISHIKAWA CELL PROLIFERATION AND INDUCTION OF APOPTOSIS

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Background: The anti-proliferative effect of progestins on the human endometrium in vivo and in vitro is well known. Previous studies suggest that at least 60 % of patients with endometrial hyperplasia respond to progestin therapy. In the present study Ishikawa cells(endometrial adenocarcinoma) were used to mimic the in vivo situation. A pharmacological analysis of the effects of progesterone, levonorgestrel and medroxyprogesterone acetate during cell culture was performed to determine sensitivity and the order of potency of these progestins/antiprogestins. Method: Progesterone,levonorgestrel,medroxyprogesterone acetate and mifepristone were added to Ishikawa cell cultures . Cell cycle analysis, detection of early apoptosis by Annexin V-FITC binding, late apoptosis detection by DNA fragmentation and detection of mitochondrial transmembrane potential were performed by flow cytometry employing a FACScan flow cytometer equipped with an argon-ion laser (488 nm). Results: Cells exposed to progesterone (0.32–95mikroM) caused a concentration-dependent reduction in cell densities. Progesterone (95mikroM) caused a reduction of 57±18% (dialyzed FCS),60 (range60-59) % (FCS) and 41±5% (stripped FCS). The respective IC50-values were 53.8±6.7mikroM, 61.5mikroM (range 53.3-69.7) and 50.6±1.0mikroM. Different progestins exhibited differing potencies for inhibition of cell growth: Levonorgestrel (IC50 = 3.9±0.4 mikroM)> medroxyprogesterone acetate (IC50=30.4±3.4mikroM)>progesterone (IC50=60.3±6.2mikroM). Surprisingly, the progesterone inhibitor, Mifepristone (0.2–23mikroM) did not antagonize the antiproliferative effect of progesterone, but had a progesterone-mimicking effect with an IC50value of 19.0±1.7mikroM. High concentrations of mifepristone (23mikroM) and progesterone (32 mikroM) had an additive effect. Both progesterone and mifepristone caused cell cycle arrest in the G1/G0 phase and induced apoptosis. Conclusion: The present study shows that pharmacological concentrations of both progesterone and mifepristone lead to cell cycle retardation and induction of apoptosis. In combination, mifepristone caused an additive effect rather than antagonizing the action of progesterone. Among the progestins tested for their anti-proliferative effect, levonorgestrel was markedly more potent than medroxyprogesterone acetate and progesterone. The present results also suggest that progesterone and mifepristone, in high concentrations, interact with non-classical molecular targets and that the antiproliferative effect is conveyed by signals having an extranuclear initiation.

OP20-4

SEX CORD-LIKE TUMOURS OF THE FEMALE GENITAL TRACT: PATTERN ANALYSIS IN 31 CONSULTATION CASES

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INTRODUCTION: Uterine tumours resembling ovarian sex cord tumours are an unusual group of stromal neoplasms that share a similar appearance with ovarian sex cord tumours. METHODS: We analyzed their clinico- and immunopathologic patterns in an extensive material (31 consultation cases). RESULTS AND CONCLUSIONS: Uterine tumours resembling ovarian sex cord tumours develop in premenopausal patients and their most frequent symptom is vaginal bleeding. Macroscopically, most tumors are intramural nodule (17 cases) or polypoid (10 cases). Some of them can be incidental findings (tumorlets) (2 cases) or may be present in ectopic endometrial tissue (2 adenomyosis, 2 endometriosis). Microscopically, tumours were evaluated for approximate proportions of sex cord, endometrial stromal and other components. Tumours with predominant (over 50%) sex cord differentiation were diagnosed as UTROSCT (22 cases) and tumours with minor sex cord differentiation (less than 50%) were diagnosed as ESTSCLE (4 cases). Four were present in ectopic endometrial tissue (2 adenomyosis. 2 endometriosis). Architecturally, the tumour cells may form trabeculae (87.09 %), nests (35,4 %) solid appearance (32,2 %), retiform pattern (19,3 %) or may be associated with luteinised cells (19,3%). Rare, the tumour cells may form pseudoglandular structures (6,4%). Pseudoglandular or trabecular patterns are usually misdiagnosed in endometrial biopsy. In some tumours, smooth muscle (1 case) or chondroid component (1 case) may be encountered. Immunohistochemically, the tumour cells of the sex cord component are usually positive for Inhibin, CD 10, CAM5.2, Vimentin and Calponin. In two cases where an endometrial biopsy was performed, an erroneous diagnosis of carcinoma was made. Although there is a marked morphologic similarity between sex-cord patterns found in tumours and the corresponding ovarian patterns (granulosa, Sertoli, retiform, etc), there are not immunopathologically identical. The microscopic aspect together with the immunohistochemical features helped us to correct diagnose these tumours and to distinguish them from other malignant epithelial tumours, mixed mullerian malignant tumours which is essential in patient's management.

OP20-5

DISCRIMINATION OF COMPLETE HYDATIDIFORM MOLE FROM PARTIAL HYDATIDIFORM MOLE AND HYDROPIC ABORTION BY IMMUNOHISTOCHEMICAL STUDY OF THE PATERNALLY IMPRINTED GENE PRODUCT P57KIP2:A STUDY OF 120 CASES OF EARLY MOLAR AND NON MOLAR ABORTIONS IN TUNISIA

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Introduction: Classification of molar gestations into complete (CHM) and partial (PHM) and their differentiation from hydropic abortions (HA) traditionally are accomplished by morphology alone. The process sometimes may be inaccurate of inconclusive

especially in early diagnosed cases. With the availability of p57kip2 immunostaining (the product of a strongly paternally imprinted gene), it may be possible to objectively classify these lesions. Aims: to assess the value of p57kip2 immunohistochemistry in the diagnosis of molar and non molar gestations. Material and method: the immunohistochemical expression of p57kip2 protein was investigated using monoclonal antibody on paraffin embedded, formalin fixed tissue sections of 120 first trimester abortus conceptions. Samples included 95 histologically confirmed molar gestations (65 CHM and 30 PHM), and 25 HA. Results: p57kip2 was strongly expressed in cytotrophoblast and villous mesenchyme in 24/25 HA, 23/30 PHM and 2/65 CHM. Immunostaining of extravillous trophoblast cells, trophoblastic cells within cell columns or islands, and interstitial trophoblast at maternofetal junctional zones was observed as internal positive controls. Syncytiotrophoblastic cells were used as negative controls in all cases. Based on the pattern of p57 staining the cases were reclassified into 25 HA (initial diagnosis (ID): 24 HA and 1 CHM), 24 PHM (ID: 23 PHM and 1 CHM) and 71 CHM (ID: 63 CHM, 7 PHM and 1 HA). Conclusion: differential expression of p57kip2 in CHM and PHM supports the evidence of misexpression of the maternally expressed, paternally imprinted gene in CHM. Therefore immunohistochemical staining with p57kip2 can be a very useful diagnostic adjunct for the differential diagnosis of CHM and PHM or HA in difficult cases. This differentiation is clinically relevant since patients with hydropic abortions do not need to be followed while patients with molar gestations do.

OP20-6

COMPARATIVE CHARACTERISTICS OF EXPRESSION OF MMPS, TIMPS, MARKERS OF PROLIFERATION AND APOPTOSIS, ESTROGEN AND PROGESTERONE RECEPTORS IN EUTOPIC AND ECTOPIC ENDOMETRIUM IN WOMEN WITH ADENOMYOSIS.

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Endometriosis is one of the most common diseases of reproductive system in women aged between 20 and 40, adenomyosis is the most frequent type of endometriosis and is found in 70 -90% of all cases. The aim of our study was to investigate the expressions of matrix metalloproteinase 1, 2, 7, 9 (MMP 1, 2, 7, 9) and tissue inhibitors of metalloproteinase 1, 3, 4 (TIMP 1, 3, 4), collagen IV, laminin, fibronectin, proliferating cell nuclear antigen (PCNA), marker of apoptosis (Apo), adhesive molecule (E-cadherin), epidermal growth factor receptor (EGFR), estrogen and progesterone receptors (ER/PR) in foci of adenomyosis and eutopic endometrium. Materials and Methods. The study was performed on biopsy samples of uterus taken from 19 women with adenomyosis. Immunohistochemical staining of tissues was performed with antibodies to MMP-1, MMP-2, MMP-7, MMP-9, TIMP-1, TIMP-4, E-cadherin (Lab Vision), PCNA, collagen IV, laminin, fibronectin (DAKO), Apo, EGFR, ER, PR (Novocastra). Reaction results were evaluated with the help of quantitative and semiquantitative methods and statistic analysis. RESULTS: Stroma in foci of adenomyosis showed higher levels of MMP1, 2, 9, TIMP1, whilst epithelium was more active in expressing MMP7, TIMP4, E-cadherin, EGFR and PR. Stroma in ectopic endometrium have similar levels of expression of MMP 1, 2, 9, TIMP-4, EGFR, PR with those in the basal layer stroma of eutopic hyperplastic endometrium. Active foci of adenomyosis with cytogenic stroma differed from silent areas with fibrotic stroma by the higher expression of MMP2, TIMP1 and e-cadherin in epithelium and MMP2 and TIMP1 in fibroblasts. CONCLUSION: The obtained data suggests that adenomyosis may result from the invasion of endometrium into the myomertium occurs due to the activity of MMPs in stromal elements and increased angiogenesis rather than the activity of glandular epithelial cells. This hypothesis may be proved by similarity of stroma in ectopic and basal layer in eutopic endometrium. Foci of adenomyosis according to their morphology and MMPs, TIMPs and E-cadherin expression may be classified as active and silent.

OP20-7

SERUM AMYLOID A: EXPRESSION AND POSSIBLE ROLE IN OVARIAN CANCER

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Background: Serum amyloid A (SAA) is an acute phase protein, whose levels in the blood are elevated in response to trauma, infection, inflammation and neoplasia. Elevated levels of SAA in the serum of cancer patients were suggested to be of liver origin rather than being produced locally in the tumor tissue. The role of SAA in human malignancies has not been elucidated. We investigated the expression and localization of SAA in ovarian serous epithelial tumors and measured the serum concentrations of SAA in patients with ovarian pathology in comparison to CA-125 and C-reactive protein (CRP). Methods: The expression of SAA mRNA was investigated by non-radioactive in situ hybridization using digoxigenin–labeled probes applied on formalin-fixed paraffin-embedded tissue sections from ovarian serous tumors of 23 patients. Sections included 10 serous carcinomas, 5 serous borderline tumors (tumors of low malignant potential) and 8 serous cystadenomas. Protein expression was studied in selected cases using immunohistochemistry. SAA levels were measured in sera of 80 patients with different ovarian pathologies using specific ELISA. Results: SAA mRNA was expressed in all the ovarian tumors, however, its intensity and pattern of expression varied with tumor type. Strong to moderate expression was observed in tumor cells of most ovarian carcinomas (90%). The staining pattern was diffuse in 60% of the carcinomas. Moderate-strong and mainly focal expression was observed in epithelial cells of all 5 borderline ovarian tumors. Weak and focal or negative staining of epithelial cells was observed in 66% of cystadenomas, and focal strong expression was found in the remaining third. Immunohistochemical staining revealed SAA protein expression that co-localized with mRNA expression. CA-125 serum levels were divided into three groups; normal, moderately elevated and highly elevated. The mean values of CA-125 within each of these groups strongly correlated with both SAA and CRP levels. Furthermore, in sera of patients with ovarian malignancies, where CA-125 values were normal SAA levels, although low, were higher than the normal values. Conclusions: Our data demonstrates enhanced expression of SAA in serous ovarian carcinomas, suggesting a role in ovarian tumorigenesis. Furthermore, SAA may serve as an additional serum tumor marker more sensitive than CA-125. Recent studies in our laboratory indicate that SAA plays a role in tumor cell migration, invasion and activity of matrix-degrading enzymes. Our findings may have diagnostic and therapeutic applications.

OP20-8

PROGNOSTIC VALUE OF THE SILVERBERG GRADING IN THE PRIMITIVE MALIGNANT EPITHELIAL TUMORS OF THE OVARY: A STUDY OF 47 CASES

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Background : Ovarian cancer impose great problems in diagnosis and prognosis. Until now, only the age, the histological type and the clinical stage have a true prognostic value. The aim of our

study is to evaluate the place of Silverberg grade in the prognosis of the ovarian carcinoma. Materiel and Methods: Our study concerned 47 cases of ovarian tumor managed between 1994 and 1998. We determined retrospectively the Silverberg grade based on architectural score, atypia and mitotic index. We defined the grade I when the score is between 3 and 5, grade between 5 and 6 and grade III between 8and 9.We made a correlation between this grade, others prognostic parameters and the survival rate (kappa test). Results: 53% of our patients were aged between 50 and 70 year with an average of 54,8. The predominant histological type was the serous type followed by the mucinous type. 81% of the tumors were diagnosed in FIGO III and IV stage. 23% were Silverberg Grade I , 23% Grade II and 32% grade III. Mucinous and endometroid tumor were respectively in 89% and 100% Silverberg Grade I-II while the clear cell carcinoma was Grade III .The tumors diagnosed in FIGO I-II were Silverberg I-II. The survival rate at 3 years was significantly correlated to Silverberg Grade. Conclusion: Silverberg Grade should be considered in the evaluation of the prognostic and the management of the ovarian tumor. This grade is easily reproducible but it is subjective in the grade II. It should be simplified in two grades; high and low grade .Studies based on large samples should be done in order to evaluate this grade by multivariate analysis.

OP21-1
MICRO-RNA IN HUMAN LUNG CANCER AND HUMAN LUNG EMBRYOGENESIS

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INTRODUCTION: Mature microRNAs (miRNAs) are small RNA molecules that act as negative regulators of genes and participate in cell regulation, embryogenesis and cancer. AIM: To analyze if the miRNAs present in lung embryogenesis are also active in lung tumors and which are differentially expressed between tumor and normal lung tissue. METHODS: We studied by Real Time-PCR 18 mature miRNAs (let-7a family, cluster 17-92, mir-155, RNU6B, RNU66) in lung tissue from twelve 7-12-week human embryos and 36 human lung samples (18 tumor and 18 normal). Data were analyzed with BRB Array tools and TIGR M-Viewer. RESULTS: The hierarchical clustering showed four groups: E7-8 embryos, E9-12 embryos, tumor and normal samples. Embryonic samples were closer to tumor samples. To determine common miRNAs during tumor and embryonic development, we analyzed the miRNAs differentially expressed between tumor and embryonic tissues versus normal. miRNAs mir-17-5p, mir-106a, mir-19a, mir-25, mir-93 and mir-98 were overexpressed in tumor and embryos samples and miRNAs let-7a, let-7b, let-7c and let-7g were underexpressed compared to normal samples. We analyzed the miRNAs differentially expressed between tumor and normal samples and we found that mir-106a, 93, 17-5p, 25, mir-98 and mir-19a were significantlly overexpressed and let7-a, let-7c, let-7g and let-7b were significantlly underexpressed. These miRNAs allows to classify a sample as tumoral or normal. CONCLUSION: Due to their similar expression miRNAs of cluster-17-92 and members of let-7 family play a role during the normal lung and tumor development. These miRNAs may be a good targets for new therapeutics strategies. FIS060087, SEPAR06.

OP21-3
IMMUNE-PHENOTYPING OF INFLAMMATORY CELLS IN BRONCHIOLAR INTERSTITIUM OF IDIOPATHIC INTERSTITIAL PNEUMONIAS: PRELIMINARY STUDY

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Background: Several studies have implicated the role of inflammation in the pathogenesis of lung damage in idiopathic interstitial pneumonias (IIPs). Investigations of inflammatory cells in IIP have show that eosinophils, neutrophils and T cells may be associated with a poorer prognosis. The aim of our study was to map, by quantitative analysis, the number of inflammatory cells in bronchiolar interstitium of patients with non-specific interstitial pneumonia/non-specific interstitial pneumonia (NSIP/NSIP), acute interstitial pneumonia/diffuse alveolar damage (AIP/DAD), and idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP). Methods and results: Inflammatory cells [macrophages, neutrophils (Elastase+), CD20 and CD3, CD4, CD8 TLs] were quantified in 8 NSIP, 18 DAD and 24 UIP surgical lung biopsies. The total density of inflammatory cells was significantly increased in DAD and NSIP when compared to UIP (p=0.04). Lymphocytes (CD3 and CD20) were increased in UIP when compared to DAD lungs (p=0.04). The other individual inflammatory cells were not significant differences between groups. Conclusion: Total density of inflammatory cells and lymphocytes present a different distribution within the pulmonary bronchiolar interstitium in AIP/DAD, NSIP/NSIP and IPF/UIP indicate evolutionary adapted responses to active parenchymal injury. Our findings not provide evidence of the participation of inflammation in the bronchiolar interstitum remodeling of IIPs.Financial Support: FAPESP, CNPq

OP21-4
ROLE OF THE MATRIX METALLOPROTEINASES IN THE PATHOGENESIS AND MORPHOGENESIS OF THE CHRONIC CAVITARY PULMONARY TUBERCULOSIS OF THE PERSONS WHO HAS BEEN LIVING IN RADIOACTIVE-CONTAMINATED ZONES OF THE SEMIPALATINSK REGION OF KAZAKHSTAN

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Aim. The object of the work is studying the role of the matrix metalloproteinases in the pathogenesis and morphogenesis of the chronic cavitary pulmonary tuberculosis of the persons who has been living in radioactive-contaminated zones of the Semipalatinsk region of Kazakhstan. Methods. 75 cases of patients with the fibrotic- cavernous tuberculosis of the lung were selected for study. 54 patients living in Kazakhstan regions with high radiation level (group I). 21 patients had been living in Kazakhstan regions (6) and Moscow (15) with normal radiation level (group II). There were 63 males and 12 females with the mean age from 17 to 74 years.. The surgical and endoscopic bronchobiopsy material was studied with light and immunohistochemistry. Paraffin-embedded microwave pretreated sections were stained immunohistichemically to detect the presence of chromogranin A (DAKO), ÌÌĞ-1; ÌÌĞ-2; ÌÌĞ-9, TIMP-1 (LabVision, USA), VEGF, CD34, CD68, Ki-67, TGF-b (DAKO). Results and conclusion. the chronic cavitary pulmonary tuberculosis in patients from Semipalatinsk region of Kazakhstan with high radiation level is characterized by accumulation of MMPs and prominent angiogenesis could be applied for the development of new and pathogenetically grounded trends in therapy.

OP21-5
MMPS AND TIMP- 4 IN REMODELING OF EXTRACELLULAR MATRIX, FIBROSIS, CELL PROLIFERATION IN DIFFERENT VARIANTS OF IDIOPATHIC CHRONIC INTERSTITIAL PNEUMONIAS (ICIP)

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The aim of this study was to evaluate the role of MMPs in pulmonary remodeling and progression of ICIP. Biopsies from 149 patients with ICIP were studied (116 with usual interstitial

pneumonia-UIP, 15 - desquamating interstitial pneumonia-DIP, 11 - nonspecific interstitial pneumonia -NSIP and 7 - bronchiolitis obliterans organizing pneumonia -BOOP). Immunohistochemistry on paraffin section was done with antibodies to MMP1, 2, 7, tissue inhibitor of metalloproteinase 4 (TIMP-4) (Lab Vision), and CD34 (Novocastra). Expression of MMPs and TIMP4 has been analyzed separately in each type of cells (epithelial cells, myofibroblast, fibroblast, macrophages and endothelial cells). Particularity of different variants of ICIP has been established with help of semiquantitative score, ranging from 0-6. Positive and negative controls were used. Results. UIP was characterized by the highest expression of all studied markers, especially MMP-2 in alveolar and interstitial macrophages (3,81± 1,28; P<0,05) as well as fibroblasts (4,50 ± 1,55; P<0,05) and endothelial cells (4,50 ± 1,37; P<0,05). Myofibroblasts, forming subepithelial foci, were positive only for MMP-2 (3,69 ± 1,54 ;P<0,05). In addition, hyperplastic alveolar and bronchiolar epithelial cells contained MMP1, 2, 7 and TIMP-4. Cells in foci of angiogenesis expressed MMP-2. BOOP differed from UIP by the highest MMP-7 expression in bronchiolar epithelial cells (4,50 ± 1,37; P<0,05). Alveolocytes of the II type covered Masson bodies were positive for MMP1, 2, 7 and TIMP-4. MMP-2 was also seen in stroma, especially in foci of angiogenesis (3,98 ± 1,80; P<0,05) . DIP cases showed predominant moderate activity of MMP-2 in alveolar macrophages (3,00 ± 1,41; P<0,05), in fibroblasts (2,00 ± 0,00; P<0,05) and endothelial cells (3,00 ± 1,41;P<0,05); MMP-7 was expressed moderately in alveolar epithelial cells (3,00 ± 1,41; P<0,05) and alveolar macrophages (3,00 ± 1,41; P<0,05) , while MMP-1 and TIMP-4 were at low level. In NSIP we registered low levels of all studied markers. Conclusion. Remodeling of lung interstitial tissue, resulting in interstitial fibrosis, develops through distinct mechanisms with activation of definite cells and production of MMPs are different in UIP, BOOP, DIP and NSIP. High activity of MMP-2 in stromal cells, the presence of hyperplastic epithelial cells and myofibroblasts as the source of MMP1, 2, 7 and TIMP-4 result in most severe reconstruction of lung tissue in UIP. The expression levels of MMPs and TIMP may relate to the differential diagnosis and distinct prognostic features of ICIP.

OP21-6
LATENCY PERIODS AND PATHOGENESIS IN ASBESTOS-RELATED MESOTHELIOMA OF THE PLEURA

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BACKGROUND Latency periods in asbestos-related mesothelioma are defined as time intervals elapsing between first exposure to asbestos and diagnosis of the tumor. The duration of the latency period is a key-point for understanding the sequence of events, that eventually result in mesothelioma. While figures of 20-40 years are generally reported in the literature, some studies indicate far longer periods. METHODS We reviewed a series of 164 malignant mesotheliomas of the pleura, diagnosed in the Trieste-Monfalcone district, northeastern Italy, in the period 2001-2006. This district (total population about 300,000) has been identified as an area with very high incidence of mesothelioma. The diagnosis was confirmed by necropsy in 91 cases. Lifetime occupational histories were obtained from the patients themselves or from their relatives by personal interviews. In 19 cases asbestos bodies were isolated from the lung after the Smith-Naylor method. Latency periods were calculated in 138 cases, in which sufficiently detailed chronological data were available. RESULTS The group, in which latency periods were calculated, included 129 men and 9 women, aged between 43 and 89 years (mean 68.8, median 69.0). A majority of patients (86 people) had been exposed to asbestos in shipbuilding industry. Other sources of exposure included various industries (16 cases), port work (11), maritime trades (9), insulation (4), various (12). Asbestos body burdens, evaluated in 15 cases, ranged between 900 and 230,000 bodies per gram of dried tissue, with nine people showing more than 10,000 bodies per gram, and five people showing more than 50,000 bodies per gram. In 109 cases (79%) latency periods were longer than 40 years. In about 20% of the cases latency periods were longer than 60 years. Mean latency periods differed in the various occupational groups, being 38.5 among insulators, 41.9 among port workers, 42.1 in the various industries workers, 46.0 in the group classified as various, 51.3 among shipyard workers, and 53.7 in maritime trades. Latent periods longer than 40 years were observed even in four patients with very high amounts of lung asbestos bodies (more than 50,000 bodies per gram). CONCLUSIONS The present findings are at variance with those currently reported. The latency periods observed in the present as well as in other series suggest that the presence of asbestos fibers might be for a longtime well tolerated. It is probable that new events (recurrent inflammatory processes? impairment in immune mechanisms?) have to occur before malignant transformation starts.

OP21-7
THE INACTIVATION OF RBM5 TUMOR SUPRESSOR GENE AT 3P21 IS A MOLECULAR PATHWAY ASSOCIATED WITH ADVANCED NON-SMALL-CELL LUNG CANCER.

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Background: 3p21 is a chromosomal region with frequent somatic genetic alterations in Non-small-cell lung cancer (NSCLC). Those changes appear to be more specific in lung cancer than in other malignancies. For many years, different putative tumor suppressor genes (TSG) at this locus has been proposed to play a major role in NSCLC. Recent studies show that RBM5 (LUCA15) located at 3p21 acts as a TSG regulating cell growth and apoptosis and inducing cell proliferation in cell lines. Materials and methods: ARN was extracted from 28 NSCLC frozen tissue samples and hybridized with the CNIO Oncochip 2001 (9216 spots anlyzing 6386 genes): 14 SCC and 14 adenocarcinomas, Stage I –II (n=18) y III-IV (n=10) . A different series of 112 NSCLC paraffin embedded cases: stage I-II (n=95) y III-IV (n=17). Median follow-up 76 months. A tissue microarray was constructed with 0.6-mm cylinders. Immunostaining for VEGF, EGFR, Her2/neu, Cox-2, p21, p27, p16, p53, rb, p120, survivin, caspase3, bcl2, ki67, TopoisomeraseIIa, ECadherin, G-catenin, Mdm2, CDK1, CDK2, Ciclins D1, D3, A, E, MLH1,MSH2, Timidilate sintase, MMP1, MMP9 and in situ hybridization with APOPTAG were performed. Results: Analysis of cDNA microarrays with POMELO tool showed that advanced stages overexpressed cytochrome p450, ubiquitins, TNF. The first genes with differential loss of expression between advanced and early stages were RBM5, nucleoplasmin 3, phosphatase DVSP22, ARF6 and AKT2. Smoker patients showed loss of MFTC and EPB41 expression and FLJ10211 overexpression. Cytokeratin10 differentiates SCC and adenocarcinomas. The immunohistochemical study of a different series of NSCLC showed that the only markers associated with overall survival and tumor progression were CyclinE and CDK2. Discussion: We previously demonstrated LOH at 3p21 in 48% of NSCLC. RBM5 is located at 3p21. RBM5 functions as a TSG downregulating the CyclinE and CDK2 complex that is crucial at the end of G1 phase in cell cycle. Conclusions: Inactivation of RBM5 by deletion and/or other types of mutations as shown by mARN analysis may originate over-expression of both CyclinE and CDK2 stimulating cell proliferation. Our study suggests that this molecular pathway is more frequent in advanced stages of NSCLC.

OP22-1
FLOW CYTOMETRIC DNA ANALYSIS OF GASTROINTESTINAL STROMAL TUMORS

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BACKGROUND: Gastrointestinal stromal tumors (GISTs) are considered to be the most common gastrointestinal mesenchymal tumor.It is generally agreed that the most powerful prognostic factors are size and mitotic counts. GISTs are classified as very low, low, intermediate and high risk groups for their estimated potential for aggressive clinical behavior. DNA aneuplody has been claimed to be discriminative between benign and malignant tumors of gastrointestinal tract, but conflicting results are also existing. The aim of this study was to examine the type of DNA content in risk groups of GISTs. METHOD: We analysed the DNA content of the parafin-embedded tumor specimens of 195 patients selected from the database of GIST working group of Turkey. Tumors were representing four different risk groups. We divided the tumors into two groups as high risk (high risk) and low risk (very low, low, intermediate risk). RESULTS: 88 cases were in low risk group and 107 cases were in high risk group. DNA aneuploidy was detected in 126 cases (67 in high risk, 59 in low risk). There was no significant relationship between DNA content and risk groups ($p>0.05$) We compared the patterns of aneuploidy of high and low risk groups. Hyperdiploidy was detected in 57 cases of the high risk group and 39 cases of the low risk group. Hyperdiploidy was associated with the high risk group ($p<0.05$). Hyperdiploid aneuploidy was also associated with spindle and mixed cellular group. CONCLUSION: The results suggest that DNA aneuploidy presents no difference between the risk groups, whereas aneuploidy patterns (hypo or hyperdiplody) may differ within the risk groups.

OP22-2
TWO PATHWAYS OF CARCINOGENESIS IN PATIENTS WITH COLORECTAL CANCER LESS THAN 45 YEARS OLD

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Background: Colorectal cancer (CRC) arising from a complex series of molecular changes that involve at least in two different pathways. These include microsatellite instability (MSI) pathway and chromosomal instability (CIN) pathway. The aim of this study was the determination of predominant pathway involved in carcinogenesis of patients with CRC less than 45 years old with and without family history (FH) of CRC. Method: In our study surgical pathology specimens of 108 patients with CRC less than 45 years old were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2. Beta-catenin and P53 were also examined for CIN pathway. Results: Totally 108 patients with median age of 40(20 to 45) were evaluated. Fifty seven patients were male and 51 were female. The site of tumor in 84 patients was colon and in 14 were rectum. Among 96 patients with known family history, 33(34.4%) had positive FH. The overall rate of abnormal immunostaining were MLH1 8.3%, MSH2 18.5%, MSH6 8.3%, PMS2 11.1%, P53 74.1% and beta catenin 35.2%. Meanwhile abnormal staining for hMSH2 and hMSH6 were significantly more seen in patients with positive family history ($p=0.008$ and $p= 0.032$ respectively). Patients with positive FH for CRC had significantly more abnormal MMRP (54.5% vs. 20.6%, $p=0.001$) and less positive p53 (54.5%vs. 81%, $p=0.006$) than patients with negative FH. Patients with early T, N stage tumor had at least one more abnormal MMRP than advance T, N stage ($P=0.050$ for T and $P=0.030$ for N stage). Among different factors abnormal hMSH2 had significant association with lower cancer related death ($P=0.060$). Patients with rectal cancer had more abnormal MMRP than patients with colon cancer but not significantly (35.7% vs. 29.8%, $p=0.655$) and positive p53 staining for rectal and colon cancer were 71.4%and 72.6% respectively. Both in colon and rectal cancer patients with negative family history had more prevalent positive p53 (80.4% vs. 56.7%, $p=0.022$ for colon and 81.8% vs. 33.3%, $p=0.099$ for rectal cancer). Conclusion: Our study indicate that even in CRC less than 45 years old, the main pathway for carcinogenesis in patients with negative family history is CIN, but in positive family history MSI is as effective as CIN.However main pathway in both colon and rectal cancer is CIN .

OP22-3
ADAM17 AND TIMP3 ARE DIFFERENTIALLY REGULATED IN INFLAMED INTESTINAL EPITHELIUM: ROLE NEUTROPHIL TRANSEPITHELIAL MIGRATION

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Background: Inflammatory bowel diseases (ulcerative colitis and Crohn's disease)(IBD) in humans are chronic disorders inducing intestinal inflammation. These inflammatory diseases are mainly characterized by a large afflux of PMNL into the mucosa causing the release of several cytokines such as TNF alpha. This latter cytokine requires cleavage of a transmembrane precursor protein to release the biologically active form. The protease responsible for this cleavage is the TNF alpha-converting enzyme (TACE, ADAM17) which is mainly regulated by TIMP3. Aims: to determine the status of ADAM17 in intestinal epithelium during the transepithelial migration of PMNL and in inflamed mucosa of patients with Crohn’s disease and then to elucidate the molecular mechanism (s) of such modulation. Methods: we used both an in vitro model of human neutrophils migration (T84 model) and an ex vivo approach from a collection of human digestive tissues.

ADAM17 expression was checked by RT-PCR, Western blotting and immunohistochemistry. Results: ADAM17 was up-regulated during PMNL transepithelial migration and in digestive biopsies associated with acute phase of IBD showing strong afflux of PMNL and crypt abcesses, independently of NOD2 mutations. Timp3 expression into the intestinal cells was decreased after 4 h of migration, but decreased or increased in digestive biopsies, depending of the disease activity (presence or not of afflux of PMNL and of crypt abcesses). In the absence of acute phase of inflammation, digestive biopsies showed a weakly expression of ADAM17. Finally, proinflammatory cytokines induced ADAM17 upregulation in T84 cells. Conclusion: Taken together, these results strongly suggest an early upregulation of ADAM17 in intestinal epithelial cells during PMNL transepithelial migration linked to proinflammatory cytokines production, whereas Timp3 is down regulated during the early phase of neutrophil migration.

OP22-4

CARDIAC RATHER THAN INTESTINAL-TYPE BACKGROUND IN ENDOSCOPIC MUCOSAL RESECTION SPECIMENS OF PRIMARY ESOPHAGEAL ADENOCARCINOMA

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Background: The most important complication of Barrett's esophagus is adenocarcinoma, and recently the incidence of Barrett's adenocarcinoma has been increasing very rapidly, especially in male Caucasians in Western countries. It is widely accepted that intestinal-type mucosa in the columnar-lined esophagus (CLE) is the probable common precursor of adenocarcinoma. Many publications focusing on the background or original mucosa of Barrett's adenocarcinoma have maintained that adenocarcinoma arises in intestinal-type mucosa with goblet cells in the CLE, and this has become a central dogma. Materials and Methods: The mucosa on each side of a series of 151 small esophageal adenocarcinomas endoscopically resected was recorded as the background mucosa. The mucosae were classified as squamous, fundic-type, cardiac-type, and intestinal-type on the basis of routine histopathology, mucin staining (alcian blue, pH 2.5) and immunohistochemical staining (MUC-2, human gastric mucin, MUC-6, CD10). Histologic evaluation was performed by five Japanese and German pathologists. The type of mucosa on both sides, immediately adjacent to the adenocarcinoma, was recorded. The 151 adenocarcinomas were classified on the basis of Japanese criteria, according to the Japanese Classification of Gastric Carcinoma, 2nd English edition. Results and Discussion: The present joint pathologic examination of the background mucosa of Barrett's adenocarcinoma conducted by Japanese and German pathologists found that about 70% of primary early and small adenocarcinomas of the esophagus were adjacent to cardiac-type rather than intestinal-type mucosa. In other words, there was no evidence to support the previously held view that Barrett's adenocarcinoma is nearly always accompanied and preceded by intestinal-type mucosa. Conclusion: Follow-up is recommended irrespective of whether intestinal-type or cardiac-type mucosa is found in biopsy specimens from CLE. Also, it seems better to define Barrett's esophagus as metaplastic CLE alone, without requiring the presence of goblet cells.

OP22-5

CONFLUENT BASAL APOPTOSIS IN COLONIC ADENOMAS: MORPHOLOGY, POTENTIAL FUNCTIONAL SIGNIFICANCE, AND PRELIMINARY REVERSE PHASE MICROARRAY FINDINGS FOR APOPTOSIS-RELATED PROTEINS

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BACKGROUND: Apoptosis extent and pattern differs among colonic adenomas,and may correlate with progressive alterations in cell proliferation,growth,and increasing genomic instability.We(Brodie et al 2004)have reported that the confluent basal pattern of apoptosis very significantly correlates with small([LTEQ]5mm)adenoma size,whereas the crypt luminal apoptosis pattern correlates with larger adenomas and high grade dysplasia,and is the typical apoptosis pattern of adenocarcinoma. In this study we extend observations on confluent apoptosis and present preliminary tissue proteomics results for apoptosis/cell proliferation proteins in colonic tumourigenesis. METHODS: Fifty sequential colorectal biopsies of tubular adenomas with low grade dysplasia ([LTEQ]5m) were assessed as previously described by Brodie et al (2004). The definition of confluent basal apoptosis was expanded to include those crypts in which clusters of basal apoptotic bodies were discontinuous but overall measured at least the equivalent of the crypt diameter.Fresh tissue from separately processed colonic tissue samples (normal colonic mucosa,adenomas, and colonic adenocarcinomas) was subjected to laser capture microdissection and processed for reverse phase protein microarrays(Paweletz, 2001).Lysates were probed for 17 apoptosis related proteins,including those of the MAPK cell proliferation and differentiation pathway. RESULTS: All small adenomas had basal apoptosis. Twenty-one (42%) small tubular adenomas had confluent basal apoptosis, and 29 (58%) had scattered basal apoptosis.Confluent basal apoptosis was conspicuous but focal in each adenoma section, often sparing crypts.It was most prominent at crypt convolutions and branching points.Preliminary tissue proteomics results: There was differential expression of several probed proteins e.g. pAkt,pFAK,MEK,pMEK in adenocarcinomas.In 2 large adenomas there was differential expression of EGFR and pAKT. However,there were no consistent alterations in small adenomas. CONCLUSION: Variations in tumour morphology often reflect specific molecular or pathogenetic mechanisms, some of which may become the basis of new therapies.Confluent basal apoptosis may be considered a typical but very distinctive feature of early tubular adenomas and tubulovillous adenomas. Because it is prominent in convolutions and branch-points we will investigate whether it represents anoikis(apoptosis due to cell-cell or cell-matrix detachment),using reverse phase arrays to probe integrins and other proteins on lysates prepared from paraffin sections and confocal microscopy.

OP22-6
PROGNOSTIC AND PREDICTIVE VALUE OF THYMIDYLATE SYNTHASE EXPRESSION IN COLON CANCER

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Background: Surgery is the primary treatment of colon cancer, resulting in cure in approximately 50% of patients, while recurrence following surgery remains the major problem. Treatment with 5-fluorouracil (5-FU) significantly improves patients' survival rate. The antitumor effect of 5-FU has been ascribed to a number of mechanisms including a competitive inhibition of thymidylate synthase (TS, EC 2.1.1.45), an enzyme responsible for DNA synthesis. Aim of our study was to examine the clinical importance of TS expression in colon cancer, and to correlate it with various clinicopathological parameters and patients´ outcome. TS expression was also correlated with tumor cells' proliferative capacity and the expression of cell cycle related molecules (cyclin D1, cyclin E, pRb, p16 and p21). Methods: TS expression was examined immunohistochemically on paraffin-embedded tissue sections obtained from 71 patients, who underwent surgical resection due to colon cancer without preoperatively administration of chemo- or radiotherapy. TS positivity, was correlated with patients' age and gender; tumor location, Dukes' stage, histological grade, and proliferative capacity (Ki-67 immunohistochemical expression); as well as with patients' final outcome. Additionally, TS expression was correlated with that of cyclin D1, cyclin E, pRb and p21, detected immunohistochemically. Result: Positivity for TS was found in 51 out of 71 (71.83%) colon cancer cases, being statistically significantly correlated with patients´ gender (P=0.012) and tumor histological grade (P=0.032). TS expression was also positively correlated with the expression of pRb and p16, (P=0.001 and P=0.001, respectively), while a positive trend for cyclin E (P=0.042) was noted. The overall five years survival rate was 40% for TS positive patients and 68.6% for TS negative ones (P=0.0134). In patients aged more than 70 years, the five years survival rate was 30% for TS positive and 77.8% for TS negative ones (P=0.0008). In multivariate analysis of survival (9 variables, including TS), TS expression proved of prognostic significance (P=0.0174). Conclusion: TS was expressed in the majority of colon cancer cases examined, being statistically significantly correlated with clinicopathological parameters and the expression of cell cycle-related molecules. The prognosis of TS positive patients was worse than that of TS negative, especially in patients older than 70 years. Multivariate analysis confirmed that TS expression can be used as an independent prognostic risk factor for colon cancer patients.

OP23-1
HUMAN PAPILLOMAVIRUS (HPV) PROFILES OF VULVAR LESIONS: POSSIBLE IMPLICATIONS FOR THE CLASSIFICATION OF VULVAR SQUAMOUS CELL CARCINOMA PRECURSORS AND FOR THE EFFICACY OF PROPHYLACTIC HPV VACCINATION

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Background: The term vulvar intraepithelial neoplasia (VIN) was introduced by the International Society for the Study of Vulvovaginal Disease (ISSVD) in 1986 and incorporates three grades of usual VIN (u-VIN I-III) and the differentiated VIN (d-VIN). While u-VIN is associated with the human papillomavirus (HPV) infection, d-VIN belongs to the HPV negative pathway of vulvar carcinogenesis. In the revised ISSVD 2004 classification, the u VIN I category was abandoned and u-VIN II and III were merged. Further, an alternative Bethesda-like terminology presenting the term vulvar intraepithelial lesion (VIL) was proposed. To analyze the validity of the newly introduced classifications of vulvar precancerous lesions and to assess the presumable efficacy of the prophylactic HPV vaccination, we correlated histopathological features and HPV profiles of various vulvar non-neoplastic, precancerous and neoplastic lesions. Method: The total of 269 vulvar excisions representing lichen sclerosus (n=35), lichen simplex chronicus (n=14), condylomata acuminata (CoA; n=57), d-VIN (n=12), all grades of u-VIN (n=82) and squamous cell carcinomas (SCC; n=69) were subjected to the HPV typing by use of GP5+/6+ PCR and reverse line blot hybridization. Detected HPV types were stratified into low-risk (LR), high-risk (HR), probably high-risk and undetermined-risk groups. Histopathological categorization of vulvar lesions was correlated with their HPV profiles. Results: High prevalence of HPV DNA was found in CoA (94.7%) and u-VIN (98.8%) being lower in SCCs (42%). While LR-HPV types prevailed in the HPV positive CoA (96.3%), HR-HPV types (especially HPV 16, 33 and 45) were detected in the majority of the HPV positive u-VIN (97.6%) and SCCs (93.1%). A tendency to the multiple-type HPV infection was observed in CoA (17.5%) and u-VIN II (41.7%). Importantly, our results showed differing HPV profiles, as well as the frequency of multiple-type HPV infection and the age structure in patients with u-VIN II and III. The biological heterogeneity within the u-VIN II group was also demonstrated. U-VIN I was distinguished as a rare disorder associated with HR-HPV. Conclusion: The ISSVD 1986 terminology seems to be optimal for the classification of vulvar precancerous lesions. The spectrum of HPV types found in vulvar SCCs indicates that the efficacy of HPV vaccination in the prevention of vulvar cancer might be decreased in the studied population, because prophylactic vaccines are targeted against a limited number of HPV types. Acknowledgement: Supported by IGA MZ CR NR/8852-3 and VZ FNM 00000064203.

OP23-2
FASCIN IMMUNOHISTOCHEMICAL INVESTIGATION IN ENDOMETRIAL STROMAL SARCOMAS AND PRIMARY UTERINE LEIOMYOSARCOMAS

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Background: Endometrial stromal sarcoma (ESS) of the uterus is a rare uterine neoplasm. It accounts for approximately 10-13% of uterine sarcomas and 0.2% of all uterine malignancies. There are two types of ESS: low-grade ESS (LGESS), and high-grade ESS (HGESS). Primary uterine leiomyosarcomas (LM) comprise approximately 1% of uterine malignancies. Often issues of differential diagnosis arise when a pathologist encounters a tumor (especially in endometrial biopsy material) with high cellularity, increased mitotic index and areas of necrosis. Recent reports indicate that immunohistochemistry can be useful in affording a

distinction. In the present study we assessed the usefulness of fascin in distinguishing these two neoplasms Methods: 17 ESS and 11 LM cases were collected from 4 different Institutes for the period 1998-2006. Representative sections from FFPE tissue blocks of the tumors were re-cut and stained in automated staining system using the mAb NCL-Fascin. Results: The mean age of the patients with ESS was 52 years (range 25-71), while for LM was 63 years (48-89). 12/17 ESS was of low histologic grade. Fascin expression was detected in 16/17 ESS (12/12 LGESS; 5/6 HGESS) and in 8/11 LM. Conclusions: As our data indicates fascin is widely expressed in both investigated groups of sarcomas (94% for ESS vs 73% for LM). Given what is currently known about fascin, the widespread immunoexpression of fascin in primary uterine sarcomas of stromal and smooth muscle differentiation may indicate a potential factor in tumor cell infiltration and a risk factor of pelvic recurrence.

OP23-3

COXSACKIEVIRUS ADENOVIRUS RECEPTOR (CAR) EXPRESSION IN ENDOMETRIAL ADENOCARCINOMA

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Background: Coxsackievirus adenovirus receptor (CAR) functions as the primary receptor for both coxsackie B viruses and adenoviruses, playing a crucial role for their entry into host cells. CAR expression on target tumoral cells is correlated with their sensitivity to adenoviral infection. Tumoral cells lacking or expressing low CAR levels are resistant to adenovirus infection and to efficient oncolysis by recombinant adenoviruses. The aim of our study was to examine CAR expression on clinical material of endometrial adenocarcinoma and to correlate it with clinicopathological parameters, tumors' proliferative capacity and patients' survival. Methods: CAR expression was detected immunohistochemically on paraffin-embedded tissue sections of 41 endometrial adenocarcinoma cases. CAR positivity, overexpression (expression in more than the mean percentage value), and intensity of immunostaining were correlated with patients' age and FIGO stage; tumors' histopathological type (adenocarcinoma with or without squamous elements) and grade of differentiation, proliferative capacity (Ki-67 immunohistochemical expression); and patients' survival. Results: CAR positivity was noted in 23 out of 41 (56%) endometrial adenocarcinoma cases and CAR overexpression in 8 out of 23 (35%) positive cases. CAR intensity of immunostaining was classified as mild in 11 (48%), moderate in 10 (43%) and intense in 2 (9%) out of the 23 positive cases. CAR positivity was statistically significantly correlated with tumors' histopathological grade of differentiation (p=0.036). CAR intensity of immunostaining was statistically significantly correlated with tumors' histological type (p=0.016), as cases possessing squamous elements presented more frequently intense CAR immunostaining. CAR overexpression also presented a trend of correlation with tumors presenting increased proliferative capacity (p=0.057). CAR positivity, overexpression and intensity of immunostaining were not statistically significantly correlated with the other clinicopathological parameters examined and patients' survival. Conclusion: Our data reveal, for the first time, the expression of CAR protein in clinical material obtained from patients with endometrial adenocarcinoma in correlation with clinicopathological parameters. As CAR appears to modulate the proliferation and characteristics of cancer cells, playing a crucial role in malignant transformation, its expression is considered of possible clinical importance in the future.

OP23-4

EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF CYCLIN D3 IN OVARIAN ADENOCARCINOMAS

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Background: Abnormal expression of cell cycle regulators may contribute to malignant transformation. Cyclins are prime cell cycle regulators which play a key role in the control of major checkpoints of cell cycle progression. Recently, experimental data have been published implying that the relationship between D-type cyclins and oncogenesis may not be as simple as initially thought, given the emerging dual role of cyclin D1 and D3 as regulators of both cell cycle progression and arrest. For example, cyclin D1 overexpression is associated with unfavourable prognosis in pancreatic carcinoma and serous ovarian adenocarcinoma, but with a better survival in breast, hepatocellular and lung carcinoma Moreover, the clinical significance of the expression of cyclin D3 in ovarian cancer remains undefined. We therefore conducted a retrospective investigation to address the role of this cell-cycle protein in this tumour. Methods: In our study, paraffin-embedded tissue from 109 non-benign epithelial ovarian tumours, including 17 tumours of low malignant potential and 92 primary adenocarcinomas, was stained immunohistochemically for cyclin D3 by using a mouse monoclonal anti-cyclin D3-antibody and a sensitive polymer-based system. Most of the cases had previously been stained for pRb, p21Cip1, p27Kip1, p53, and Ki-67 antigen. Expression of cyclin D3 was correlated with clinicopathological features, the expression of other cell cycle regulators, and postoperative survival of patients. Results: Cyclin D3 levels were significantly higher in tumours of low malignant potential than in adenocarcinomas (P = 0.0002). In the latter group, cyclin D3 expression decreased with increasing grade (P = 0.0004) and advancing stage (P = 0.0315). Cyclin D3 expression positively correlated with pRb, p21Cip1, and p27Kip1 levels (P = 0.0021; P = 0.0036; P G 0.0001, respectively) and negatively with p53 and Ki-67 (P = 0.0003; P G 0.0001). Absent cyclin D3 expression was an important indicator of poor survival in univariate analysis in the entire cohort (P 9 0.00010) and in the platinum-treated patients (P = 0.001) and in multivariate analysis (P = 0.044). Conclusions: Our results demonstrate that absent or decreased cyclin D3 expression is adversely related to several clinicopathological indicators of aggressiveness in ovarian adenocarcinomas and is combined with a better prognosis, suggesting that cyclin D3 may have a biological role distinct from that of other G1 cyclins which is possibly regulated through interaction with other cell cycle genes.

OP23-5

EXPRESSION OF AMP-ACTIVATED PROTEIN KINASE (AMPK) IN OVARIAN CARCINOMA: CORRELATION WITH TUMOR GRADING AND PROGNOSIS

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Background: Adenosine monophosphate (AMP)-activated protein kinase (AMPK) plays a central role in regulating the energy metabolism in cells. In addition, activated AMPK is known to inhibit the mTOR pathway in cancer cell lines, which leads to an attenuation of cell growth and proliferation. The recently identified upstream kinase of AMPK is LKB1, a known tumor suppressor in Peutz-Jeghers syndrome. However, little is known on AMPK expression in human malignant tumors. Method: To address this apparent lack of translational information, we

investigated AMPK expression in normal ovaries (n=5) and in tissue microarrays (TMA) of ovarian tumors (n=84) by immunohistochemistry. We correlated our findings with clinicopathological patient characteristics and progression free as well as overall patient survival. Results: We observed loss of cytoplasmic AMPK expression in 44.3% of ovarian carcinomas. A decrease in AMPK expression correlated with higher tumor grade (p=0.009). High expression of AMPK was significantly associated with prolonged overall survival time in patients with advanced tumor stages (p=0.016) as well as in patients with serous ovarian tumors (p=0.037). Conclusion: Our study shows that loss of AMPK is more prevalent in poorly differentiated ovarian carcinomas and might serve for an important and large subgroup of ovarian cancer patients as a predictor of poor survival, which underscores its biological significance. This hints on AMPK as an attractive target for novel antineoplastic treatment strategies.

OP23-6

FALLOPIAN TUBE EPITHELIUM: THE IGNORED HYPOTHETIC ORIGIN OF GYNECOLOGIC SEROUS CARCINOMAS

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BACKGROUND: Tubal inner surface epithelium, due to its histological resemblance to the neoplasm, has been suggested as one of the possible tissues of origin for serous female tumors for a long time. We aimed to make an immunohistochemical and morphological comparison between normal and abnormal looking tubal epithelium of two different serous tumor groups (ovarian and peritoneal) . METHOD: Fallopian tube samples from 26 cases of ovarian, 28 cases of peritoneal serous carcinomas were included.Tissue array blocks containing 2 to 4 samples, from each case were prepared. For immunohistochemical study p16, p53, WT1, CD24, calretinin and Ep-CAM antibodies were used. For all antibodies, cases were evaluated for staining rate, intensity and pattern seperately. Cytoplasmic and membranous staining for CD24, membranous staining for Ep-CAM, nuclear and/or cytoplasmic staining for p16, p53 and WT1 were taken into evaluation. RESULTS: The intensity of p16 staining was strong in ovarian (57,7%) and the peritoneal group (60,7). Peritoneal carcinoma group had the highest number of diffusely positive cases for p16 (35,7%-10/28). It was noted that strong and diffuse p16 and p53 positivity always accompanied the obvious atypical changes in the tubal epithelium.Two peritoneal cases were found to display p16 and p53 positive atypical changes in the tubal epithelium. CD24 showed strong and diffuse positivity in both groups. CD24 showed cytoplasmic staining in 15,4% of ovarian and 21,4% of peritoneal cases. Ep-CAM displayed similar staining intensity and rate with CD24. Over 57,1% of the tubal epithelium of the ovarian and peritoneal cases showed strong and diffuse positivity with WT1.Calretinin was mostly negative and weak in intensity in both groups. CONCLUSION: Existence of atypical changes associated with strong and diffuse expression of p16 and p53 in the tubal epithelium of both the ovarian and peritoneal serous carcinomas and variable expression of these markers in normal-looking tubal epithelium is an important finding. Also, in a few areas, the cytoplasmic expression of CD24 was noted instead of the expected membranous staining. These observations may support the hypothesis of common tubal origin for ovarian and peritoneal serous carcinomas. Prospective studies in which the whole fallopian tube is sectioned are required for further support.
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OP24-1

DIAGNOSIS OF BACTERIAL INFECTIONS IN HUMAN LUNG DISEASES : POTENTIAL USEFULNESS OF FLUORESCENT IN SITU HYBRIDIZATION (FISH) TECHNIQUE IN FIXED AND PARAFFIN EMBEDDED TISSUES

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An important problem in the diagnosis of lung bacterial infection is the evaluation of microbiological results. Moreover, histological approach of bacterial pneumonia does not allow to make an etiological diagnosis, since a precise identification of bacilli and cocci is impossible in paraffin sections, most of the time. In situ hybridization is gaining popularity and with the development of automated and more standardized methods, it is becoming more widely available and more cost effective. This method has been used to detect the presence of a variety of microorganisms in tissue sections. Although it is most commonly used to detect viral targets, in situ hybridization can also been used to detect bacteria. The aim of this work was to identify different bacteria species in lung tissues using FISH method and to compare the signal obtained in different specimens fixed in formalin and in alternative preservation methods (RCL2, Excell plus, Glyofix, Finefix solutions). We used a series of 10 patients with bacterial pneumonia and the following probes: the oligonucleotide probe EUB338, which binds to the 16S RNAs of all eubacteria, Spn pB-00350 (Streptococcus peumoniae), PseaerA pB 00384 (Peudomonas aeruginosa), Sau pB 00349 (Staphylococcus aureus), and EC01167-ECO 45A pb-00115 (Escherichia coli). The results obtained by fluorescence microscopy can be summarized as follows: i) hybridization with the positive control (EUB338) revealed that all eubacterial cells are visualized by this technique without any difference according to the fixative, ii) 5 cases showed different associated bacteria species (S. pneumoniae and P. aeruginosa: 3 cases; S. pneumoniae and E. coli: 2 cases), iii) 2 cases revealed the presence of S. aureus, and iv) 3 cases were negative with the specific probes used in this study. In conclusion, FISH method using probes against bacteria can be performed after formalin fixation and different alternative preservation methods. This method increases the specificity and in some instances may increase the sensitivity of histopathologic tests. FISH method may be particularly useful when bacteria are present in low numbers, stain poorly, are uncultivable, or exhibit an atypical morphology.

OP24-2

MOST LUNG CARCINOMAS ASSOCIATED WITH IDIOPATHIC PULMONARY FIBROSIS SHOW FEATURES OF BRONCHIOLAR DERIVATION

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The association of lung cancer and idiopathic pulmonary fibrosis (IPF) is a well known event, but the pathogenesis of this association is poorly understood. According to recent data, the pathogenesis of IPF is not inflammatory, but related to abnormal tissue remodelling following chronic damage. In previous studies we proposed that abnormal bronchiolar proliferation is a major event in IPF, triggered by molecular abnormalities (wnt/beta-catenin, p53/p21waf1 pathways) involving progenitor basal cells at the bronchiolo-alveolar junction. In this study we have investigated the morphologic and immunophenotypic features in a series of lung carcinomas developed in patients with IPF. Material and Methods. Paraffin blocks of 17 cases of lung cancer arising in patients with IPF were retrieved from the files of our Institutions and analyzed using a panel of markers useful for characterising different epithelial cells and airway (basal cells) and alveolar (pneumocyte type II) precursor cells. Results. Overall there were 6 peripheral squamous carcinomas, 8 adenocarcinomas, 2 large-cell carcinomas and 1 small-cell carcinoma. Among adenocarcinomas 3/8 exhibited a peculiar and rare immunophenotype consistent with metaplastic-enteric type carcinoma as defined by the expression of the intestinal markers cdx-2, MUC2, and villin. 2 cases were classified as mucinous-BAC, and expressed at high level MUC5, a mucin expressed by goblet bronchiolar cells. Other 2 were conventional adenocarcinomas lacking surfactant protein-A (SPA). Only two cases (one adenocarcinoma and one large-cell carcinoma) showed focal expression of the Clara-cell CC10 antigen and SPA. A single large-cell carcinoma showed lymphoepithelioma-like features with focal expression of squamous markers such as CK5 and DN-p63. Abnormal p53 expression (>50% positive cells, with concomitant low/absent p21waf1) was demonstrated in 4/6 squamous carcinomas, both large-cell carcinomas, and 2/8 adenocarcinomas. Conclusions. Striking divergence from carcinoma cases observed in non-IPF patients, including the prevalence of rare entities, is shown. The peculiar features of our series suggest that most cases have a “bronchiolar” derivation, in line with the proposed model of IPF pathogenesis. In particular, the expression of pneumocyte-related markers was rare, whereas the expression of an enteric-type “metaplastic” phenotype was exceedingly represented (37,5%). It is possible to speculate that also the peripheral squamous carcinomas can be considered as “metaplastic”, arising from transformed bronchiolar basal cells.

OP24-3

INFLAMMATORY MYOFIBROBLASTIC TUMOUR OF THE LUNG – MORPHOLOGY DECEIVES HISTOGENESIS

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Inflammatory myofibroblastic tumours (IMT) are represented by spindle cells, lymphocytes, plasma cells, macrophages and variable collagen deposition. Children and adults present this tumour that arises mainly in lung and also in mesentery and retroperitoneum. Being a rare tumour, 19 cases were collected from the archives of five Departments of Pathology to be studied under an immunohistochemical and genetic point of view to follow the pass of histogenesis. The patients had been submitted to lobectomy (14 cases) or pulmonary resection (5), there were 10 females and 9 men with ages ranging from 19 to 65 years. The immunohistochemical panel was chosen in order to determine the properties of the tumour spindle cells and also to allow differential diagnosis with the other spindle cell tumours of the lung; vimentin was demonstrated in all cases indicating the validity of this study based in formalin-fixed paraffin-embedded tissue; actin and calponin were revealed in 18 cases; CD68 was a constant marker in spindle cells. The antibodies directed to search dendritic cell differentiation (CD35; CK8 and CK18) were weakly positive in 4 cases. The ALK antibody applied (ALK1, DAKO) was revealed in 5 cases. FISH analysis was performed in all cases, corresponding to paraffin-embedded tissue, by the application of the Vysis LSI ALK probe assay. Only 6 cases showed till 5% of cells with the split signal indicating a translocation of the ALK gene. In these cases it was not possible to reveal the ALK antibody by immonohistochemistry. In this study it was clear an immunohistochemical macrophagic differentiation of the spindle cells in all the 19 cases of IMT of the lung classified according with the criteria defined in WHO 1999/2004 classification. It indicates that the histogenesis of this particular tumour is related with the concept of the histiocyte as a tissue macrophage that sometimes and somewhere acquires genetic alterations not yet demonstrated. Being the lung an organ of antigen contact and defence and according with the results of this study, IMT is a tumour of the monocytic – macrophagic system (related to the classical reticulum – endothelial system as complementary auto-immune defence). IMTs may be understood as a predominantly low grade tumour of monocytic – macrophagic origin (in parallel with the clinical behaviour of MALT lymphoma).

OP24-4

PROGNOSTIC IMPACT OF INTRATUMORAL AND STROMAL LYMPHOCYTIC INFILTRATE IN NON-SMALL-CELL LUNG CANCER

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Background: Lung carcinoma is the most common cancer in the world (12.6% of all new cancer, 17.8% of cancer deaths), with an overall 5 years survival of 15%. The identification of events that are predictive of the rate of progression towards metastatic cancer, and of new treatment modules, including immunotherapy,

remains a big challenge. The immune system has a paradoxical role during cancer development; activation of the adoptive immune system can eradicate malignant cells, whereas activation of various types of innate immune cells might promote tumor development. However, the significance of tumor infiltrating lymphocytes, especially in the stroma, as a prognostic factor in non-small cell lung carcinoma (NSCLC) is controversial and not well studied. The aim of this study is to elucidate the prognostic significance of intratumoral and stromal lymphocytes in NSCLC. Methods: Tumor samples from 335 resected NSCLC, stages I to IIIA were obtained and tissue microarrays were constructed from duplicate cores of viable tumor tissue and surrounding stroma. Immunohistochemistry was used to evaluate the infiltrate of CD20, CD4, CD8 lymphocytes. The disease-free survival curves were calculated by the Kaplan-Meier method and compared using the Log-rank test. Multivariate analysis was carried out by Cox's proportional hazard method, and Chi-Square test for correlation with the clinicopathological variables. Results: In univariate analyses, a high number of CD8 T lymphocytes in the tumor (P = .023) and in the stroma (P=.002) was significant positive prognostic indicators for a better disease-specific survival (DSS). No such relation was noted with CD4 and CD20. The most significant correlation between stromal CD8 lymphocytic infiltration and DSS was observed in patients with T2 stage and with squamous cell carcinoma. Furthermore, a low level of stromal CD8 lymphocytes is associated with an increased incidence of angiolymphatic invasion (P=.032). In multivariate analyses, high CD8 cells in the stroma (P = .003) was an independent positive prognostic factor for DSS. Conclusion: A high number of CD8 positive lymphocytes in the stroma of NSCLC is a favorable independent prognostic factor; its significance is higher than that of tumor grade and angiolymphatic invasion. It is also associated with a lower incidence of angiolymphatic invasion.

OP24-5

CATHEPSIN-K IMMUNOHISTOCHEMISTRY IN THE SENSITIVE AND SPECIFIC DETECTION OF MICRO-GRANULOMAS IN HYPERSENSITIVITY PNEUMONITIS

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Background: Cathepsin-K (Cath-K) is a cysteine protease with high matrix-degrading activity that plays an important role in osteoclast function. Its expression is regulated by Mitf transcriptional factor family. Cath-K is expressed also in epithelioid cells and in multinucleated giant cells but not in resident macrophages, suggesting that it could be a marker of macrophage activation and differentiation. Hypersensitivity Pneumonitis (HP), also known as Extrinsic Allergic Alveolitis, is a diffuse interstitial granulomatous lung disease that represents an immunologic reaction to inhaled organic antigens or to chemicals. Histologically, small noncaseating granulomas and mononuclear cell infiltrates are diagnostic criteria of HP, but detection of small aggregates of epithelioid cells and distinction from alveolar macrophages is at times difficult. Different macrophage markers can be used to detect and quantify granulomas and epithelioid cells (CD68, CD45RO, CD16 and others) but none of these allows a specific and sensitive evaluation. In this work we aimed to study expression of Cath-K in interstitial lung diseases and its utility in diagnosis of HP. Method: We studied Cath-K expression in 20 HP cases and in control cases: 3 normal lung tissue samples, 7 pulmonary Wegener Granulomatosis, 3 Sarcoidosis, 3 Airway-Centred Interstitial Fibrosis (ACIF) and 25 cases of other interstitial lung diseases. Granulomas were quantitatively evaluated scoring Cath-K expression as follows: negative: no immunoreactivity; 1+: scattered small granulomas; 2+: scattered large granulomas; 3+: numerous large granulomas. Results: 16/20 HP (80%) contained Cath-K-positive granulomas: in 4/20 cases (20%) score was 3+; in 4/20 (20%) 2+ and in 8/20 (40%) 1+. In 4 cases (20%) granulomas were absent. In control cases, Cath-K was strongly expressed in granulomas of Wegener Granulomatosis and Sarcoidosis but was negative in alveolar macrophages of normal lung tissue and in all interstitial lung diseases, including cases with large collections of macrophages (RB-ILD, DIP, alveolar haemorrhage). No granulomas were detected in the 3 cases of ACIF. Conclusion: In our study Cath-K was strongly expressed in epithelioid and giant cells of granulomas (80% of HP had Cath-K-expressing granulomas) but was negative in intraluminal alveolar macrophages, confirming Cath-K as a good marker for sensitive and specific detection of small interstitial granulomas in Hypersensitivity Pneumonitis and as a useful tool in diagnostic routine of interstitial lung diseases.

OP25-1

TUMOURS WITH PERITONEAL METASTASIS OF COLORECTAL OR APPENDICEAL ORIGIN ARE A SPECIFIC SUBGROUP

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BACKGROUND: A specific subgroup of colorectal and appendiceal neoplasms progress with widespread peritoneal metastasis rather than the more common metastatic spread to the liver. A histologic classification of peritoneal metastasis in the clinical context of pseudomyxoma peritonei (PMP) according to Ronnett et al. has been shown to predict survival. This study investigates if a similar classification is useful for all cases with peritoneal metastasis of colorectal or appendiceal origin. And if these cases represent a specific subtype of colorectal and appendiceal tumours. METHODS: In the Netherlands Cancer Institute patients with peritoneal metastasis from primary colorectal or appendiceal origin are treated with Hypertermic Intraperitoneal Chemotherapy (HIPEC). Distant metastasis are an exclusion criterion for this treatment. Histology of peritoneal metastasis of all patients (n=269) treated with HIPEC in this institute between 1995 and 2006 was evaluated using a semi quantitative analysis of mitotic activity, atypia, cellularity, and the mucinous component. Using these criteria peritoneal metastasis were classified according to Ronett in DPAM (Diffuse Peritoneal Adeno Mucinosis), PMCA (Peritoneal Mucinous Carcinomatosis) and an intermediate subtype (PMCA-i). Metastasis consisting for less than 50% of mucin were classified as PCA (Peritoneal Carcinomatosis). Furthermore, tumour type was described according to WHO standards. RESULTS: Peritoneal metastasis classified as DPAM (n=101) had a significant better outcome, originated in 97% from the appendix and were more common in women (F:M = 64%:36%). The other peritoneal metastasis were mucinous in 44% (PMCA or PMCA-i). Of PCA, 26% was partly mucinous. These partly mucinous PCA seemed more common in men. PMCA and PMCA-i originated from the appendix in 41%, whereas PCA was only in 14% of appendiceal origin. Of all primary colorectal tumours 37% had mucinous peritoneal metastasis, whereas 16% were partly mucinous. Of all cases 15% were of a non-intestinal tumour type (signet-ring or goblet) CONCLUSION: DPAM seems to be a separate disease with a specific clinical outcome that should be recognised within the group of patients with peritoneal metastasis of colorectal or appendiceal origin. Peritoneal metastasis of the non-DPAM type are mucinous relatively often in contrast to what is known of the incidence of mucinous tumours of colorectum and appendix. This suggests a specific genetic pathway for the development of peritoneal metastasis.

OP25-2
IMMUNO-EXPRESSION OF PRO- AND ANTI-APOPTOTIC PROTEINS IN H. PYLORI INFECTION ASSOCIATED WITH GASTRITIS AND DUODENAL ULCER

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Background: H. pylori infection induces apoptosis in the gastric epithelium, which might be related to atrophy development. There are no data about apoptosis in H. pylori gastritis associated with duodenal ulcer (DU). Most data on record point to involvement of the intrinsic pathway, but over-expression of Fas and Fas-ligand (Fas-L) proteins was reported. The aims of this study were to evaluate the expression of proteins of the intrinsic and extrinsic apoptotic pathways in patients with H. pylori gastritis and in relationship to DU and atrophy development. Method: The expression of bcl2 family proteins, FAS and FAS-L was studied by immunohistochemistry (LSAB) in the antral mucosa of 57 H. pylori infected patients, 18 with DU and in 14 non-infected patients with normal mucosa. The immuno-expression was scored considering the intensity (0-negative; 1-weak; 2- moderate; 3- strong) and extension (1:<10%); 2:10-25%; 3:25-50%; 4:50-75%; 5:>75%) of the reaction in the superficial epithelium and foveolae; the final result in each region and in each case was the sum of these scores. Results: Bax was significantly over-expressed in H. pylori gastritis, both in the superficial epithelium and foveolae and considering the reaction intensity and the percentage of stained epithelium, in comparison to cases with DU ($p \leq 0.03$) and non-infected patients ($p \leq 0.2$). Bak was over-expressed in the superficial epithelium of non-infected and in DU patients in comparison to those with gastritis ($p=0.04$, $p=0.048$, respectively). However, in the final evaluation of the antral mucosa, the reaction intensity was significantly higher in gastritis than in DU. Bcl-x was significantly over-expressed in patients with gastritis compared to non-infected ones ($p \leq 0.04$) and to those with DU ($p=0.03$). Fas expression was negative or weak in most of the cases and the expression of Fas-L was higher in non-infected mucosae than in gastritis ($p=0.05$). Bax, Bak and Bcl-x were over-expressed in the foveolae of cases with atrophy compared to non-atrophic ones ($p \leq 0.05$) and the percentage of superficial epithelium expressing Bcl2 was higher among atrophic gastritis ($p=0.03$). On the other hand, Fas-L was significantly over-expressed in non-atrophic cases ($p=0.05$). Conclusion: Apoptosis in H. pylori infection was mainly related to the intrinsic pathway. The expression of pro-apoptotic proteins (Bax/Bak) was significantly higher in patients with gastritis than in those with DU, which might be related to atrophy development in the former group. Financial support: CNPq, CAPES.

OP25-3
LYMPH NODE ANALYSIS IN COLORETAL CANCER: THE IMPACT OF THE USE OF CLEARING FAT SOLUTION

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Background: Lymph node (LN) involvement is one of the most important criteria for staging colorectal cancer (CRC). Special procedures have been recommended in order to improve LN analysis and metastasis detection. The aims of this study were to evaluate the impact of using a fat clearing solution in LN dissection. Method: We analysed prospectively 74 CRC (35 men, median age 62.9 years-old). After routine (conventional - CM) LN dissection, the fat was emerged in a mixture containing 65% alcohol, 20% ether, 5% acetic acid and 10% formalin (at 10%) for 24 hours (2 changes). A new LN search was performed. LN diameters were measured using a computerized system (KS400, Karl Zeiss, Germany). The number of positive and negative LN with <4mm was determined. The number and measures of the LN obtained by both methods were compared and analysed in respect to patients and tumour characteristics. Results: All CRC were adenocarcinomas, 81% moderately differentiated and 69% pT3; 3167 LN (42.8/case) were dissected: 1814 (24.5/case) by the CM and 1355 (18.3/case) after the use of the clearing solution (CS). Metastasis were detected in 196 LN: 170 (86.7%) by the CM and 26 (13.2%) after CS; 59% of the LN dissected conventionally had <4mm (n=1068, 14.4/case; range 0.4-23mm); 93% of the LN dissected after CS had <4mm (n=1261, 17/case; range 0.2-11mm). Among the 170 positive LN (LN+) conventionally dissected, 39 (22.9%) had <4mm; 73% of the LN+ dissected after CS measured <4mm ($p=0.000$). The use of the CS increased the LN number in 74.7% ($p=0.000$), the detection of small LN in 118% ($p=0.000$), and metastasis in 15.3% of the cases ($p=0.003$). The number of LN dissected in both groups was correlated ($r=0.3$; $p=0.004$) and the number of LN+ after CS was higher when at least one metastasis was previously detected ($p=0.007$); pN changed in 5 cases ($p=0.03$); 90% of the LN+ cases were detected only after the dissection of 40 LN. Patient's age ($r=-0,2$; $p=0.04$), LN diameter (smallest: $r=-0.3$;$p=0.01$; biggest: $r=0.3$;$p=0.004$) and the number of <4mm LN ($r=0.9$; $p=000$) were significantly associated with LN number. The number of LN was independently predicted by the pT, the number of <4mm LN and the diameter of the biggest LN. Conclusion: A large number of small LN must be dissected to adequately stage CRC. Fat clearing solutions simple to apply and neither expensive nor time consuming, might be useful for this propose. Financial support: CNPq

OP25-4
ALTERATIONS OF MDR1 AND MRP1 FUNCTIONAL ACTIVITY OF COLORECTAL MALIGNANCIES

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BACKGROUND: The ABC-transporter MDR1 and MRP1 are expressed in the normal mucosa of gastrointestinal tract and early studies assumed elevated expression of these proteins during the colorectal carcinogenesis. Other studies found decreased expression of these proteins in the majority of colorectal cancer cells. Since these transporters are able to pump out various drugs from cells and thus can alter the chemosensitivity of tumor cells, the role of ABC-transporters in the moderate chemoterapeutic response of colorectal carcinoma cells has been investigated by several groups by immunohystchemistry or real-time PCR. Posttranslational modifications influence the functional activity (MDR Activity Factor: MAF) of MDR proteins, so the determination of functional activity could bring new insights in understanding the controversial data and connection between colorectal cancer and MDR. Furthermore, in case of the upcoming treatment modalities which brought better survival in CRC (like irinotecan and oxaliplatin) and which are substrates of the MDR-transporters, MAF might also have impact on treatment and survival. METHOD: Our aim was to determine and compare the functional activity of MDR1 and MRP1 transporters in surgical samples from normal mucosa and primary and metastatic colorectal cancers. Cancer tissue and blood samples were taken after informed consent (98 primary colorectal cancers, 38 hepatic metastases and 23 healthy mucosae) and brought into one-cell suspension with collagenase treatment. MDR1/MRP1 functional activity (MAF) of viable epithelial cells were determined according to the modified protocol of the calcein-assay (MDQuant). MAF-values of blood leukocytes were determined according the original calcein-assay protocol. RESULTS: The mean MDR1 functional activity of primary CRC was significantly lower than in normal mucosa. MDR1-MAF significantly increased in metastases altought didn't reach the activity of normal mucosa. MRP1 activity was similarly low in

healthy mucosa and in primary CRCs and only slightly elevated in metastatic tumors. The elevation of MAF in metastatic cases was also found on peripheral blood leukocytes. CONCLUSION: In contrast to the common dogma of the majority of the scientific litterature, our results showed that primary CRCs have lower mean MDR1 functional activity than normal mucosa cells. The relative increase in MDR activity in metastases may be induced by the applied chemotherapy which took place after the resection of primary tumors. This finding is further supported by the increase of MAF-values of the PBLs.

OP25-5

PROGESTERON INDUCED BLOCKING FACTOR – PIBF AS A POTENTIAL TUMORMARKER

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BACKGROUND: PIBF was first described in the blood and urine of pregnant women. It is secreted by lymphocytes and cells of placenta upon progesterone stimulation and causes a Th1>Th2 shift, which leads to decrease in cell mediated activity of the immune system. This creates a favorable condition for the embryo and in healthy pregnancy PIBF urine levels continuously increase from the first trimester until the end of pregnancy. The elevation of PIBF in the urine samples correlated to the outcome of the pregnancy: higher levels of PIBF predicted normal delivery, while lower PIBF levels predicted preterm delivery. Other investigations showed that PIBF production is also elevated in rapidly proliferating cells and might be in connection with tumor progression, since decreased NK-cell-activity is favorable also for tumor growths. METHOD: Our aim was to investigate PIBF expression in different malignancies. Clinical samples have been obtained from patients with colorectal and pancreatic cancer and a smaller number of other malignancies: mamma, lung, stomach, bile duct cancers, lymphomas, etc. PIBF-levels in the urine samples were determined by one-plate competitive ELISA and statistical analysis of the collected 80 tumor samples, and the 19 normal control and 15 samples from inflammatory diseases confirmed the potential use of PIBF as a tumor marker. RESULTS: The PIBF level in the urine sample of cancer patients was significantly higher than in healthy individuals. In case of colorectal and pancreatic cancers (25 samples each) the significant difference in PIBF levels compared to control samples was confirmed also separately respectively. Patients with inflammatory diseases had higher PIBF levels than in healthy people, but still much lower than in tumor patients. The protein expression of PIBF was simultaneously investigated with more polyclonal and monoclonal antibodies by immunohistochemistry in the tissue blocks of surgical samples of tumor patients. Specific strong positive cytoplasmic-staining was found in cancer cells. CONCLUSION: The investigated surgical samples and clinical urine samples of different malignant diseases showed strong positivity with PIBF-antibodies by ELISA and IHC and strengthened the possibility of applying PIBF as a tumor marker. Further studies are in progress with other malignancies and for the evaluation of PIBF as a tumor progression and monitoring marker.

OP25-6

THE CRUCIAL ROLE OF PATHOLOGIST IN DETERMINING THE PROGNOSIS OF COLORECTAL CARCINOMA CASES: AN ANALYSIS OF 502 CASES WITH COLORECTAL CARCINOMA BY TURKISH NATIONAL WORKING GROUP OF GASTROINTESTINAL PATHOLOGY

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BACKGROUND: Lymph Node evaluation is an important prognostic factor in colorectal cancer (CRC). An accurate gross and microscopic examination of the surgical specimen is very important for the correct assesment of the lymph node (LN) status and other pathological parameters of tumor. We performed a retrospective analysis to determine the clinicopathological parameters associated with poor prognosis in CRCs. METHOD: A total of 502 CRC patients diagnosed and treated with surgery in 14 different hospital located in different cities in Turkey, were reviewed. The clinical and pathological parameters considered in statistical analysis were age, gender, tumor size and location, depth of invasion (AJCC,TNM-2002), histological grade, the number of examined LN, LN metastasis, lymphatic vessel invasion (LVI), blood vessel invasion (BVI), perineural invasion (PNI). Patients were divided in three groups depending on the number of examined LNs; group I (n=190) patients had <9; group II (n=117) had 10-14 and group III (n=195) had ≥15. RESULTS: Patients in our study group were constituted by 294 men, 208 women had a mean age of 61,8 and mean follow-up of 42,7 months (24-122) . The mean number of examined lymph node was 13,8 (range 1-57). Two hundred eight (41,2%) of the patients had LN metastases with mean number 4,1 (range 1-31). Depth of invasion was T1 in 9 (1,8%); T2 in 61 (12,1%); T3 in 275 (54,5) and T4 in 143(28,3%) of cases. There was strong correlation between depth of invasion and lymph node metastasis (p<0,001). Cox regression analysis revealed that BVI had the most significant prognostic effect in disease-free survival (p=0,001); LN involvement (p=0,007) and depth of invasion (p=0,028) had the highest prognostic effect in overall survival in our study group. In Kaplan-Meier analysis, mean survival time(month±SD) was significantly lower in group I (45,44±2,9) than in both groups II (59,6±5,8) and III (75±4,79), (p=0,018). The patients with 4 or more LN metastasis (36,1± 3,7) had poorer prognosis than for those with ≤3 LN metastasis (40,6 ±3,1) and with no metastasis (66,20±3,79), respectively (p<0,001). CONCLUSION: LN metastasis is associated with poor outcome in CRC cases in whom accuracy of staging and survival can be improved with increasing the number of LN examined by pathologists. A standardization of harvesting and reporting in lymph nodes is extremely necessary.

OP25-7

MUCINOUS TUMOURS OF THE COLORECTUM: RIGHT VERSUS LEFT IN TERMS OF MOLECULAR MECHANISMS

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Background: The majority of colorectal cancer cases arise in a background of chromosomal instability, a mechanism associated with loss of heterozygosity leading to tumour suppressor gene inactivation. A small proportion of CRC, however, progress through microsatellite instability (MSI) – "serrated" pathway and these tumours are often right-sided and present a mucinous phenotype. The molecular characteristics of right and left sided mucinous adenocarcinomas (MAC) and conventional adenocarcinomas (CA) in terms of MSI status, KRAS and APC mutations were determined in the present study. Method: A total of 37 MAC (17 left, 20 right sided) and 37 sex and age-matched CA (19 left, 18 right sided) were studied. Mutation analysis was performed using PCR and sequencing exon 2, exon 3 of KRAS and mutation cluster region of APC (part 1-5). Microsatellite instability status was determined using primers for BAT25, BAT26, D5S346, Mfd15, D2S123 BAT40, Mycl, D18S58, D10S197 and D13S153. MSI-high (MSI-H) cases showed mutation in two of five markers whereas in MSI-low (MSI-L) phenotype only one of five markers was unstable and in microsatellite stable (MSS) cases none was mutated. "MSI tumours" were defined as those that showed MSI-H. Immunohistochemical detection of mismatch repair gene proteins was performed using antibodies to hMLH1, hMSH2, hMSH6 and PMS2. Tumour cells without nuclear staining, in the presence of positively stained stromal cells were considered to have an abnormal pattern of expression. Chi-square and Mann-Whitney U tests were used for statistical analysis. Results: Our results revealed that, MSI tumours were significantly more frequent in MAC (43,2%) then in CA (18.9%) ($p<0.05$). In MAC, MSI-H tumors were significantly ($p<0.05$) more frequent in the proximal colon (55%) than in distal colon (29.4%). Compared to CA, more MAC presented with expansive margins (45 vs 0%, $p<0.001$), tumour "serration" (37,8 vs 5.4%, $p<0.01$) and hyperchromatic nucleus (56.8 vs 24,3%, $p<0.01$). APC mutation was significantly higher in CA when compared to MAC (55,9% vs 32,4%, $p<0,05$). No significant difference was found for KRAS mutation between tumour types. Immunohistochemically hMLH1, hMLH2, hMSH6 and PMS2 yielded a sensitivity of %32, %11, %4 and %4 respectively, for the detection of MSI-H tumours. Conclusions: In accordance with the morphologic characteristics of MAC which are suggestive of "serrated pathway" in their neoplastic progression, they showed higher MSI in right colon tumours, in particular, and fewer APC and KRAS mutations compared to CA.

OP25-8

THE RELEVANCE OF MULTIPLEX PCR AND HETERODUBLEX ANALYSIS ON DIFFERENTIATING THE REACTIVE VERSUS NEOPLASTIC T CELLS IN THE SPECTRUM OF GLUTEN SENSITIVE ENTEROPATHY – ENTEROPATHY ASSOCIATED T CELL LYMPHOMA.

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Enteropathy associated T cell lymphoma (EATCL) is a rare agressive lymphoid neoplasm of intestine. Gluten sensitive enteropathy (GSE) and EATCL are at the two ends of one spectrum. Some of the cases which are refractory to gluten free diet may represent the early phase of neoplastic transformation to EATCL. Multiplex PCR (MPCR) by Biomed-2 primer sets for T cell clonality analysis are very sensitive. The aim of this study is to examine the diagnostic value of MPCR and hetereodublex analysis (HD) by using Biomed-2 primers for differentiation of the entities within the GSE-EATCL spectrum. Intestinal biopsies of 23 GSE patiens diagnosed by clinical and histopathological parameters, 8 cases of EATCL were studied in the disease group. Nine duodenal biopsies with normal histology were studied as the disease free negative control group. The immunophenotypic characteristics of the samples of the disease group were recorded. The control biopsies of 8 patients in GSE group with no or partial response to gluten free diet were and the non-tumoral segments of EATCL were studied. Multiplex PCR and HD analysis for examination of the clonal rearrangement of T cell receptor beta, gamma and delta genes were performed for every sample. Clonal TCR rearrangement was present in the tumoral area of all EATCL cases whereas the the non tumoral mucosa were found clonal in 75% of the cases. In the negative control group 7 cases were clonal for TCR gamma gene whereas 2 for beta gene. TCR gamma clonality was present in 46% of the responders. Only 6% had clonal TCR beta rearrangement. Except one case the immunophenotype of intraepithelial T lymphocytes (IETL) were CD8 positive in the refractory group. TCR gamma gene was clonal in 65%, TCR beta was 25% in non responders. Only one case was composed of CD8 and CD4 negative IETL and clonal rearrangement in TCR beta and gamma in this group. Conclusion: MPCR and HD with Biomed-2 primers is a sensitve method for detecting the clonal T cell population in the lesions of GSE-EATCL. The value of TCR gamma for clonality analysis are not enough for the decision of the neoplastic T cells. Examination of TCR beta gene rearrangement together with gamma decrease the rate false positivity. Relying on molecular methods alone for the cases in this disease spectrum may cause overdiagnosis if not combined with the immunophenotypic and clinical data.

OP25-9

GASTROINTESTINAL AND PANCREATIC NEUROENDOCRINE TUMORS (GEPNETS), ANALYSIS OF A MULTICENTRIC STUDY ON 191 SURGICALLY REMOVED TUMORS

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Background: GEPNETs are relatively rare tumors with heterogeneous histological, clinical and biological properties. In this preliminary multicentric study, a large series of GEPNETs were evaluated according to the WHO classification along with some other macroscopical and histopathological features. Method: A national database network was developed and ten different centers registered a total of 191 cases of GEPNETs. Only surgically resected pure endocrine tumors immunostained with at least two neuroendocrine markers were included in the study. All tumors were classified according to the 2000 WHO criteria. Results: Our series consisted of 72 well-differentiated benign GEPNETs (37,7%), 32 well-differentiated GEPNETs with uncertain behavior (16,8%), 61 well-differentiated malignant GEPNETs (31,9%) and 26 poorly-differentiated endocrine carcinomas (13,6%). There were 102 female (53,4%) and 89 male (46,6%) patients. The most common tumor location was pancreas (32,5%), followed by appendix, stomach, ileum, duodenum, rectum, jejunum, cecum and colon (23%, 17,3%, 8,3%, 6,8%, 4,1%, 3,1%, 2%, 2%, respectively). Benign GEPNETs were more frequent in women and younger patients while poorly-differentiated carcinomas were more frequent in men and older patients (p=0,001). Multicentricity was a common finding in well-differentiated tumors (19%), but not in poorly differentiated ones (3,8%). Necrosis when found grossly or microscopically was a strong indicator of malignancy (benign vs malignant, p=0,001) though there was no statistically significant difference between benign and uncertain group. Parameters such as tumor size, mitotic index, and Ki-67 index were all statistically significant between benign-uncertain, benign-malignant and well differentiated-poorly differentiated malignant tumors (p=0,001). Conclusion: The 2000 WHO classification provides a standard evaluation of GEPNETs which will improve our understanding of biology and natural history of these rare tumors.

OP25-10

CLINICOPATHOLOGIC AND MOLECULAR CHARACTERISTICS OF 523 TURKISH GASTROINTESTINAL STROMAL TUMOR CASES: A MULTICENTER STUDY

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Background: It is important to make the differential diagnosis of gastrointestinal (GI) stromal tumors (GIST) from other GI mesenchymal tumors (GIMT) because of the opportunity of molecular-targeted therapy for GISTs. This study was designed to describe the pathologic, immunohistochemical (IHC) and molecular features of cases with a possible diagnosis of GIST and to identify KIT immunostaining profile by using two different antibodies. Method: Cases of GIMTs, identified from the database of 14 diagnostic centers in Turkey between 1986-2007, were analyzed. A total of 523 cases were represented on seven tissue microarray (TMA) parafin blocks by using manual arrayer. Immunohistochemistry was performed using CD117, CD34, SMA, Desmin, S100, and h-caldesmon. Two different antibodies for CD117 were used. RNA in situ hybridization (ISH) was performed to identify KIT and platelet-derived growth factor receptor alpha (PDGFRA) m-RNA. Mutation testing for KIT and PDGFRA genes will be performed. Results: Tumors were located in the stomach (41%), small intestine (32%), colorectum (10%), abdominal cavity (16%), and esophagus (1%). Histologically, the tumors had spindle cell (61%), epithelioid (12%), and mixed type (27%) of morphology. Immunohistochemically, 78% of cases were positive for monoclonal CD117 antibody (T595, Novocastra) whereas 77% of cases were stained with polyclonal CD117 antibody (polyclonal, A4502, DAKO). There was statistically moderate to good agreement on CD117 0,001). CD117 staining<0,037, p±=0,743κstaining with two different antibodies (by IHC and by ISH was not comparable because of the preparation differences of parafin blocks from different centers. Mutation testing for KIT and PDGFRA will be performed for 95 KIT negative cases either by IHC or by ISH. Conclusion: The accurate diagnosis of GIST is dependent on clinicopathological and immunohistochemical criteria. It is

important to perform appropriate antibody optimization and validation, and to interpret KIT immunostaining in the clinical decisions of treating with Imatinib. This study has an importance of being the review of the largest series of Turkish GIST cases with pathological, phenotypic and molecular characteristics

OP25-11
GASTROINTESTINAL EPITHELIAL DYSPLASIA/NEOPLASIA: WHERE DO TURKISH PATHOLOGISTS STAND - EAST OR WEST?

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Background: Interpretation of gastrointestinal biopsies for epithelial neoplasia/dysplasia is one of the most difficult tasks of pathologists since there is no objective method. Despite two international consensus meetings there are still discrepancies between east and west in the interpretation of dysplastic lesions. Pathologists with special interest in gastrointestinal cancer precursors have proposed different nomenclatures to identify such lesions, both in western countries and in Japan. Vienna classification has largely resolved this problem by adopting both architectural and cytologic criteria. However, this classification scheme has not gained much interest from the practising pathologists despite its clinical considerations. We, therefore, decided to evaluate the approach of a group of pathologists to gastrointestinal epithelial lesions in terms of diagnostic criteria and also to assess interobserver agreement on the diagnosis of preneoplastic lesions of the gastrointestinal tract. Method: Twenty four Turkish pathologists and four Japanese pathologists, all experienced in gastrointestinal pathology, reviewed 20 gastric and 8 colonic lesions (11 endoscopic biopsies, 6 polypectomy, and 10 operation specimens). The specimens were evaluated according to Japanese classification and Vienna classification using both architectural and cytologic criteria. The extent of diagnostic agreement between the observers with Western and Japanese viewpoints was assessed by kappa statistics. Results: Turkish pathologists gave a diagnosis of cancer in 32.1% of cases while Japanese pathologists gave a diagnosis of cancer in 71.4% of the cases. The agreement between Turkish and Japanese pathologists had a kappa value of 0.597 for Japanese classification while kappa was 0.508 for Vienna classification between the two groups of pathologists. Overall interobserver agreement did not differ between Japanese and Vienna classification (kappa: 0.357, kappa: 0.332, respectively). The disagreement between biopsy diagnosis and EMR or resection was 59.5% for Turkish pathologists, and 45.8% for Japanese pathologists. Conclusion: Turkish pathologists seem to be more conservative in their interpretation of dysplasia. The concept of noninvasive carcinoma does not seem to be widely accepted by Turkish pathologists who stand closer to the West in their approach to gastrointestinal epithelial dysplasia-neoplasia. There is stil much to be done to improve our diagnostic skills and to standardize our criteria in the diagnosis of gastrointestinal epithelial dysplasia.

OP26-1
THE VEGF-SIGNALLING PATHWAY IN PULMONARY SMALL AND NON-SMALL CELL CARCINOMAS – AN IMMUNOHISTOCHEMICAL COMPARATIVE STUDY

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Context: Angiogenesis is pivotal for the survival of tumor cells. VEGF (Vascular Endothelial Growth Factor) is understood to be a major stimulator of angiogenesis. Recently VEGF-targeted therapies have been identified as a promising therapeutic approach in solid tumors, including lung tumors. Our study aims to analyze the differences and similarities of factors of the VEGF-pathway (Tie2, VEGF, VEGFR2, VEGFR3, PI3K) in different types of Small and Non-Small Cell Lung Cancers. Design: We used tissue microarrays consisting of 32 Small Cell Carcinomas (SCLC), 27 Large Cell Neuroendocrine Carcinomas (LCNEC), 75 Adenocarcinomas (AC), 67 Squamous Cell Carcinomas (SQCC) and 53 Large Cell Carcinomas (LC), respectively (each represented by an average of 5 punches and 1 punch of normal adjacent lung parenchyma). Immunohistochemistry was performed according to standard protocols. The mean value for percentage and intensity and a composite score (percentage multiplied by intensity) of stained tumour cells were evaluated statistically using the Goeman`s Global Test. Results: Whereas factors of the VEGF-pathway are frequently expressed in LC, AC, SQCC and LCNEC (in descending order), they reveal a positivity in only a small amount of cases in SCLC. We found striking differences regarding the expression of VEGFR2 and VEGFR3: VEGFR2 was frequently and strongly expressed in LC and AC, whereas it was negative or weak in SCLC, LCNEC and SQCC. In AC and LC we observed an intense nuclar positivity of VEGFR3, and also SCLC and LCNEC revealed a nuclear positivity of VEGFR3, but to a much lesser extent. VEGFR3 was negative in SQCC. Conclusion: Our results suggest that the inhibition of angiogenesis by blocking of VEGF- VEGFR- pathway might lead to a better outcome in the group of Non-Small Cell Lung Carcinomas. In contrast angiogenesis might be mediated by another pathway than the VEGF-pathway in Small Cell Lung Carcinomas. The frequently observed event of nuclear staining of VEGFR3 might point towards another, hithertho unknown function.

OP26-2
SUPEROXIDE DISMUTASE POLYMORPHISMS AND EXPRESSION IN IDIOPATHIC PULMONARY FIBROSIS

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Background The inflammatory theory in the pathogenesis in idiopathic pulmonary fibrosis (IPF) has been largely confuted. However, markers of oxidative stress have been consistently

reported in lavage samples form IPF patients, and are responsible of fibrogenic TGFb activation. Increased reactive species of oxygen and nitrogen could depend from reduced scavenger activity of enzymes such as superoxide dismutases (SOD). Two enzyme isoforms are mainly implied in antioxidant activity: intracellular MnSOD and extracellular (Ec)SOD. Both proteins show genetic variants that influence the activity and/or stability of the enzyme. Methods We investigated by means of PCR amplification the Arg213Gly polymorphism in EcSOD and the Ala16Val polymorphism in MnSOD in 70 patients with clinical and instrumental diagnosis of IPF, in 38 different patients with confirmed histopathological and clinical diagnosis of usual interstitial pneumonia (UIP) according to ATS/ERS 2000 criteria, and in 295 normal subjects. Ec and MnSOD expression was also assessed immunohistochemically in UIP samples Results MnSOD Ala16Val polymorphism was significantly more frequent in histologically confirmed UIPs (heterozygous 39%, homozygous 44%) than in control cases (heterozygous 51%, homozygous 25%, p<0.05%). No difference was found between controls and clinical IPF series. The distribution of ARG213Gly EcSOD polymorphism was not significantly different in the 3 groups. Both Mn and EcSOD were moderately expressed in type 1 pneumocytes, in airway epithelia, macrophages, endothelia and vessel miocytes. No immunoreactivity was observed for both enzymes in fibroblastic foci, while overlying reactive pneumocytes were intensely positive for EcSOD. No difference in staining was observed in cases with or without polymorphisms. Conclusion Molecular investigations documented the significantly higher frequency in UIP patients of an MnSOD genetic variant associated with reduced antioxidant activity. Interestingly, the significant association was only observed in a series of histologically confirmed UIP cases, but not in a series of clinically diagnosed IPF. This result stresses the importance of basing molecular association studies on carefully selected patient series, in particular in the complex field of pulmonary fibrosis, and underlines the relevance of histological classification of fibrosis. Negative antioxidant activity in fibroblastic foci, together with the molecular results, suggest that foci can be the site of increased oxidative stress.

OP26-3

PDGFR BETA, A POTENTIAL TARGET FOR MOLECULAR CHEMOTHERAPY, IS EXPRESSED IN MESOTHELIOMA

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Background In the search for drugs effective in controlling malignant mesothelioma (MM) clinical trials have recently been performed employing tyrosine kinase (TK) inhibitor imatinib mesylate, as single drug or in combination with other molecules. While monotherapy did not show appreciable advantage, the synergistic combination with gemcitabine or pemetrexed seems to be more promising, both in experimental and in as yet unpublished clinical settings. Imatinib blocks a family of TK associated receptors including c-kit/CD117 and PDGFRβ by inhibiting kinase binding with ATP and activation. PDGF autocrine production seems to play a relevant role in mesothelioma cell growth, and its receptors are differentially expressed in normal mesothelium (expressing PDGFRβ) and in MM cell lines (expressing PDGFRβ). Furthermore, one of the most common genetic abnormalities observed in MMs involves chromosome 22q13, where PDGFRβ gene is mapped. CD117 has been reported to be expressed in 1/3 of MM, while PDGFRβ was documented in MM cell coltures but it has never been investigated in MM sample series. Methods We investigated with immunohistochemistry the expression of PDGFRβ (Rb-1692, Labvision, Freemont CA) and CD117 (A4502, Dako Cytomation, Carpinteria, CA) in a series of 138 MMs and correlated the results with clinical and tumor data (histological type, patient survival, type of exposure to asbestos). Results 105 cases (76%) were moderately or intensely reactive for PDGFRβ; 33 (24%) showed weak or absent immunoreactivity. Consistent expression of CD117 was observed in 16 cases (11,5%). All 16 cases were also reactive for PDGFRβ. CD117 expression was significantly associated with epithelioid histology (p<0,01). No association with histological type was observed for PDGFRβ. TK receptor expression was not associated with survival. Interestingly, PDGFRβ expression was significantly associated with professional exposure to asbestos (subjects working in cement factory, railways, naval industry), as compared to ambiental exposure (subjects living in neighbourhood of cement factories) or to undetermined exposure. Conclusion The present study confirms for the first time in vivo in a large series of MM cases the expression of PDGFRβ, further supporting the promising therapeutic strategy combining TK inhibitor imatinib mesylate with antifolates or gemcitabine.

OP26-4

EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF PTEN, P27, CKS1 AND SKP2 IN NON-SMALL CELL LUNG CANCER

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Background: p27 inhibits cell cycle progression and is frequently down-regulated in various neoplasms. p27 levels are regulated by degradation from a SCF complex. Cks1 and Skp2 are important components of the SCF complex and they are upregulated in various neoplasms. PTEN increases p27 levels, due to inhibition of Skp2-mediated degradation. This study investigates the expression of PTEN, p27, Cks1 and Skp2 in human non-small cell lung carcinomas (NSCLC) and correlates them with clinicopathologic factors and survival. Method: 128 surgically resected, formalin-fixed, paraffin-embedded NSCLC specimens were subjected to immunohistochemistry for PTEN, p27, Cks1 and Skp2 proteins. Adjacent non-neoplastic lung parenchyma was also evaluated. For each case, a % value for each marker was obtained (labeling index-LI), by dividing the positive stained (epithelial) nuclei by the total number of cells. Specimens containing <50% stained cells were considered as having downregulated PTEN and p27. The cut-off level used for Cks1 and Skp2 was 10%. Follow-up data was available for 91 patients (follow-up time 1-78 months, mean: 19). Results: The median LIs of PTEN, p27, Cks1 and Skp2 were 75%, 60%, 15% and 12%, respectively. PTEN and p27 were always highly expressed in non-neoplastic lung (median LI:95%) whereas Cks1 and Skp2 were never expressed in normal tissue. PTEN and p27 were downregulated in 30.4 and 39.1% of the cases, respectively. Induced expression of Cks1 and Skp2 was noted in 58,5 and 57% of the cases. Statistical analysis revealed a significant reduction of PTEN and p27 expression in neoplastic compared to normal lung tissue (p<0.0001). Additionally, PTEN and p27 were more frequently expressed in T1 compared to T3-T4 neoplasms (p=0.033 and 0.028, respectively). A positive correlation between PTEN expression and prolonged survival was noted for T1 tumors (p=0.034) and albeit not reaching statistical significance, PTEN tended to be an independent prognostic factor for overall survival in T1 tumors (p=0.095). Cox regression analysis revealed stage, age and gender as independent prognostic factors for overall survival(p=0.011, 0.013, 0.014, respectively). Cks1 and Skp2 expression levels correlated to each other but not with p27, stage or survival. Conclusion: PTEN and p27 seem to be

involved in the progression of lung neoplasia. PTEN emerges as an important prognostic factor in T1 neoplasms, albeit its prognostic value needs further investigation. Finally, Cks1 and Skp2, despite their parallel expression, do not seem to account for p27 loss in lung neoplasms.

OP26-5
DECREASED FOXP3 EXPRESSION IN COPD PATIENTS

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Background. Chronic obstructive pulmonary disease (COPD) is a frequent disorder characterized by mostly irreversible airway obstruction that develops predominantly in susceptible smokers and is characterized by persistent airway inflammation and remodelling. CD4+CD25+ FOXP3 positive T regulatory cells have been shown to play an important role in controlling immune and inflammatory reactions. We hypothesize that these cells can be involved in the pathogenesis of COPD. The aim of our study was to characterise the expression of FOXP3 in large and small airways of nonsmokers, smokers with normal lung function and COPD patients. Methods. 19 nonsmokers, 20 smokers with normal lung function and 16 smokers with moderate COPD were enrolled in the study. Immunohistochemical methods were used to evaluate FOXP3 expression in airways. Results. Obtained results have shown that patients with COPD have decreased number of FOXP3 cells in small airways compared to nonsmokers (10 ± 7 versus 21 ± 10 cells/ mm2, p=0.001). Furthermore, the positive correlation between FOXP3 T cell density and airways obstruction was observed (r=0.35; p=0.02). However, our results have shown that the total number of FOXP3 immunoreactive cells in large airways did not significantly differ between nonsmokers, smokers with normal lung ventilation function (137 ± 14 versus 74 ± 12 cells, p>0.05) and COPD patients (304 ± 51 versus 74 ± 12 cells, p>0.05). Conclusion. To conclude, COPD is characterized by downregulation of FOXP3 positive Tregulatory cells in small airways that correlates with airways obstruction.These results highlight a novel, potentially relevant, pathogenic mechanism of chronic obstructive pulmonary disease

OP26-6
LUNG INJURY AFTER SURFACTANT TREATMENT – EXPERIMENTAL STUDIES

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Background. The use of exogenous pulmonary surfactants formulas was accepted with very good results in treatment of respiratory distress syndrome in newborns. Several attempts were also done for acute respiratory insufficiencies treatment in adults. Unfortunately results of such efforts remain still unsatisfactory. The aim of the present study was the evaluation of effects of therapeutic dose of semi-natural surfactant on the morphology of lung in an experimental model. Method. Pathogen-free rats under anesthesia were given a single dose (150mg of lipids/kg of b.w.) of commercially available surfactant (Curosurf). After 1, 6, 24 hours, 5, 10, 21 and 42 days animals were sacrificed (at least 5 in each group) and lungs were taken and prepared in standard way for paraffin and epoxy resin blocks. Tissue slides were examined by light and transmission electron microscopy as well morphometric studies on aeration of the lung were performed. All results were statistically evaluated. Results. In controls average air-less areas (AALA; corresponding to lung parenchyma) oscillated between 13.86 and 21.73%. In experimental lungs AALA were fluctuating during observations, but in all groups they were larger than in controls ($p<0.0001$). In lungs taken short after treatment we found a progressive raise of AALA from 28.15% (at 1h) to 35.31% (at 24h; $p < 0.001$) and than a decrease. At first time intervals in experimental lungs there were collapsed alveoli with parenchymal and intraalveolar edema. At 1h observations in some samples only about 10% of area was aerated (vs controls 82%; $p<0.0001$). Since 24h observations enormous, inflow of pulmonary macrophages into areas with abundance of exogenous surfactant was found. Moreover in such areas we found proliferation of type II pneumocytes. At 21 and 42 days of observations alveolar septa were widened by the proliferation of fibroblasts as well as deposits of extracellular components (mainly collagen). At 10th day of experiment average area occupied by lung parenchyma was the lowest in experimental groups (28.41% vs 17.61% in controls; $p<0.002$) and than it raised again. Although the maximum AALA at 24h in experimental animals decreased during observations it never became same as for controls. In experimental lungs at 42nd day of observations AALA was 30.57% vs 21.73% in controls ($p<0.001$). Conclusion. Our results suggest that surfactant use has possible destructive effects while reacting with healthy lung. It could cause a rapidly developing changes which are partially reversible. Unfortunately it might lead to lung fibrosis.

OP26-7
EVALUATION OF TKTL1 EXPRESSION IN PRIMARY NON-SMALL CELL LUNG CANCER IN RELATION TO CLINICO-PATHOLOGICAL DATA: WHAT DO AUTOMATED ANALYSES ADD TO HUMAN PERFORMANCE?

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Introduction: Malignant tumours are able to degrade glucose to lactose even in the presence of oxygen. This so called Warburg effect is controlled by transketolases converting ribose-5-phosphate, which is usually utilized for nucleic acid synthesis, into C5- and C3-carbohydrates. These can now be degraded into lactate for energy generation. Transketolase like Protein 1 (TKTL1) is supposed to be one of the key-molecules in this shunt from the pentose-5-phosphate pathway to the Embden-Meyerhoff pathway. Material and Methods: Upon this theoretic background we investigated the expression of TKTL1 in 201 primary non small cell lung carcinomas operated with curative intent especially in regard to clinico-pathological data and patients' over all survival by immunohistochemistry using a monoclonal TKTL1 antibody. Results: 90 tumours (44.7%) showed no or mild TKTL1 expression, whereas in 111 tumours (55.3%) it was overexpressed. TKTL1 overexpression correlated with tumour-type ($p= 0.02$) and histological grading (G2: 61% vs. G3: 76%; $p= 0.034$). Overexpression of TKTL1 was significantly associated with poor survival ($p= 0.0077$). This prognostic effect remained significant in multivariate analysis with overexpression of TKTL1 being the second most important marker for patients' survival in our study ($p< 0.0223$). Furthermore, patients with favourable clinicopathologic stages (i. e. $G < 3$, N0, adeno- or squamous cell carcinoma) showed a poorer outcome if the tumours were overexpressing TKTL1 ($p < 0,039$). Conclusion: The data suggest that TKTL1 overexpression relative to the corresponding benign epithelial cells is a new predictor of patient survival in primary non small lung cancer especially for patients with a favourable prognosis. Since inhibition of transketolase enzyme reactions has recently been shown to effectively suppress tumour growth TKTL1 may represent pharmaceutical target.

OP26-8
SEQUENCING OF THE MITOCHONDRIAL D-LOOP REGION AS A TOOL TO DISCRIMINATE BETWEEN SYNCHRONOUS OR METACHRONOUS PRIMARY LUNG TUMOURS AND INTRAPULMONARY METASTATIC SPREAD

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Four to 10 percent of patients with non small cell lung carcinoma (NSCLC) present with two nodules or develop a second nodule after the initial diagnosis. Tumor stage is the most important prognostic factor in lung cancer and, therefore, the differential diagnosis between synchronous or metachronous primary lung neoplasms (pT1m) and intrapulmonary metastasis of (NSCLC) (pT4 or pM1 according to their location) is crucial for patient management. The large number of mitochondrial mitochonodrial DNA (mtDNA) copies present in each tumour cell and the high frequency of mtDNA mutation rate in tumours suggest that sequencing of the highly polymorphic D-loop mtDNA region may be useful to discriminate synchronous/metachronous primary tumours from intrapulmonary metastases (1). We studied a retrospective series of 20 patients underwent to surgery for synchronous and metachronous NSCLC. The charts from each patient were reviewed and the tumours were staged according the current WHO and classified as multiple primary or metastases according Martini and Melamed (2). Direct DNA sequencing of the mitochondrial D-loop region was performed in all samples and the genetic distance between the multiple tumours was evaluated. Our results show that in 72% of the casess the tumours were genetically unrelated and thus should be considered as independent multiple primary cancers. It is noteworthy that in this group, in up to 65 per cent of patients, the histological type was the same in all nodules analyzed (most frequently adenocarcinoma, acinar type). In the remaining 28% of cases the mtDNA sequencing revealed the same mutation pattern, compatible with origin from a single neoplastic cell clone. Thus, our study indicates that the present clinicopathologic criteria (site, histological type, etc) for staging patients with multiple nodules of NSCLC may not be fully reliable and that molecular analysis may represent a very useful tool to discriminate multiple primary tumours from intrapulmonary metastases.

OP27-1
INVERTED PAPILLOMA AND RESPIRATORY EPITHELIAL ADENOMATOID HAMARTOMA; CONFUSING TERMINOLOGY AND OVERLAPPING FEATURES

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Background: At least some of the inverted papillomas (IP) and respiratory epithelial adenomatoid hamartomas (REAH) have overlapping histopathological features. Cases with a diagnosis of IP or REAH, along with a number of sinonasal polyps and nasopharyngeal biopsies were morphologically evaluated in order to describe the overlapping and differential histological features of these cases. Methods: The REAH and/or IP cases included 36 biopsies from 24 patients. All slides were reevaluated according to the pertinent literature by three pathologists in order to classify as IP, REAH or with features of both REAH and IP. Also 55 nasal biopsies from 30 patients and 26 nasopharyngeal biopsies from 10 patients were evaluated for invagination of the surface epithelium with or without branching as well as squamous metaplasia. Results: Twenty eight (% 77.7) biopsies from 17 (70,8%) patients were IP, 4 (16,6%) biopsies from 4(% 11.1) patients were REAH, 4 (16,6%) biopsies from 3 (% 12,5) patients demonstrated a mixture of the microscopic features of IP and REAH. Six patients had recurrent disease. Of these 4 were IP, 1 was REAH and 1 case had features of both IP and REAH. Invagination of the surface epithelium was identified at 45 (82%) of the 55 nasal polyps and 17(65,4) of the nasopharyngeal biopsies, but squamous metaplasia was not identified. Conclusions: We found that only squamous metaplasia was a feature of IP but not of REAH. The invagination of the surface epithelium was a very common feature of the sinonasal region that mimics REAH in many biopsies of the sinonasal region, but smaller. So herein we propose that hamartoma term should be reserved for only cases with components other than epithelial. And IP cases might be grouped as glandular (respiratory epithelial) IP, glandular and solid IP, solid (squamous metaplastic) IP. Probably in this setting the solid (squamous metaplastic) IP will be the group most frequently associated with neoplastic transformation.

OP27-2
THE DIAGNOSTIC ACCURACY OF REVERSE TRANSCRIPTION-PCR QUANTIFICATION OF

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PURPOSE: The main goal of sentinel lymph node (SLN) detection in head and neck squamous cell carcinomas is to limit neck dissections to pN+ cases only. However, intraoperative + diagnosis cannot be routinely done using the current gold standard, serial step sectioning with immunohistochemistry. Real-time quantitative reverse transcription-PCR (RT-PCR) is potentially compatible with intraoperative use, proving highly sensitive in detecting molecular markers. This study postoperatively assessed the accuracy of quantitative RT-PCR in staging patients from their SLN. EXPERIMENTAL DESIGN: A combined analysis on the same SLN by serial step sectioning with immunohistochemistry and quantitative RT-PCR targeting cytokeratins 5, 14, and 17 was done in 18 consecutive patients with oral or oropharyngeal squamous cell carcinoma and 10 control subjects. RESULTS: From 71 lymph nodes examined, mRNA levels (KRT) were linked to metastasis size for the three cytokeratins studied (Pearson correlation coefficient, $r = 0.89$, 0.73, and 0.77 for KRT 5, 14, and 17 respectively; $P < 0.05$). Histopathology-positive SLNs (macro- and micrometastases) showed higher mRNA values than negative SLNs for KRT 17 ($P < 10(-4)$) and KRT 14 ($P < 10(-2)$). KRT 5 showed nonsignificant results. KRT 17 seemed to be the most accurate marker for the diagnosis of micrometastases of a size >450 mum. Smaller micrometastases and isolated tumor cells did not provide results above the background level. Receiver operating characteristic curve analysis for KRT 17 identified a cutoff value where patient staging reached 100% specificity and sensitivity for macro- and micrometastases. CONCLUSION: Quantitative RT-PCR for SLN staging in cN(0) patients with oral and oropharyngeal squamous cell carcinoma seems to be a promising approach.

OP27-3
HYPOXIA INDUCIBLE FACTORS HIF1A AND HIF2A ARE LINKED TO POOR RESPONSE OF HEAD-NECK CANCER TO RADIOTHERAPY

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Background: Hypoxia is a major factor defining resistance of cancer cells to ionizing radiation. HIF1α and HIF2α are key transcription factors that are accumulated intracellularly under hypoxic stress, triggering the transcription of more than 50 genes involved in angiogenesis, glycolysis and apoptosis. Materials and Methods: In this study, we assessed the expression of HIF1α and HIF2α in formalin-fixed paraffin-embedded tissues from 37 patients with locally advanced inoperable head-neck cancer (HNC) treated with radical radiotherapy combined with platinum chemotherapy. Radiotherapy was given with hypofractionation (3.5Gy/day). Results: HIF1α and HIF2α were consistently expressed in the nuclei and cytoplasm of cancer cells, but only occasionally in the tumor associated stroma. Using a grading scale that combined the subcellular patterns, the extent and the intensity of staining, we identified groups of low and high HIF reactivity. High expression of HIF1α and HIF2α was noted in 20/37 (54%) and 17/37 (46%), respectively. HIF1α was significantly associated with poor LRFS (p=0.01), whilst HIF2α was not related to local relapse. Cases with low HIF1α expression had a 24month local progression free survival of 73% vs. 46% of patients with high HIF1α reactivity. Conclusion: The expression of HIF1α in HNC predicts resistance to platinum to hypofractionated raditoherapy. Whether HIF2α expressing tumors are more sensitive to larger radiotherapy fractions, compared to standard radiotherapy fractionation, should be further investigated. Molecular inhibition of HIF function appears as a promising therapeutic approach aiming to improve the efficacy of radiotherapy.

OP27-4

OVEREXPRESSION OF CORTACTIN IN HEAD AND NECK SQUAMOUS CELL CARCINOMA IS ASSOCIATED WITH POOR PROGNOSIS AND HIGHER LOCAL RECURRENCE RATES

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Background : Despite recent advances in cancer treatment, the overall-5-year survival rate for patients with squamous cell carcinoma of the head and neck (HNSCC) has not improved over the last 30 years. Thus identifying a biological marker that correlates with recurrence would provide more accurate information on prognosis. Cortactin is a ubiquitously expressed Src substrate that engages in several protein-protein interactions that may be functionally relevant to tumoral progression. Aim : We have investigated expression of cortactin in a large series of HNSCC using tissue microarray (TMA) technology and have correlated this expression to the outcome of these patients. EGF receptor (EGFR) staining was performed to investigate the potential linkage between cortactin and EGFR receptor overexpression. Methods: Cortactin and EGFR expression was retrospectively evaluated by immunohistochemistry in a TMA composed of 176 HNSCC with a mean follow-up time of 5 years. Results: Cortactin immunoreactivity was weak to absent in normal epithelial tissue. Overexpression of the protein in 77 of 176 tumors (44%) was associated with more advanced Tumor-Node-Metastasis stage and higher histologic grade. Cortactin overexpression was associated with increased 5-year local recurrence rates (49 versus 36 %), decreased 5-year disease-free survival (11 versus 48%) and decreased overall survival (24 versus 51%), independently of the EGFR status. In multivariate analysis, cortactin expression status remained an independent prognostic factor for local recurrence, disease-free survival, and overall survival. A subset of patients with cortactin-overexpressing tumors that displayed low EGFR levels had a survival rate that equalled that of patients with tumoral overexpression of both EGFR and cortactin. Conclusion: In patients with HNSCC, overexpression of cortactin as determined by immunohistochemistry is associated with significantly poor prognosis and higher local recurrence rates.

OP27-5

CELL CYCLE PROTEIN EXPRESSION PATTERNS IN HPV-CONTAINING AND –LACKING TONSILLAR SQUAMOUS CELL CARCINOMAS AND ASSOCIATION WITH PROGNOSIS: A ROLE FOR P21?

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Background. Tonsillar squamous cell carcinoma (TSCC) may be induced by chemical carcinogens as a result of, e.g., tobacco and alcohol consumption, or by oncogenic human papillomavirus (HPV). HPV presence has been reported to be associated with a favorable patient survival. This study was undertaken because controversial data exist on the expression of key cell cycle proteins in the retinoblastoma (pRb) and p53 pathways in HPV-containing and –lacking TSCC and their impact on patient survival. Method. Formalin-fixed, paraffin-embedded tissue sections (4 µm thick) of 77 TSCC were analyzed for ki-67-, p16INK4A-, cyclinD1-, pRb-, p14ARF-, MDM2-, p53-, p21CIP1/WAF1- and p27KIP1 expression by immunohistochemistry. The presence of biologically associated HPV16 was determined by HPV-specific fluorescence in situ hybridization. Results were correlated with each other and with patient survival. Results. 75 of 77 (97%) TSCC showed strong, nuclear Ki67 expression in 30-90% of tumor cells. 32 (42%) TSCC were HPV16-positive. The presence of HPV was significantly associated with overexpression of p16INK4A ($p<0.0001$), p14ARF ($p<0.0001$) and p21CIP1/WAF1 ($p=0.001$), and downregulation of pRb ($p<0.0001$). p53 and MDM2 accumulation tended to be associated with absence of HPV ($P<0.082$). Accumulation of nuclear cyclinD1 and p27KIP1 was detected in 43% and 28% of the tumors, respectively, irrespective of HPV-status. Univariate Cox regression analysis revealed a favorable survival rate for non-smokers ($p=0.006$), as well as for patients with TSCC, which are classified as T1-2 ($p<0.0001$) or show either low/no expression of cyclinD1 ($p=0.028$), HPV presence ($p=0.024$) or overexpression of p16INK4A ($p=0.029$), p14ARF ($p=0.02$) or p21CIP1/WAF1 ($p=0.004$). Conclusion. HPV 16-associated TSCC exhibit overexpression of p16INK4A, p14ARF and p21CIP1/WAF1 and downregulation of pRb. Tumor size and p21CIP1/WAF1 immunostaining are the most optimal indicators for favorable prognosis in TSCC. This study was supported by the Research Foundation of the University Hospital Maastricht.

OP27-6

ASSOCIATION OF INTERLEUKIN-6 (IL-6) EXPRESSION WITH PROGRESSION OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK IS NOT DEPENDENT ON IL-6 -174 G>C POLYMORPHISM

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Background: Recent studies showed increased levels of interleukin-6 (IL-6) and IL-6 receptor (IL-6R) playing a role in development/progression of different cancers including oral carcinoma where oncogenesis was associated with IL-6-174 G>C promoter polymorphism. We wanted to determine whether IL-6 polymorphism affects IL-6 and IL-6R expression in squamous cell carcinomas of the head and neck (HNSCC) and how these factors are interrelated. Methods: DNA was isolated from tumor and adjacent normal tissue from 67 HNSCC patients using standard procedure. The allelic discrimination was performed with TaqMan™ technology on ABIPrism® 7900 HT (Applied Biosystems, USA). Formalin fixed tissue samples were stained immunohistochemically with antibodies against proteins IL-6 and IL-6R. Percentage of positive tumor and adjacent non-cancerous squamous epithelial cells was determined. Correlation with disease parameters and survival analysis was performed with SPSS ver. 14. Results: HNSCC patients were mostly male (60; 89,6%) with moderately differentiated (43; 64,2%) laryngeal cancers (37; 55,2%). Homozygosity for normal IL-6 promoter allele (GG) was found in 21 (31,3%), heterozygosity (GC) in 34 (50,8%) and CC homozygosity in 12 (17,9%) cases. Allele frequency in 67 HNSCC patients didn't differ from control population of 93 healthy persons. It correlated significantly with IL-6 expression in adjacent mucosa (p=0,004) but not tumor cells. Significant correlation was present between IL-6/IL-6R expression in tumor cells (p=0,025) and IL-6 expression/disease progression (N stage p=0,002; stage grouping p=0,044; recidivant disease p=0,043). Survival analysis attested prevalent prognostic value of tumor based prognostic markers, since for other variables only non-significant trends were noted. Conclusions: Our results proved coordinated expression of IL-6 and IL-6R in HNSCC cells. Irrespective of no statistical significance between IL-6 promoter polymorphism and protein expression in tumor cells there was positive correlation to IL-6 expression in adjacent mucosa. This finding suggests that promoter polymorphism may play a role in activation of autocrine/paracrine activating loop mediated by IL-6 and other cytokines appearing in the area of field cancerisation, leading to HNSCC as an ultimate result. Moreover, significant association of IL-6 expression to disease progression not only confirms its significance in tumor proliferative and immune suppresive activity but suggests a possible role of immunohistochemistry in research and different diagnostic or treatment procedures.

OP27-7

PREVALENCE AND TYPING OF HUMAN PAPILLOMAVIRUS IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS IN GREECE

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BACKGROUND AND OBJECTIVE: The incidence of head and neck squamous cell carcinoma (HNSCC), varies between geographic regions, and tobacco usage and alcohol consumption are the main risk factors. Over the past twenty years however, it has been shown that high-risk types of HPV may be a risk factor for developing HNSCC, and particularly for tonsillar cancer, where HPV is most commonly found and where HPV-16 is by far the most frequently identified subtype. Moreover, recently in the Stockholm area, we have shown that between the years 1970-2002, there has been a 3-fold increase both in the proportion of HPV positive tonsillar cancer (from 23-68%) and the incidence of tonsillar cancer. The increase in tonsillar cancer incidence has been observed in spite of a decreased incidence in smoking, suggesting that HPV is an independent causative factor for tonsillar cancer. Furthermore, patients with HPV positive tonsillar cancer were with regard to median age 10 years younger that patients with HPV negative cancer. The incidence of smoking in Greece is much higher than in Sweden, and for this reason we decided to examine for the presence of HPV in head and neck cancer and particularly in tonsillar cancer in a Greek material for comparison. MATERIALS AND METHODS: Ninety-three squamous cell carcinoma samples (18 tonsillar, 38 tongue, 41 oral cavity tumours) collected between 1986-2006 were analysed by PCR. DNA was extracted from paraffin embedded biopsies. To confirm the presence of PCR amplifiable DNA a PCR for the house-keeping gene S14 was run. For the detection of HPV, PCR with HPV consensus primers GP5+/6+ and CPI/IIG were used. For detection of HPV 16, a PCR with HPV 16 type specific primers was used. RESULTS AND CONCLUSIONS: In total 9/93 tumours were HPV positive. Eight of the 18 tonsillar cancer samples (44%) were HPV positive and notably, within the same material, 7/12 (58%) of the tonsillar cancer samples obtained between years 2000-2006 were HPV positive. Seven (88%) out of the eight HPV positive tonsillar tumours were HPV 16 positive. Of the tongue cancer samples 1/38 (3%) were HPV positive, while none of the 41 oral cavity cancer samples were HPV positive. In addition, patients with HPV positive tonsillar cancer were with regard to median age 5 years younger than patients with HPV negative tonsillar cancer. In summary, almost half of all the Greek tonsillar cancer patients had presence of HPV in their tumours, and HPV-16 was the dominant type. In addition, patients with HPV positive tumours were also younger than patients with HPV negative ones

OP27-8

DEVELOPING HUMAN MINOR SALIVARY GLANDS: MORPHOLOGICAL RELATIONSHIP BETWEEN THE EXPRESSION OF TGF-BETA ISOFORMS AND CYTOSKELETAL MARKERS OF DIFFERENTIATION

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Background: Development and complete differentiation of salivary glands is a complex process that involves a large number of coordinated events. Synchronization between cell proliferation, polarisation and differentiation, which are dependent on epithelial-mesenchymal interactions and on the microenvironment are a requirement. Hormones and growth factors mediate many of these highly orchestrated biological processes and transforming growth factor-beta (TGF-beta) appear to be relevant. TGF-beta is a multifunctional growth factor that has several biological effects, including control of cell growth and differentiation, cell migration, lineage determination, motility, adhesion, apoptosis, synthesis and degradation of

extracellular matrix. Method: Using immunohistochemistry and immunofluorescence we have mapped the distribution of TGF-beta 1, 2 and 3 and compared it with the expression of differentiation markers in human salivary glands obtained from foetuses ranging from weeks 4 to 24 of gestation. Results: TGF-beta 1 first appeared during canalisation stage in the surrounding mesenchyme, and, in the more differentiated stages was expressed in the cytoplasm of acinar mucous cells throughout adult gland. TGF-beta 2 was detected since the bud stage of salivary gland differentiation and its expression was observed in epithelial ductal cell, which increased along gland differentiation, TGF-beta 3 was detected from canalisation stage of salivary gland differentiation being weakly expressed on epithelial ductal cells and it was the only factor detected on myoepithelial cells. Conclusion: The data suggest that TGF-beta have a role to play in salivary gland development and differentiation.

OP28-1

PRIMARY CULTURES FOR PROTEOMIC STUDY IN COLORECTAL CARCINOMA: DO CELLS SURVIVE?

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A novel approach in the proteomic study of tumours, breast cancer in particular, was developed to identify proteins present in fluid collected from primary cultures. A possible aim is to compare the secreted proteomic profile with the plasma proteome, to highlight candidate markers for the diagnosis. In our study we cultured samples of tissue from healthy colon and from colorectal cancer and we evaluate the different expression between proteins derived from the lysis of cells, obtained from traditional tissue homogenization, and those secreted from vital cells in primary culture. Surgery samples were brought in ice to the department of pathology within 20 minutes from resection; they were accurately washed in water and sample from neoplastic and healthy tissue limited to the mucosa far from the zone of necrosis were collected. Tissue samples were posed in sterile Petri dishes, fragmented with sterile knifes, then cultured for 1 to 4 hours in sterile physiologic solution into a CO2 humidified incubator at 5% CO2 and 37 °C. Supernatant collected by centrifugation underwent Western-Blot analysis against Ku80, a nuclear protein involved in DNA repair, and HSC70, involved in secretive pathway. Supernatants were also analyzed to deeper investigate possible modifications in the protein expression profiles. Results achieved show an actual enrichment of the secreted protein within the primary colture media, with a parallel decrease of the amount of the nuclear protein, both for healthy and neoplastic tissues, compared to whole tissue lysates. We can conclude from these preliminary data that cells maintain their secretory activity at least for 4 hours after the resection; the minor alterations observed in the protein profiles point out small modification in cell activity, but suggest also to limit the colture time.

OP28-2

IMPACT OF CELL SENESCENCE AND RAS PATHWAYS IN BREAST CANCER PROGRESSION

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Background: Breast cancer is the major cause of death among women. Many studies on gene expression have identified, by microarrays, expression profiles and gene sets that are prognostic, predictive or both for breast cancer patients. The major concern related to the use of microarrays platform is the requirement of high-quality RNA, generally derived from frozen samples. Such tumor sets are often small and lack clinical information, whereas formalin-fixed paraffin-embedded (FFPE) materials are abundant and gave the possibility to select samples related to patients with long follow-up periods. Although RT-PCR-based methods from FFPE samples are achieving clinical application, genome-wide microarray analysis has proven difficult. Methods: By using quantitative RT-PCR, we explored the mRNA expression level of 23 genes belonging to RAS pathway (EGFr1, Her2, k-RAS, RAF-1, ERK1, PI3K, AKT), cell cycle regulation/senescence (Cyclin A, D1 and E1, CDK2, CDK4, CDK6, p16, p130-RB2, Retinoblastoma, CDC25a), telomerase complex (mRNA coding for catalytic component and RNA component) and differentiation/metastasis suppression genes (NM23, SEL-1L, cytokeratin 8) by quantitative RT-PCR in 80 patients with breast cancer and 19 metastic lymph nodes. Patients submitted to this study were diagnosed between 1971 and 1989 and were only treated with Halsted radical mastectomy. Our main goal was to investigate whether the gene expression profiles of the reported molecular markers in the primary tumor is predictive of its metastatic potential. Results: The prognostic contribution of the analysed molecular makers in conjunction to age, tumor stage and grade were submitted to Cox proportional hazard regression analysis. Our results showed that PI3K/AKT signalling is related to patients' survival via HER2. Patients with higher expression level of HER2 and/or PI3K, but lower expression level of AKT seem to have worst outcome. Moreover, RAF-1 and CDK2 higher expression seem to have a worst influence on patients' outcome. Among the analysed markers Cytokeratin 8 and Retinoblastoma seem to have a protective effect, in fact higher expression level were associated to better prognosis. Conclusions: In this study we identified a panel of 7 genes that seems to have an influence on patients' survival. The final phase of these markers evaluation would include a prospectively conducted randomised case study to show that the use of these assays yields better clinical outcome than current decision making without molecular tests.

OP28-3

POTENTIAL OF MICRORNA PROFILING FOR THE DIAGNOSIS OF PAPILLARY THYROID CARCINOMA

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MicroRNAs (miRNAs) are a new class of non-protein-coding, endogenous small RNAs. They are important regulatory molecules and plants. miRNA regulates gene expression by translational repression, mRNA cleavage, and mRNA decay initiated by miRNA-guided rapid deadenylation. Previous studies showed that some miRNAs regulate cell proliferation and apoptosis processes that are important in cancer formation. So, a current challenge is to elucidate the function of miRNAs in normal physiologic processes and in disease states, since several clues have recently pointed to the potential role of miRNAs in

cancer and suggest that aberrations in miRNAs may be crucial in tumor progression. Thyroid cancer poses a significant clinical change, and our understanding of its pathogenesis is still incomplete. Papillary thyroid carcinoma (PTC) is the most common malignancy in thyroid tissue accouting for around 80% of all thyroid cancers. Apart from alterations in the BRAF-RAS-RET/PTC pathway, little is known about he genetics of PTC. The aim of our study was to determine the regulation of miRNAs in different thyroid tumors, including PTC, by using an home-made miRNA microarray. For this purpose, 8 follicular adenomas, 10 PTC, and 3 follicular carcinomas were included. Total RNA was extracted with TRIzol solution and the integrity of RNA was assessed by using an Agilent Bioanalyzer 2100. Following extraction, hybridization of miRNAs was then carried out on our miRNA microarray chip, which contained more than 500 mature miRNA probes spotted in quadruplicate. Obtained results were the more significative in PTC tumors. Analysis showed that eight miRNA genes were significantly overexpressed in PTC with fold changes superior to 2.5 in all patients. Among these miRNAs the following were strongly upregulated: miRNA-146, miR-221, miR-21, miR-155, and miR181. Interestingly, the expression of miR-221 was also detectable in normal thyroid tissue adjacent to PTC tumors from all patients. Overexpression of miR-221 in PTC tumors and in normal thyroid tissue adjacent to PTC tumors was confirmed by both semiquantitative RT-PCR and Northern blot. In conclusion, these data indicated that the upregulation of miRNAs in PTC tumors may represent a distinct miRNA signature. Moreover, increased expression of miR-221 in normal tissue might be also an early genetic event in PTC carcinogenesis. Altogether, these datas showed that RNA-based regulatory mechanisms involving miRNAs characterise PTC.

OP28-4

GENE EXPRESSION ANALYSIS IDENTIFIES NOVEL RBL2/P130 TARGET GENES IN ENDEMIC BURKITT'S LYMPHOMA CELL LINES AND PRIMARY TUMORS

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Background: Burkitt lymphoma (BL) is a B-cell tumor whose characteristic gene aberration is the translocation t(8;14), which determines c-myc over expression. Several genetic and epigenetic alterations, other than c-myc over expression, have also been described in BL. It has been demonstrated that the RBL2/p130 gene, a member of the retinoblastoma family (pRbs), is mutated in BL cell lines and primary tumors. Methods: The aim of this study was to investigate the biological effect of RBL2/p130 in BL cells and its possible role in lymphomagenesis, as we previously observed that this gene is often mutated in BL. Therefore, we reintroduced a functional RBL2/p130 in BL cell lines. Results and conclusions: Our results demonstrated that RBL2/p130-transfected cells regain growth control, suggesting that RBL2/p130 may control the expression of several genes, which may be important for cell growth and viability. Gene expression analysis revealed a modulation of several genes, including CGRRF1, RGS1, BTG1, TIA1 and PCDHA2, upon RBL2/p130 reintroduction. We then monitored their expression in primary tumors of endemic BL as well, demonstrating that their expression resembled those of the BL cell lines. These data suggest that, as RBL2/p130 modulates the expression of target genes, which are important for cell growth and viability, its inactivation may be relevant for the occurrence of BL. Therefore we checked whether it could represent the second hit, together with c-myc over expression, for the onset of BL. To check whether these two events were sufficient per se to confer the cells a transformed phenotype and to mimic a BL, we engineered a human non-transformed lymphoblastoid B cell line to over express c-myc and we then silenced RBL2/p130 by RNAi. Our preliminary results indicate that RBL2/p130 silencing strengthens the growth advantage due to c-myc over expression, suggesting that its inactivation may concur to Burkitt lymphomagenesis.

OP28-5

VEGF AS AN AUTOCRINE STIMULATOR OF PROLIFERATION AND MOTILITY IN ASTROGLIAL CELL LINES

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Background: Glioblastoma multiforme is characterized by exuberant angiogenesis, a key event in tumor growth and progression. Of all solid tumors, those of the brain show the highest degree of vascular proliferation. The neovascularization in glial tumors correlates directly with their biological aggressiveness, degree of malignancy and inversely with post-operative survival. Inducibility of Vascular Endothelial Growth Factor (VEGF) through growth factors such as epidermal growth factor, platelet derived growth factor and basic fibroblast growth factor in vitro has been demonstrated in astroglial cell lines. Paracrine effect of VEGF secreted by glioma cells on tumor vasculature was supported by several lines of evidence, but lesser is known about its autocrine function in glioma progression and invasivity. Method: RT-PCR and immunohistochemistry were used to confirm co-expression of VEGF and VEGF receptors (Flt-1 and KDR) in astroglial cell lines (A172, U87MG, T98G, U118MG, SPF126, U251G, Gli-6 and U373MG). MTT proliferation test and F-actin staining were performed to investigate effect of VEGF on cell proliferation and motility. Following stimulation with exogenous VEGF, cells were washed and incubated for 24 hours to detect exogenously induced VEGF secretion using VEGF ELISA Kit (R&D Systems). Protein extracts from VEGF-treated cell lines were used for Western blotting to detect phosphorylation levels of key members of Ras/MAPK and PI3K signalling pathways. Results: VEGF and VEGF receptors were found to be co-expressed in all cell lines tested, both on RNA and protein level. Stimulation of these cell lines with exogenous VEGF lead to significant increase in proliferation, as well as massive re-organization of F-actin and stress fibers formation implicating enhanced cell motility. These cellular effects were based on preferential activation of Ras/MAPK in co-operation with PI3K signalling pathway. ELISA testing of VEGF-treated astroglial cell lines confirmed autocrine loop between VEGF ligand and VEGF receptor, since exogenous VEGF was capable of enhancing VEGF secretion comparing to VEGF-untreated control. Conclusion: Our results indicate indispensable role of VEGF not only as paracrine stimulator of tumor angiogenesis, but also as important autocrine factor stimulating glioma growth and migration. Because diffusely infiltrating gliomas are mostly resistant to current chemo- and radiotherapies, the research of angiogenesis as well as autocrine role of VEGF is of special interest. This work was supported by grants VZMSM6198959216 & IGAMZCR & NR/8370-3/2005.

OP28-6

PRESENCE OF VIP SYSTEM AT THE NUCLEAR CELL MEMBRANE IN DUCTAL BREAST CARCINOMA IN SITU

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BACKGROUND: Ductal carcinoma in situ (DCIS), responsible for 25% of newly diagnosed breast carcinomas, is considered an early step in the progression to invasive cancer. The rationale for treating DCIS is to prevent progression into an invasive disease. Vasoactive intestinal peptide (VIP) may function as autocrine/paracrine growth factor in breast cancer tissue. Recently, it has been hypothesized that VIP receptors ($VPAC_1$ and $VPAC_2$) may constitute a dynamic element in plasma and nuclear membranes. The aim of this work was to detect the different compartmentalization of VPAC receptors supporting the role for both exogenous and endogenous VIP. METHOD: Tissue samples were obtained by total or partial mastectomy and embedded in paraffin. They included benign proliferative diseases, and in situ and infiltrative carcinomas. The expression of the mRNAs for both VPAC receptors was detected in normal and pathological situations by means of quantitative RT-PCR assays. In order to evaluate VIP expression, quantitative RT-PCR and enzyme immuno-analysis assays were performed. RESULTS: All the specimens studied showed $VPAC_1$ immnunoreactivity mainly located at nuclear level and showing a differential expression. $VPAC_2$-immunoreactivity was absent. We observed overexpression of VIP mRNA, and two to four-fold higher VIP levels in DCIS samples as compared to normal tissue. CONCLUSION: This is the first demonstration of nuclear location of $VPAC_1$ receptor in human breast tissue. It suggests another role, which is still unclear, for this receptor in cell signalling involved in malignant transformation processes. In addition, these findings contribute to previous observations of our group on the intracrine mode of action of VIP on the genomic regulation of target genes such a vascular entothelial growth factor. Furthermore, these features support further studies on the potential therapeutic usefulness of available VIP receptor antagonists for breast cancer.

OP28-7

LOSS OF HETEROZYGOSITY (LOH) FOR THE 17Q22-24 LOCUS, PRKAR1A GENE MUTATIONS AND MICROSATELLITE INSTABILITY (MSI) IN BASAL CELL CARCINOMA (BCC)

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Background: Basal cell carcinoma (BCC) is the most common malignant human neoplasm; it is characterized by slow growth, virtual absence of metastases and strong stroma-dependency. It is estimated that the incidence of cutaneous basal cell carcinoma is increasing worldwide. Microsatellite instability (MSI) constitutes an alternative -to the chromosomal instability- pathway of carcinogenesis for certain tumor types with prognostic and therapeutic significance for the respective patients. Protein kinase A regulatory subunit type 1α (R1α) is encoded by the PRKAR1A gene located on chromosome 17q22-24. PRKAR1A is mutated in more than half of Carney complex (CNC) patients; loss of heterozygosity (LOH) can be demonstrated in tumors from CNC patients. Molecular studies also revealed R1α down-regulation, PRKAR1A-inactivating mutations and/or 17q22-24 LOH in certain sporadic tumors. In this study, we investigated whether changes in the PRKAR1A gene and the expression of the R1α protein are related to the MSI status of BCCs and involved in the pathogenesis of BCC. Methods: The presence of MSI was investigated in 75 BCCs using mononucleotide microsatellite markers, BAT-25, BAT-26 and the repetitive sequences within the target genes TGF-beta-RII, IGFIIR, hMSH3, hMSH6 and Caspase5. Additionally, 3 dinucleotide markers were analyzed on 20 cases of BCC with matched normal tissue. DNA analyses for the 17q22-24 locus (LOH) and the PRKAR1A gene mutations were also performed on 20 cases of BCCs with matched normal tissue. For LOH studies, 12 microsatellite markers (MM) were examined around the 17q22-24 locus. The coding PRKAR1A gene sequence was checked in all samples. Results: MSI was detected in 16% of the cases exhibiting alterations only in the mononucleotide markers. The rate of High-MSI (H-MSI) was <5%: two cases, 1 multifocal superficial and 1 nodular BCC showed H-MSI at three markers (BAT-25, BAT-26 and hMSH3) and at two markers (BAT-25 and TGF-beta-RII) respectively. The rate of LOH for 17q22-24 was 15%; 3 of the 20 matched BCC cases showed extensive 17q22-24 LOH. The analysis of the PRKAR1A gene is pending. Conclusion: No association could be elicited between LOH for the 17q22-24 locus and the MSI status of the BCCs. Results of immunohistochemistry (IHC) with R1a antibody as well as the results of PRKAR1A mutations will elucidate the contribution of PRKAR1A in the pathogenesis of BCC.

OP28-8

THE FUSE/FBP/FIR/TFIIH-SYSTEM IS A MOLECULAR MACHINERY EQUIPPED TO CONTROL THE EXPRESSION OF THE C-MYC PROTO-ONCOGENE BY SENSING DNA-CONFORMATION OF THE PROMOTER AND FINE-TUNING TRANSCRIPTIONAL OUTPUT

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Background: De-Regulation of the c-myc proto-oncogene can be carcinogenic. A continuous stream of activating and repressing signals is processed by the transcription complex at the promoter of the c-myc gene. The general transcription factor IIH (TFIIH) is held at promoters prior to promoter escape, and targeted by the single-stranded DNA-binding transcription factors FarUpStream element (FUSE) binding protein (FBP) and FBP-interacting repressor (FIR). Since transcpritionally generated torsion melts FUSE, FBP and FIR bind dependent on FUSE melting, and both regulate transcription through TFIIH, the FUSE/FBP/FIR/TFIIH-system is well situated to modulate the input of activators and repressors of the c-myc gene. Methods: Chromatin immunoprecipitation (ChIP) reveals that FBP and FIR establish a dynamically remodelled loop with TFIIH, which is lost in cells from xeroderma pigmentosum B, a cancer-prone syndrome caused by mutations of p89, the largest subunit of TFIIH (XPB). Using in vivo-footprinting and nuclear run on-experiments, structural, compositional, and functional differences are demonstrated in transcription complexes at the native c-myc promoter between XPB cells, and cells complemented with wild-type p89 (XPB/wt-89). Remarkably, although the mean levels of c-Myc are only modestly elevated in XPB cells compared to XPB/wt-p89 cells (determined by RNase protection assay, western blot, and FACS analysis), the range of expression and the cell-to-cell variation of c-Myc are shown to be markedly increased. Results and Conclusions: Our data indicate that TFIIH integrates inputs from multiple signals, regulating transcription at multiple steps between initiation and promoter escape. By utilizing dynamic changes of DNA-conformation, the FUSE/FBP/FIR/TFIIH system helps to suppress the intrinsic

noise of transcription and to ensure a steady transcriptional output of c-myc, which is necessary for cellular homeostasis.

OP29-1

E -CADHERIN LOSS AND DELTA-NP73L EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMAS

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BACKGROUND: During the last years studies based on DNA array have evidenced that oral squamous cell carcinoma (OSCC) with metastases has a gene expression profile different from OSCC devoid of metastases. This lead to the formulation of the "metastatic gene expression profile" hypothesis. Accordingly primary OSCC harboring metastases carry genetic alterations favoring the metastatic process. Purpose of the present study is to investigate parameters useful to predict lymph node metastases. METHOD: Fifty-eight cases of surgically resected OSCC were studied with an immunohistochemical method for the adhesion molecule E-Cadherin and the truncated dominant-negative isoform of p63 (delta-Np63). In addition p63 gene expression profile was evaluated by Real Time-PCR to disclose the presence of the truncated variant delta-Np73L. Data obtained were correlated with lymph node metastases detected at presentation and during follow-up. RESULTS: E-Cadherin expression is the most powerful parameter related to the presence of lymph node metastases at presentation. Twenty four cases out of 38 (63%) showing low E-Cadherin expression developed lymph node metastases as compared to 5 out of 20 (25%) cases($P<0.01$) showing high E-Cadherin expression. The predictive value increases when low expression of E- cadherin is associated to immunohistochemical expression of delta Np63. When this occurs, lymph node metastases are seen in 21 out of 28 (75%) of the cases, as opposed to 17 out of 30 (57%) of the remaining cases. The association between E-Cadherin low expression and delta-Np73L is highly predictive for developing lymph node metastases even in small sized tumours (T1\T2). When this association occurs, metastases develop in 5 out of 8 (62.5%) cases as compared to 5 out of 31 (16.1%) that don't show this association. CONCLUSION: the present study shows that low E-Ccadherin expression is useful to predict lymph node metastases in cases of OSCC. The predictive value is enhanced when low E-Cadherin positivity is associated to delta-Np63 and delta-Np73L expression.

OP29-2

HTERC TELOMERASE GENE AMPLIFICATION AT 3Q DEMONSTRATED WITH FISH ANALYSIS STRATIFIES LARYNGEAL PRECURSOR LESIONS

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Background: HPV DNA is detectable in 25 to 43% of laryngeal carcinomas. HPV-associated invasive cervical carcinomas invariably carry extra copies of chromosome arm 3q. The demonstration of this specific genomic imbalance using FISH analysis is considered as a valuable tool to identify precursor lesions with potential to progress to invasive carcinoma. FISH analysis includes a series of six BAC clones that cover the human telomerase gene (hTERC) which maps to chromosome 3q. We analysed the gain at 3q in HPV-positive or negative laryngeal precursor lesions using FISH technique in order to stratify the potential to progress. Method: Buffered formalin-fixed routine samples of laryngeal precursor lesions were classified according to the Ljubljana criteria (WHO 2005). 11 laryngeal precursor lesions (4 atypical hyperplasias and 7 carcinomas in situ) were analysed for HPV by PCR technique. PCR primers GP5 and GP6 detecting the HPV L1 open reading frame were used for general HPV detection. Typing was performed using type-specific primers for HPV 6, 11, 16 und 18. Additionally, all samples were analysed concerning gains at 3q using FISH technique and the CERVIxCYTE kit following manufacture's protocol (Cancer Genetics, USA). Results: None of the 4 atypical hyperplasias were positive for HPV, but 3 out of 4 cases demonstrated a 3q amplification in FISH analysis. In 3 out of 7 carcinomas in situ HPV DNA was detected and typed as HPV 16. The 3 HPV 16-positive cases carried multiple signals of the telomerase gene. Conclusions: The increase of telomerase gene signals in the HPV-positive laryngeal carcinoma in situ is in accordance with reports of cervical intraepithelial neoplasia (CIN) 2/3. Consequently, it can be assumed that the telomerase gene amplification visualised via FISH technique is suitable to stratify HPV-associated laryngeal precursor lesions concerning the potential risk to progress. An increase of telomerase activity and protein expression in laryngeal precursor lesions is well documented. The significance of increased telomerase FISH signals in the HPV-negative atypical hyperplasias may be discussed in this relation.

OP29-3

CHROMOSOME INSTABILITY AS DETECTED BY FISH PREDICTS PROGRESSION OF PREMALIGNANT LESIONS OF THE LARYNX

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Background: The dilemma in managing patients with precursor lesions of the head and neck mucosa is of deciding which lesions or mucosal areas are potentially malignant and will progress to carcinoma. In a previous study on a small series of laryngeal precursor lesions we showed that chromosomal instability (CIN), indicated by the presence of chromosome imbalances and/or polyploidization using single-target brightfield in situ hybridization (ISH) for chromosome centromeres 1 and 7, strongly predicted its malignant progression (J. Clin. Oncol. 2000, 1644-1651). To further substantiate these data for clinical application, we have examined retrospectively a new series of 69 patient specimens by a double-target fluorescence ISH (FISH) approach using both centromere probes. Method: Paraffin-embedded tissue sections of laryngeal precursor lesions with dysplastic features of 69 patients were used for histopathological reclassification and FISH analysis. Medical records were screened for patient follow-up data (development of carcinoma (in situ)), and results were statistically evaluated. Twenty cases, which received radiotherapeutic treatment directly after the histopathological diagnosis of carcinoma in situ, were omitted in Kaplan Meier analysis. Results: FISH analysis showed an increasing level of CIN with increasing histopathological grade. The disease-free survival in lesions with CIN was significantly worse than in lesions without CIN ($P=0.0014$). Routine histopathology was unable to predict malignant progression in precursor lesions of the larynx ($P=0.13$). Its value, however, strongly improved after reclassification of the specimens by an experienced pathologist ($P=0.0007$). In the group of low grade lesions (hyperplasia, mild and moderate dysplasia) only CIN

strongly predicted malignant progression (P=0.0004 versus P=0.1134 for histopathological diagnosis). Conclusion: Laryngeal tumorigenesis is associated with the development of CIN, which can be used for reliable identification of lesions at risk for progression. This study was supported by the Research Foundation of the University Hospital Maastricht and the Dutch Cancer Society.

OP29-5
HIGH PREVALENCE OF HPV INFECTION IN HEAD AND NECK SQUAMOUS CELL LESIONS.

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Background: Cytopathic modifications due to human papillomavirus (HPV) infection are difficult to recognize from artefactual changes in small, crushed or electrocoagulated samples and from cytologic atypia of neoplastic cells. Having noticed the presence of simil-cytopathic modifications in nearly all squamous cell lesions of the head and neck, we collected a small consecutive series of hyperplastic, preneoplastic and neoplastic lesions and investigated the prevalence and the genotype of HPV infection. Materials: 30 samples were examined including 5 reactive hyperplasias, 16 squamous cell papillomas, 3 dysplasias and 6 squamous cell carcers. HPV infection was assessed with the INNO-Lipa HPV-Genotyping V2 kit (Innogenetics NV, Ghent, B) that recognizes 10 low-grade (LG) and 15 high-grade (HG) viral types. P16 expression was immunohistochemically determined to document viral genome integration. Results: All samples tested but one papilloma harboured HPV DNA. HG-HPV was detected in 3 (60%) hyperplasias, 6 (37,5%) papillomas, 2 (66%) dysplasias and 5 (83,3%) neoplasias. 27 different HG-HPV infections were observed in 16 samples. HPV 16 was the most frequent (26%), followed by less common types 31, 51 and 52 (18.5% each), 18 (7,4%), and 33, 39,68 (3,7% each). According to disease site, oral, oropharyngeal and nasal lesions showed an equal number of HG and LG type infections; laryngeal lesions harboured in 7 cases HG and in one case LG HPV. One reactive hyperplasia with HG-HPV infection recurred repeatedly with cancer development; 1 of 9 papillomas with LG infection and 3 of six with HG recurred repeatedly. Follow-up of dysplastic lesions was too short to assess recurrences. P16 expression was observed in 75% of samples with HG infection, including those recurring, and in none of those with LG infection. One heavy smoker and 2 drinkers were present in the series, all harbouring HG HPV infection. Conclusions: The high prevalence of HPV infection in a small series of squamous cell lesions of the head and neck with suspected cytopathic changes suggests that in our geographic area HPV diffusely infects high airways. Molecular tests recognizing a wide spectrum of viral types are probably in part responsible for the higher prevalence observed in the present study with respect to literature data, most focusing on types 16 and 18. HG infection in benign lesions seems to increase the risk of recurrence/progression. Further tests to assess prevalence of HPV in normal subjects and the association with heavy smoke/drink are in progress.

OP29-6
CO-EXPRESSION OF EGFR AND CYCLIN-D1 PREDICTS RADIOCURABILITY OF T2N0 SQUAMOUS CARCINOMA OF THE LARYNX

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Modulators of the cell cycle are considered important in carcinogenesis and may influence the response to radiotherapy. Many previous studies of potential interactions between tumour biomarkers in head and neck cancers have produced inconsistent results due to a mixture of primary sites, stages and treatment modalities. This study has examined the immunocytochemical expression of Ki-67, epidermal growth factor receptor (EGFR), cyclin D1 and retinoblastoma protein in diagnostic biopsy material, in relation to primary site recurrence rates of T2N0 laryngeal carcinomas in 50 patients treated with curative intent using a standard radical radiotherapeutic protocol. Five carcinomas showed loss of retinoblastoma protein expression and the median indices of immunocytochemical markers were 50% for Ki-67, 21% for cyclin D1 and 47% for EGFR. There was a positive correlation between the expression of EGFR and that of cyclin D1 (p=0.03). On univariate analysis, relatively low Ki-67 expression (<50%) showed a trend for association with tumour recurrence but this association was not statistically significant. When interactions were examined using median values for a cut off between high and low expression and multiple logistic regression analysis with categorical modelling, it was found that a combination of high EGFR expression and high cyclin-D1 expression was associated with a high risk of tumour recurrence at the primary site (odds ratio 5.32, 95% CI +/- 0.41). This study illustrates the value of examining molecular interactions in a group of clinically well-defined cancers treated with a uniform protocol. If confirmed, the results may provide assistance in deciding between appropriate treatment options for patients with squamous cell carcinomas of the larynx.

OP30-1
MOLECULAR H&E

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Traditional quantitation techniques for anatomical or molecular pathology applications involve either processing Hematoxylin-Eosin (H&E) bright field microscopy color images (containing red, green, and blue image channels), or multiple channel molecular images taken by a fluorescence microscopy. Simultaneous imaging of both microscopy modalities has been difficult due to optical and chemical effects of the H&E dyes. We propose a new sequential imaging and registration technique that overlays the molecular image information from fluorescence microscopy with the color H&E images, hence increasing the number of data channels. First the tissue is labeled with molecular biomarkers, and imaged through a fluorescence microscope, and then the tissue is re-labeled with H&E dyes, and imaged again. We present robust image registration techniques to overcome the sparse distribution of small cell nuclei across different modalities. Once the images are registered then image analysis and quantitation techniques can benefit from the extra information channels. Multi-dimensional clustering and segmentation algorithms can detect different cell compartments from the registered multi-channel images. By merging transmitted brightfield channels with monochromatic fluorescence channels, we can utilize the advantages of each imaging modalitiy such as, high signal-to-noise ratio, and specificity in fluorescence images, and unscattered light in bright-field images. For diagnosis, the pathologist can easily superimpose the molecular biomarker images on the digital H&E images. We demonstrate superimposition of ER, AR, Her2 and

p53 marker images of immunohistochemically stained breast tissue micro arrays on the H&E image of the same tissue.

OP30-2

AUTOMATED IMAGE ANALYSIS FOR HIGH-THROUGHPUT QUANTITATIVE DETECTION OF ER AND PGR EXPRESSION LEVELS IN LARGE-SCALE CLINICAL STUDIES: THE TEAM TRIAL EXPERIENCE

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BACKGROUND: Automated image analysis is an attractive solution to rapid, accurate, and reproducible evaluation of tissue biomarkers in large clinical trials, which to date has not been validated for standard immunohistochemical (IHC) stains in large numbers of samples due to perceived difficulties in complex tissue analysis. By taking a pathology-based approach to cell and compartment discrimination we have validated the Ariol Image Analaysis platform for nuclear ER and PgR histoscore analysis against manually-scored samples. METHODS: 397 cases of breast cancer (UK TEAM trial, part of the multinational TEAM pathology study) were arrayed on two tissue microarrays (TMAs), in six replicates. 56 full sections of corresponding tumours were cut for comparison with TMAs. Slides were stained for ER and PgR by standard IHC techniques. All cores were scored to produce a semi-quantitative histoscore (0-300). One half of the cohort was used as a training set and the other as an independent test set. Slides were scanned on the Ariol SL-50 Image Analysis System, tumour epithelium marked for analysis, and marked areas quality assured by a pathologist. The system was trained on the basis of the cellular characteristics of epithelial, stromal, and infiltrating non-tumourous cells to produce a trained classifier applicable to all nuclear stains. Correlations were assessed using Pearson's correlation coefficient (PCC) and inter-class correlation coefficient (ICCC) for continuous variables. RESULTS:The PCCs for full section and TMA PgR manual histoscores were equal for both three and six cores (0.94). PCCs and ICCCs for automated and manual scores for PgR training TMAs were 0.96-0.97 and 0.88-0.93 when <3 cores were analysed and 0.97-0.98 and 0.94-0.96 when >3 cores were analysed. The PCC and ICC for the PgR test set was 0.98 and 0.96, respectively. PCCs and ICCCs for ER training sets were 0.95 and 0.82 while for the ER test set were 0.98 and 0.84. CONCLUSIONS:Full section and TMA scoring shows excellent concordance for both ER, and the more heterogeneously staining PgR. There is no significant improvement in correlation when more than 3 cores are analysed. The PCCs and ICCCs for PgR are always excellent (>0.93, >2 cores), while the ICCCs for ER show good concordance (0.8-0.9). The lower ICCC values for ER reflect better discrimination of darkly stained nuclei, and an improvement over manual scoring. Automated image analysis can replace manual scoring for ER and PgR performed on TMAs in clinical trials.

OP30-3

MOLECULAR PROFILING OF SIGNAL PATHWAYS IN FORMALIN FIXED CANCER TISSUES USING PROTEIN LYSATE MICROARRAYS

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Histopathological diagnosis in most hospitals around the world is based upon formalin fixed and paraffin embedded (FFPE) tissues. Recently, we established a very efficient and easy-to-use technique for extraction of high amounts of full length, non-degraded and immunoreactive proteins from routinely processed FFPE tissues for Western blot and protein lysate microarray analysis. As defective, hyperactive or dominating signal pathways may drive cancer growth, survival, invasion, and metastasis, we evaluated in this study whether phosphorylated proteins can be detected and quantified in routine clinical tissues. Monitoring the total and phosphorylated proteins in a diseased tissue may allow us to infer the activity levels of the proteins in a particular pathway as starting point for the design of individual therapy regimens. We first compared the extraction efficiency of phosphorylated proteins between formalin fixed and unfixed cells in culture. Epidermal growth factor (EGF) receptor-positive cell lines were stimulated with EGF for various time points and either fixed with formalin or left unfixed. Subsequently, proteins were extracted and analysed by Western blot. We were able to extract and identify all phosphorylated proteins analysed, including phospho-EGFR, phospho-ERK, and phospho-Akt. There were no differences between formalin fixed and unfixed cells. In a first proof-of-principle study we analysed 31 formalin fixed primary human cancers (20 breast and 11 endometrial cancers) and quantified 6 pairs of phosphorylated and non-phosphorylated proteins (EGFR/pEGFR, p38MAPK/pp38MAPK, Akt/pAkt, HER2/pHER2, ERK/pERK, GSK3beta/pGSK3beta). Proteins were extracted from formalin fixed histological tumour sections and printed on nitrocellulose coated glass slides. The resultant protein lysate microarrays were probed with 12 antibodies against the total or the phosphorylated proteins, respectively. We were able to identify all proteins of interest; thus, quantitation of protein phosphorylation in formalin-fixed tissues is possible. The first result of our analysis is the striking degree of patient heterogeneity in the activity of the signalling molecules within each breast cancer patient. In contrast, the endometrial cancer patients did not show this marked heterogeneity. Our study demonstrates the utility of protein lysate microarrays from formalin fixed tissues for the multiplexed analysis of critical phospho-specific endpoints and suggest that pathway activation profiles in breast cancer may be patient-specific.

OP30-4

MOLECULAR EVALUATION AND CLINICAL CORRELATION OF T(14;18)(BCL-2/IGH) IN 26 CASES OF FOLLICULAR LYMPHOMA: THE IMPACT OF DIFFERENT MOLECULAR APPROACHES

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Follicular lymphomas (FL) are among the most common lymphomas, characterized t(14;18) (q32;q21) in more than 80% of patients. As a result bcl-2/IgH rearrangement occurs leading to overexpression of bcl-2. Detection of this translocation is essential either for the diagnosis or the follow-up of the minimal residual disease. The aim of this study was to determine the positivity of t(14;18) by detection of mbr or mcr in 32 paraffin-embedded tissue samples and four peripheral blood samples of consecutive FL patients who were diagnosed and followed-up between 1999-2006 and compare the results with clinical outcomes. After extracting the DNA, t(14;18) bcl-2/IgH rearrangements were detected via different PCR methods. In order to detect the mbr breakpoint real-time PCR with the LightCycler t(14;18) Quantification Kit, multiplex PCR, and

semi-nested PCR was performed. For the determination of the mcr breakpoint, real-time PCR was performed with specific primers and the LightCycler-Fast Start DNA Master SYBR Green I Kit. For the detection of t(14;18) by fluorescence in situ hybridization (FISH), nuclei from paraffin-embedded tissue sections were extracted and used together with LSI IGH Green/BCL2 Orange probes. The success rates for DNA and nuclei isolation of 12 B5-formalin-fixed tissues was 42% and 33%, respectively. More successful isolation rates were obtained on 20 tissues fixed in formalin only, 95% and 60%, respectively. The DNA of blood samples was isolated in all four, but a 50% success rate was obtained in nuclei isolation. In DNA of 24 tissues and four blood samples, mbr positivity was detected 82.14% with semi nested PCR, 53.57% with multiplex PCR, and 28.57% by real-time PCR. mcr rearrangement could not be detected in any of the samples. Of 19 patients whose nuclei were isolated, all but one were t(14,18) positive with FISH. Fourteen of 18 patients with Grade I FL, the only one case of Grade II FL, and four of five patients with Grade III FL were mbr-positive. Kaplan-Meier curves demonstrated that the mean estimated overall survival in mbr (+) and (-) patients were 59.0 and 91.5 months, respectively. As a result, semi-nested PCR has been found to be superior for the detection of mbr positivity, compared to all the other methods used in this study. In addition it has been shown that mbr positivity has an important impact on survival and is essential for the follow-up of minimal residual disease even in the status of clinical remission.

OP30-5
OPTIMISATION OF DNA EXTRACTION AND AMPLIFICATION FROM LASER MICRODISSECTED PARAFFIN EMBEDDED BREAST CARCINOMA FOR ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (ACGH): PROBLEMS AND SOLUTIONS

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Background: Array comparative genomic hybridization (aCGH) is a powerful cytogenetic technique for assessing unbalanced genomic alterations and has successfully been used in determining putative oncogenes and tumour suppressor genes in breast cancer. There is a need to apply aCGH to small quantities of DNA obtained from laser capture microdissected (LCM) archival formalin-fixed paraffin-embedded (FFPE) tissue. Our objective was to develop a DNA extraction and amplification protocol compatible with aCGH for investigating small foci of FFPE putative preinvasive breast cancer lesions. Material and Methods: A number of DNA extraction and amplification techniques were compared using whole FFPE and frozen tissue sections, including degenerate oligonucleotide-primed (DOP) PCR, Phi29 (GenomiPhi), restriction and circularization aided rolling circle amplification (RCA-RCA), GenomePlex whole genome & single cell amplification (WGA-2 & WGA-4) techniques. Following amplification, three proprietary kits were compared for their ability to retain high molecular weight DNA. The DNA quality was assessed using a multiplex PCR-based technique. Subsequently, an optimised technique was employed to investigate DNA derived from LMD FFPE tissue with aCGH, utilizing the Breakthrough Breast Cancer Research Centre 16K BAC array platform. Results: We found that incubation of FFPE sections in NaSCN at 37oC overnight prior to DNA extraction was associated with improved DNA quality. Although DOP-PCR and GenomiPhi amplification gave high DNA yield, we found that a modified GenomePlex amplification (WGA-4) technique was more robust in producing higher molecular weight DNA fragments as assessed by the multiplex PCR QC assay, in conjunction with the use of the MinElute kit. Prediction of successful PCR QC was found to be independent of the FFPE sample age. Successful aCGH was achieved only in the cases that had passed the multiplex PCR QC assay. Conclusion: We present a protocol for DNA extraction and amplification of archived LMD-FFPE tissue compatible with aCGH. Using our technique, successful aCGH can be accomplished from as little as 10ng of FFPE-derived DNA; further work is needed to assess amplification fidelity. This technique facilitates the investigation of small/microscopic lesions, including breast pre-invasive lesions, breast cancer precursors and micrometastases.

OP31-1
NEURONAL ALTERATIONS IN RAT HYPOTHALAMIC TYPE HYPOTHYROIDISM

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Background: Thyroid hormones (THs) are important in the development and maturation of the central nervous system (CNS). The significant actions of THs during CNS development occur at the time when TH levels are lower than those in the mother and the hypothalamic-thyroid (HPT) axis is not fully functional. In the developing rat nervous system, primarily cerebellum the first three postnatal weeks represents a period of significant sensitivity to thyroid hormones. Method: Hypothyroid Sprague-Dawley rats were obtained from a colony maintained at Texas A&M University, USA. Thyroid hormones (T3, T4, and TSH) were analyzed from blood serum samples by a radioimmunoassay procedure. Frozen brain sections were saved at -80C for western blot and RT-PCR assays. Rats were perfused with 4% paraformaldehyde via the left ventricle and tissues were saved for routine histology, immunohistochemistry and electron microscopy studies. Results: This study presents a spontaneous, inherited recessive hypothyroidism in Sprague Dawley rats with devastating functional consequences to the development of the CNS. The clinical signs develop around 14 day?s postnatal (dpn) and are characterized by ataxia, spasticity, weight loss and hypercholesterolemia. The afflicted rats died at 30 days due to severe neurological deficits. The deterioration affects the entire CNS and is characterized by progressive neuronal morphological and biochemical changes, demyelination and astrogliosis. The cerebellum, brain stem, neocortex, hippocampus and adrenal gland medulla appear to be most affected. Thyroid Stimulating Hormone (TSH), T3 and T4 levels are significantly lower in hypothyroid rats than control. Immunohistochemistry and RT-PCR demonstrated a reduction of Thyrotropin Releasing Hormone (TRH) in the hypothalamus of hypothyroid rats. Western blots demonstrated elevation of ERK1/2, pro- NGF and GFAP. The HPLC analysis of brain samples from hypothyroid rats showed elevation of glutamate and decrease in GABA. The weight of both thyroid and pituitary glands are significantly less in hypothyroid rats than the corresponding normal littermate controls. Transmission electron microscopy demonstrates consistent postsynaptic dendritic, synaptic and spine alterative changes in the brain of hypothyroid rats. Conclusions: This data suggests that we discovered a tertiary form of inherited hypothyroidism involving the hypothalamus. In this neonatal inherited tertiary hypothyroidism rat animal model there are widespread CNS changes with fatal consequences to the host.

OP31-2
THE IMPACT OF IGF-1 AND VEGF ON CELLULAR PROLIFERATION AND INVASIVENESS OF PITUITARY ADENOMAS

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Introduction: Pituitary tumors comprise 10 to 15% of all intracranial tumors. The factors related to tumor progression, proliferation and invasiveness in pituitary tumors have not been fully explained. In the recent studies it was shown apparently that proliferative, antiapoptotic and mitogenic effects of IGF-1 in epithelial and mesenchymal tumors. VEGF is a specific mitogen of vascular endothelium what is more is its well documented role in angiogenesis of diabetic retinopathy. Additionally, it is a fact that tumors those of capable of secreting VEGF behave more aggressive clinically. Methods: In this study, formalin fixed-paraffin embedded tissue blocs were specifically stained. Endocrine active (GH, PRL and mammosomatotrop) and endocrine inactive (null cell) and invasive pituitary adenomas were grouped according to their expressivity of IGF-1, IGF-1 receptor, VEFG and MIB-1 LI's. Tumors were classified according to demographic (age, gender, site) and hormonal immunoreactivities. Proliferative capacities of each individual tumor were assessed by MIB-1 in addition to other factors, i.e., IGF-1, IGF-1 receptor, VEFG. Results: In almost all groups it was apparent that IGF-1 receptor reactivity decreased and VEFG expressivity increased. Besides, it was apparent that statistically significant correlation between IGF-1 and VEGF existed. In contrary, although there was a trivial increase in MIB-1 LI in comparison with control group, but no significant correlation between histological subtypes. Conclusion: It seemed that no correlation between VEGF and tumor cell proliferation was existent. However, proliferating elements of vascular infrastructure for pituitary tissue could be suppressed by IGF-1 so that these neoplastic lesions could have been kept under control without necessitating surplus therapeutic irradiation. Also, the use medical IGF-1 treatment relentlessly decrease incidence of post radiotherapy pituitary failure syndrome. Hitherto, invasive pituitary adenoma group shared similar immunophenotypical properties with that of non invasive group. These findings were most likely demonstrating that some additional factors controlling not only invasion but also migration might have been action. So, this emphasizes the uncertainty in this field necessitating further compelling and meticulous studies. Abbreviations: LI: Labeling index / IGF-1: insulin-like growth factor / IGF-1 receptor: insulin-like growth factor / MIB-1 (anti-Ki-67) /

OP31-3
PROGNOSTIC EFFECT OF Ki-67 PROLIFERATION INDEX IN GLIOBLASTOMA MULTIFORME

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Aim: Studies investigating the proliferation markers and outcome of patients with glioblastoma multiforme (GBM) have generated controversial results. In this study, we aimed to assess the prognostic significance of Ki-67 proliferation index (PI) in a cohort of 85 GBM patients treated with surgery and radiotherapy. Material and Methods: Patients were treated in a single institution between 1997 and 2004. Median age was 59 years (range 22-79) and male/female ratio 55/30. Type of surgery was gross total resection in 43%, subtotal resection in 29% and stereotactic biopsy in 28%. All patients received median dose of 60 Gy radiotherapy (range 10-72 Gy). MIB-1 (MS-123-R7, Neomarkers) against the Ki-67 antigen in paraffin-embedded tissue sections was used to determine Ki-67 PI. Results: Median overall survival (OS) was 9 months, and 1 year OS rate was 37% for all patients. Ki-67 PI ranged between 0 and 96 (median=13, mean=17.86). In univariate analysis, age<50(p=0.02), male gender (p=0.039), KPS score >/=70 (p=0.037), symptom duration >/= 2 months (p=0.035), multicentricity (p=0.034) and surgical resection (p=0.0003) were favourable prognostic factors for improved survival. Patients with Ki-67 PI >/= 13 showed a trend for better prognosis (p=0.07). In multivariate analysis, age (p=0.006), Ki-67 PI (p=0.008) and surgical resection (p=0.008) were observed to be predictive for survival. Conclusion: In our GBM patients series younger age, surgical resection and Ki-67 PI proved to be independent prognostic factors for survival. Since all patients received radiotherapy, we suggest that tumors with a high proliferation rate were more responsive to treatment.

OP31-4
VASCULOGENESIS IN HUMAN GLIOMAS

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Neovascularization is a prerequisite for the growth and invasion of a malignant tumor mass. Traditionally vasculogenesis, a process defined as the recruitment and differentiation of circulating bone marrow-derived endothelial progenitor cells (EPCs) into mature endothelium, was thought as solely occuring in the embryo, but it appears that postnatal vasculogenesis under specific circumstances may also occur in adults. EPCs largely reside in the adult bone marrow and the fraction of circulating EPCs in the peripheral blood of healthy people is very low. Upon triggering by cytokines or the release of angiogenic growth factors this fraction increases. Subsequently, the EPCs may enter extravascular tissues where they promote de novo vessel formation by integrating into existing blood vessels and by growth factor signalling. So far vasculogenesis, as a hypothesized mechanism of tumor neovascularization in glioma, has only been investigated in animal models by injection of exogenous bone marrow-derived stem cells followed by tracking these cells in an artificially induced tumor. Whether such data truthfully mirror the situation in human tumors like gliomas is questionable. It is well known that gliomas are among the highest vascularized tumor types. We hypothesized that EPCs are present in glioma tissues and also that patients with gliomas have raised levels of circulating EPCs due to increased release of these cells from bone marrow. We quantified the fraction of circulating EPCs by FACS-analysis using anti-CD34, -CD133 and -KDR-monoclonal antibodies in unselected peripheral blood samples of 32 glioma patients. As control groups served 39 patients with other CNS tumors or diseases; 10 patients with recent ischemic strokes and 19 healthy blood donors. The presence of the EPCs in blood samples and the gliomas was confirmed by mmunophenotyping of cells in cytosmears of the blood buffy-coat and glioma tissue sections. We found that the percentage of CD34+CD133+KDR+ EPCs in the blood of glioma patients is significantly higher than that in the blood of patients with other CNS tumors or diseases (p=0.002), stroke patients (p=0.005) or healthy donors (p=0.013). The presence of identical cells in the cytosmears and in the tumor tissue of the same patients further supports the notion that the EPCs significantly contribute to glioma vasculogenesis. Further investigations on the diagnostic value of these elevated blood levels of EPCs in glioma patients is indicated.

OP31-5
IDENTIFICATION OF GLIOMA NEOVASCULARISATION-RELATED PROTEINS BY USING MALDI-FTMS AND NANO-LC FRACTIONATION TO MICRODISSECTED TUMOR VESSELS

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The identification of angiogenesis-related proteins is important for the development of new anti-angiogenetic therapies, and such proteins are potential new biomarkers for gliomas. The aim of this study was to identify proteins which are exclusively present in glioma neovasculature and not in the vasculature of normal brain. We combined advanced proteomic techniques to compare the expression profiles of microdissected blood vessels from glioma with blood vessels of normal control brain samples. We measured the enzymatic generated peptide profiles from these microdissected samples by matrix-assisted laser desorption\ionization Fourier transform mass spectrometry (MALDI-FTMS). Subsequently, the samples were fractionated by nano-LC prior to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF). This combined approach enabled us to identify four proteins which appeared to be exclusively expressed in the glioma blood vessels. Two of these proteins, fibronectin and colligin 2, were validated on tissue sections using specific antibodies. We found that both proteins are present in active angiogenesis in glioma, other neoplasms and reactive conditions in which neo-angiogenesis takes place. This work proves that gel-free mass spectrometric techniques can be used on relatively small numbers of cells generated by microdissection procedures to successfully identify differentially expressed proteins.

OP31-6
INTERLEUKIN (IL)-8 EXPRESSION AND MICROVASCULARITY IN DIFFUSE ASTROCYTOMAS

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Background: Interleukin-8 is a member of the chemokine family which plays a critical role to the progression of various malignancies, including those that arise in the CNS. Accumulating evidence highlights IL-8 mRNA and protein expression in grade II, III, and IV astrocytomas and the potent mediative role of IL-8 in angiogenesis. Nevertheless, the accurate mechanisms by which IL-8 exerts its angiogenic properties are still under investigation. The aim of the present study was to examine the expression levels of IL-8 in astrocytomas and to explore any possible associations with microvessel morphometry. Method: 98 patients with astrocytomas were enrolled in our study. We determined the expression levels of IL-8 using immunohistochemistry in formalin-fixed paraffin-embedded tissue sections of 65 glioblastomas (WHO grade IV), 10 anaplastic astrocytomas (WHO grade III), and 23 diffuse astrocytomas (WHO grade II). Tumour specimens were categorized in three levels according to the presence of necrosis (absent, focal, extensive). Microvessels, highlighted by means of anti-CD34 immunohistochemistry, were enumerated with computer-assisted image analysis. A spectrum of microvascular parameters was estimated: major and minor axis length, total vascular area (TVA), microvessel density (MVD), perimeter, area, shape factor. Results: IL-8 expression was noted in the cytoplasm of neoplastic astrocytes in 89 cases. Positive neoplastic cells represented 1% to 40% of the neoplastic population. IL-8 expression was highest in cells around areas of pseudopalisading necrosis, within glioblastomas. IL-8 was also detectable in endothelial cells within glomeruloid vascular structures of glioblastomas as well as in inflammatory cells usually around the areas of necrosis. IL-8 expression increased significantly with the presence of necrosis and with tumour grade ($p<0,001$, respectively). We observed a significant positive correlation among IL-8 expression levels and: MVD ($p<0,001$), major axis length ($p=0,008$), minor axis length ($p=0,032$), area ($p=0,016$), perimeter ($p=0,016$), shape factor ($p=0,003$). Conclusion: To the best of our knowledge, this is the first study presenting a fundamental relationship between IL-8 expression and various microvascular parameters within astrocytomas, using immunohistochemistry. Our experimental results testify the proangiogenic capacity of IL-8 confirming its central role in the malignant transformation of astrocytes. Further studies are needed to shed light upon the prognostic significance of IL-8 expression within the heterogeneous group of astrocytomas.

OP31-7
ACTIVATION OF ENDOGENOUS REMYELINATION CAPACITY IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS FOLLOWING TRANSPLATATION OF NEURAL STEM CELLS

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Background: Neural stem cell (NSC) transplantation has been proposed as a means of cell replacement therapy for various diseases of the Central Nervous System (CNS). Transplanted NSCs improved the clinical outcome in experimental models of stroke and spinal cord trauma Although transplanted myelin-forming cells have remarkable remyelinating properties the exact mechanisms by which they react are not clear yet. In multiple sclerosis (MS), as well as in experimental allergic encephalomyelitis (EAE) which is the most reliable experimental model of MS, demyelination and inflammation-related axonal pathology lead to chronic neurological disability. Method: EAE was induced in C57Black/6 mice with myelin oligodendrocyte glycoprotein (MOG). NSCs were isolated and cultured from newborn green fluorescent protein (GFP) transgenic mice, with the same genetic background and transplanted into the lateral ventricles of mice at day 7 after induction of EAE. The control group received only the medium without NSCs. The mice were clinically evaluated daily. Brain and spinal cord immunohistochemistry was performed for glial and neuronal differentiation of transplanted cells and the sections were evaluated under light and confocal microscope. Evaluation for inflammation, demyelination and axonal injury, was also performed under standard histological protocols. Results: Transplanted cells attenuated the clinical course of the disease,

migrated into the white matter and were differentiaied mainly into glial cells. NSC transplantation down-regulated the CNS inflammatory process as indicated by a reduction in the number of perivascular infiltrates. In addition, transplanted cells reduced demyelination and axonal injury and induced the remyelination of demyelinated axons. However, remyelination was attributed to an increased production of myelin of host oligodendrocytes since there was no indication of myelin derived directly from the transplanted cells. Conclusion:Demyelination and acute axonal injury in this model result mainly from the acute inflammatory process and correlate well with the chronic neurological residua. We suggest that the beneficial effect of transplanted NSCs in chronic EAE may at least in part, be due to a down-regulation of brain inflammation and the concomitant reduced axonal injury, leading therefore to improvement of the clinical outcome.

OP31-8

EXPRESSION PROFILING IN GLIOBLASTOMA MULTIFORME IDENTIFIES SURVIVAL-RELATED GENES OF THE PHOSPHATIDYLINOSITOL 3´-KINASE SIGNALING PATHWAY

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Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults and remains extremely lethal despite the continuous technical improvements and recent therapeutic advances. High-throughput analyses of gene expression can identify new key genes, networks and pathways which may be clinically rellevant in patients with this highly malignant tumor. We investigated the pattern of gene expression in 20 primary GBMs using cDNA microarrays containing 27,454 cDNA clones, including 9,900 known genes and uncharacterized tumorigenesis-related ESTs. Differentially expressed genes detected by cDNA array analysis were further evaluated by RT-PCR, tissue microarray (TMA) immunohistochemistry, FISH, and Western blot techniques. Statistical analysis revealed 345 deregulated genes (159 upregulated and 186 downregulated). A gene ontology-based study showed that the most strongly deregulated pathways in our series were those of MAPK and actin cytoskeleton, followed by cell cycle, GnRH and calcium signaling pathways. Several genes of the PI3K pathway were also included in the signature of our GBM series. Microarray analyses showed strong upregulation of ANXA1 in 90% (18/20) of GBMs. Immunohistochemical analysis of protein expression demonstrated strong expression of ANXA1 in 86% (102/118) of the GBM in our TMA, and in 70% (129/182) of the astrocytomas (all grades), 5% (1/20) of oligodendrogliomas, and 17% (3/17) of oligoastrocytomas included in a separated TMA (ANOVA, $P < 0.05$). Distinct molecular subsets of GBM showing different gene expression patterns were detected. Survival analysis using functional clusters of genes revealed an association between the PI3K-signaling pathway and clinical outcome. TIAF1, BAX, RPS6KB2 (p70s6k) and BAD were among the genes whose expression was significantly associated with survival. Some candidate target genes, including ANXA1, BAX, USP7 (HAUSP) and STIM2 were further evaluated in GBM tumors by TMA immunohistochemistry and quantification of the expression levels of STIM2 and USP7 was tested by RT-PCR. We also analyzed the activation status of Akt and GSK3ß and demonstrated that PI3K/Akt pathway is activated in our series of GBM. Our high-throughput studies of gene expression patterns, complemented by immunohistochemistry, RT-PCR, and other validation studies confirm the distinctive expression profile of some PI3K pathway survival-related genes, which may play a significant role as prognostic markers in GBM.

OP31-9

MEDULLOBLASTOMA: CLINICOPATHOLOGIC EVALUATION OF 51 CASES.

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Medulloblastoma, which accounts for 20-25% of all childhood brain tumors, is defined as a primitive neuroectodermal tumor located in cerebellum. In this retrospective study, we reevaluated medulloblastoma cases diagnosed within the last 10 years in our pathology department. The aim of this study was to assess the histological variants and demographic findings of medulloblastomas in our region, and the prognostic value of MIB-1 in our medulloblastoma cases. There were 51 medulloblastoma cases at the period of January 1997 to December 2006. Of the 51 cases, 42 (82%) were children (aged 17 and younger), 9 (18%) adult, 30 (59%) male , 21(41%) female. The mean age of children was 9± 3 and adult 36± 10. Histologically 39 (76%) cases were classical, 11 (22%) desmoplastic (6 of them were adult) and 1(2%) large cell medulloblastoma. Immunohistochemically all of the tumors were immunoreactive for synaptophysine, 11(21%) of 51 were focally immunoreactive for GFAP. P53 immunoreactivity was found in 8 (15%) cases. The immunoreactivity of MIB-1 ranged from 10 to 95% (mean 35%). Follow-up was available for 37 patients in children group. Thirty four patients received a combination of chemotherapy (Cisplatin, Etoposid, Cyclophospamid and Vincristine) and craniospinal radiotherapy, 1 chemotherapy alone. Two patients died at the early phase of postoperative duration. Follow up period ranged from 5 to 64 months (mean 27,1 ±18,8 months). Of the 35 patients, 21 were alive without any evidence of recurrent disease, three were alive with evidence of recurrent disease and 11 died of disease during follow-up. The mean life time for these 11 patients was 21,9±10,4 months. Of the 35 cases with follow-up, 16 have 25% or least MIB-1 value, 19 have 26% or more. Of the 16 cases with a low MIB-1 value, 6 were dead of disease; of the 19 cases with high MIB-1 value, 5 were dead of disease. The statistical difference ($p=0,51$) was not significant.

PP1 - Displayed on Sunday, September 09

Breast Pathology

PP1-1
FINE NEEDLE ASPIRATION CYTOLOGY OF BENIGN FIBROEPITHELIAL TUMORS OF THE BREAST

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BACKGROUND: Benign fibroepithelial lesions of the breast, including hamartoma, fibroadenoma, and benign phyllodes tumor show proliferation of both epithelial and stromal elements. The aim of the present study was to look at common and dissimilar features in order to enable differentiation between these lesions, if possible.
METHOD: Nine fine needle aspiration cytology slides of each entity (hamartoma, fibroadenoma, phyllodes tumor), which were histopathologically confirmed, were reviewed for smear cellularity, epithelial and stromal fragments, epithelial/stromal area ratio, intact lobular units, naked bipolar stromal nuclei, adipose tissue, smooth muscle and multinucleated giant cells. We also reviewed the literature regarding the cytologic picture of adenomyoepithelioma, sclerosing lobular hyperplasia and other lesions with epithelial and stromal components.
RESULTS: Cytologic characteristics were retrospectively evaluated in a semiquantitive manner.
CONCLUSION: The finding of intact lobular units with various amounts of adipose tissue may favor the diagnosis of hamartoma over that of fibroadenoma. Cytologic diagnosis of phyllodes tumor remains difficult. The presence of a low epithelial/stromal ratio favors the diagnosis of phyllodes tumor over fibroadenoma.

PP1-2
PHYLLODES TUMOUR: LINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES OF 45 CASES

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BACKGROUND: The aim of our study was to investigate the clinical, pathological and immunohistochemical features of phyllodes tumors, in order to obtain a detailed profile of this lesion. METHOD: This study presents a clinical, pathological and immunohistochemical analysis of 45 phyllodes tumors, 11 high grade and 34 low grade. Three cases of fibrocystic breast changes were used as a control group. The immunohistochemical panel included, Ki67, p53, Bcl2, CD34, CNA42, Her-2/neu, telomerase, ER, and PR. The following detailed clinical data were collected: age; weight; height; family history of breast tumors; smoking; nicotine intake; and coffee intake). RESULTS: All patients presented with a palpable unilateral mass. Tumors ranged from 25–55 mm in diameter. Typical macroscopic and microscopic features were noted. Immunohistochemical studies showed higher immunoreactivity for CNA42, Ki67, and p53 in high grade phyllodes tumors. CD34 immunoreactivity was greater in low grade phyllodes tumors. No differences were seen between the high and low grade tumors relating to the other markers used. The detailed clinical data collected revealed no significant differences between the low and high grade phyllodes tumors. CONCLUSION: The immunohistochemical profile adds another aspect and may aid in the differential diagnosis of low and high grade phyllodes tumors.

PP1-3
IS ESTROGEN RECEPTOR NEGATIVITY USEFUL AS A MARKER FOR HIGH-GRADE DUCTAL CARCINOMA IN SITU OF THE BREAST?

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BACKGROUND: Ductal carcinoma in situ (DCIS) of the breast is a heterogenous group of non-invasive cancers which if left untreated thought to have the ability to transform to invasive carcinoma in around 30% of cases. There is a substantial difference in the frequency with which this phenomenon occurs depending on the type and grading of DCIS.There has been a sharp increase in the detection of DCIS during the last two decades,largely due to the widespread use of mammography.The aim of this study is to compare the morphological and immunohistochemical characteristics of estrogen receptor(ER) negative and ER positive DCIS of the breast in an attempt to establish a more objective criteria for detecting cases at higher risk to progress to invasive carcinoma. METHOD:60 cases of ductal carcinoma of breast which had a prominent DCIS component in the surrounding tissue and were surgically excised included in this study.All archival hematoxylin and eosin-stained sections were reviewed to confirm the diagnosis and to classifing and grading the in situ lesions.Additional sections of 60 cases were stained for ER,progestrone receptors(PR) and p53 using the immunoperoxidase technique.DCIS was graded according to nuclear morphology as outlined by the National Health Service Breast Screening Programe and Europian Economic Community(NHSBSP/EEC) into three grades.The lesions also classified according to predominent component into comedo,solid,cribriform and micropapillary types.For all markers ,a positive score was given if>10% of tumor cells a dark brown nuclear staining.Statistical analyses were carried out using the X^2 test. RESULTS:60 DCIS cases included 30(50%) high-grade,25(42%) intermediate-grade and 5(8%) low-grade cases.Twenty-two cases were ER-negative(37%) and 38(63%) were ER-positive.The ER-negative cases included 20(91%) high-grade and two(9%) intermediate-grade DCIS.All five low-grade cases were ER-positive.All ER-negative cases were PR-negative,but 16(73%) of ER-negative cases were p53 positive.The 38 ER-positive cases included 10(26%) high-grade,23(61%) intermediate-grade and all five low-grade cases(13%).Of the ER-positive cases 10(26%) were PR-negative and 4(11%) were p53 positive.ER negativity & p53 positivity were significantly associated with high nuclear grade in DCIS. CONCLUSION: There is a significant relationship between ER negativity and high nuclear grade, PR negativity and p53 positivity.Immunohistochemical evaluation of ER status may be of great help in classification of DCIS as well as in planning the management of the disease.

PP1-4
EVALUATION OF THE INCIDENCE OF ANDROGEN RECEPTOR EXPRESSION IN INVASIVE DUCTAL CARCINOMA OF BREAST

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BACKGROUND: To evaluate the frequency of androgen receptor (AR) expression in invasive ductal carcinoma of breast.
METHODS:Sections of 55 cases of invasive ductal breast carcinoma were examined using a monoclonal antibody against AR on formalin-fixed paraffin-embedded archival material.AR was considered positive when at least 10% of tumoral cell nuclei were immunoreactive.The results also were correlated with the results of estrogen and progestrone receptors(ER & PR) previously done immunohistochemically on the specimens. RESULTS: AR were positive in 24 cases(43.6%).From 55 cases,9(16%) were grade 1,33(60%) grade 2 and 13(24%) cases

were grade 3.AR were positive in 33%(3) of grade 1,45%(16) of grade 2 and 38%(15) of grade 3 tumors.ER and PR were done on 34 cases previously including 5 grade 1,18 grade 2 and 11 grade 3 carcinomas.Among the grade 1 cases 2 were AR positive which also were ER and PR positive but 2(11%) of grade 2 and 3(27%) of grade 3 tumors were AR positive and ER negative.Also 5(28%) of grade 2 and 3(27%) of grade 3 tumors were AR positive but PR negative.
CONCLUSION: Androgen receptor expression is common in invasive breast carcinomas.Some high grade carcinomas are ER and PR negative but AR positive.We suggest that immunohistochemical evaluation of AR may help in providing more information about steroid receptors in breast carcinomas and could be helpful in diagnosis of origin in metastatic high grade breast carcinomas.

PP1-5
BCL-2 ONCOGENE AND Ki-67 ANTIGEN EXPRESSION: A CORRELATION BETWEEN ESTROGEN-RECEPTOR POSITIVE AND NEGATIVE BREAST CARCINOMA

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BACKGROUND: The aim of this study was to evaluate Bcl-2 oncogene and Ki-67 antigen expression in estrogen receptor (ER) and progesterone receptor (PR) positive and negative breast carcinoma. METHOD: A retrospective study was conducted in 72 cases of infiltrating ductal carcinoma of the breast in postmenopausal women, between 1999 and 2004, divided into two groups: Group A (ER and PR positive, n=37) and Group B (ER and PR negative, n=35). Immunohistochemical analysis of Bcl-2 expression was carried out semi quantitatively based on the percentage of stained tumoral cells and the intensity of staining, while Ki-67 analysis was performed by counting the stained nuclei of 500 tumor cells in each sample. Student t-test and chi-square test were used in the statistical analysis of the data and significance was established at p [LTEQ] 0.05. RESULTS: Bcl-2 oncogene expression was statistically greater in tumors of Group A (59,5%) compared to those of Group B (8,6%), ($p<0.001$). There was no significant difference in Ki-67 antigen expression between the two groups (p=0,200). CONCLUSION: Of the markers studied, only Bcl-2 had a significantly greater expression in the estrogen and progesterone receptor-positive breast tumors compared to the negative tumors.

PP1-6
ASSESSMENT OF SKIN INVOLVEMENT IN MASTECTOMY SPECIMENS DUE TO BREAST CANCER REFERRED TO BAQIYATALLAH HOSPITAL PATHOLOGY PART - TEHRAN – IRAN DURING 2004-2006

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Objective: The goal of this retrospective, descriptive & cross-sectional study was to assess the rate of skin involvement by the invasive malignant tumoral cells in breast cancer patients who underwent mastectomy operations & thus to evaluate the feasibility & adequacy of skin- sparing operations in these patients. Materials & methods: There were 130 mastectomy specimens referred to this lab. during 1382-84. After gross examination the specimens were sampled using routine sampling procedure, including at least three sites of the nipple, areola & the closest portion of the skin to the tumoral mass as well as any grossly abnormal area, if present. Results: The peak of age incidence of breast cancer was the range of 40-49 years. From 130 received specimens, data about skin involvement & tumor type included in 126 & 124 available reports, respectively. Invasive ductal carcinoma was present in 120 specimens (97%) & invasive lobular carcinoma in 2 specimens (1.5%), while two specimens showed mixed invasive ductal & lobular carcinoma. The overall tumoral involvement of the skin, nipple & areola was seen in 12 patients (9.5%) as the following: skin: 4% (5 cases); nipple: 8% (10 patients); areola: 0.8% (one patient). Paget's disease was present in two patients whereas three other patients showed concomitant nipple & skin involvement by the ordinary tumoral cells. The areolar involvement was seen along with nipple involvement in only one patient. All of the surgical skin margins were free from the tumors. Conclusion: There was only a significant (statistical) relationship between skin involvement & nipple involvement & also between skin involvement & perineurial invasion. Regarding the lower age of peak incidence of breast cancer compared to other populations & also the overall low rate of skin, including resected margins, nipple & areola involvement in our patients, skin- sparing mastectomy operations seem to be the adequate procedures for the patients with the aim of performing reconstructive surgery at the time of mastectomy. Furthermore, nipple& areola- sparing mastectomies are also to be considered in these cancer patients. It is suggested that this study also to be performed in other pathology centers.

PP1-7
EVALUATION OF NUCLEAR GRADE IN LOBULAR CARCINOMA IN SITU (LCIS): CORRELATION WITH IMAGE CYTOMETRY

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Background: The LCIS lesions are a heterogeneous group of lesions that need further characterization and classification. Although in ductal lesions the correlation between nuclear grade and aggressive behavior is well accepted, this aspect has been disregarded in lobular lesions. The aim of this study was to demonstrate the heterogeneity of LCIS in their nuclear grade and confirm these findings by nuclear morphometry. Design: Consecutive cases of LCIS were retrieved from files; non-classic lesions were confirmed by immunohistochemical staining for E-cadherin and HMW-CK. The following histologic features were evaluated: cell type (A or B), necrosis, degree of distention of units and nuclear grading. Nuclear grading was based on nuclear size and pleomorphism, similar to that used for grading DCIS (CAP recommendations). We used a two-tiered grading scheme: low grade included grade 1 lesions and high grade included grade 2 and 3 lesions. Measurements of nuclear features were performed using the research prototype of quantitative pathology system ClearPathTM (Perceptronix, Vancouver, Canada). Nuclear grade was correlated with histopathologic features and quantitative nuclear features. Result: Of the total 33 cases, 73% were associated with invasive carcinoma (55% lobular, 18% ductal). Based on our grading system, 52% cases of LCIS were low grade and 48% were high grade. Low grade lesions exhibited classic LCIS phenotype; small cells with regular small nuclei without nucleoli, while high grade lesions showed larger nuclei, cytologic atypia, or both. Quantitative morphometry supported the grading system with significant differences observed in mean nuclear area (p=0.001) and standard deviation of area (p=0.005) between the grades. High nuclear grade was significantly associated with the presence of necrosis (p=0.04), but not with cell type, degree of distension of units, or presence of invasion.
Conclusion: We have shown that nuclear grading of LCIS is feasible and the differences in nuclear grade correlate with quantitative nuclear features measured by image cytometry.

Grading LCIS may be important to better characterize this heterogeneous group of lesions. Outcome based studies are needed to explore the prognostic significance of nuclear grade in LCIS in order to define treatment strategies needed for lesions with different levels of risk or malignant potential.

PP1-8
THE STATUS OF MDM2 GENE EXPRESSION IN PRIMARY BREAST CANCER AND IT'S RELATIONSHIP WITH PROGNOSTIC FACTORS

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Introduction: The breast cancer is the most common malignancy in women. MDM2 expression is determined in some carcinomas, sarcomas, leukemias,and breast cancer. The purpose of this study is to determine the expression of MDM2 gene in primary breast cancer and its relationship with grade, stage and axillary lymph node involvement. Material and Methods: the study is the cross-sectional one that was performed on 75 samples of patients with breast cancer admitted in Mostafa Khomeini hospital (2000-2005). After preparing the samples, a tissue section from each sample was obtained. One of the tumoral sections and one of the lymph node sections were stained by H&E. We determined the type of the tumor, the number of lymph nodes, the stage and the grade of the tumor.We studied MDM2 with polycholonal antibody by IHC. Result: Our study showed that 69.3% of patients had lymphoid involvement. 32% of samples were positive for MDM2, 58.7% of samples were in stage II and the most of patients (42.7%) were in grade III. In this study, we didn't find any relationship between MDM2 & grade. We found a relationship between MDM2 & stage ($p<0.002$), a relationship with lymph node involvement ($p<0.002$) that the incidence of MDM2 is increased with lymph node involvement. Conclusion: According to the results of previous studies and our study, it seems that MDM2 is a prognostic factor in breast cancer. But a further investigation with more specimens and long-time follow-up is required to clarify the exact role of MDM2 in prognosis of breast cancer.

PP1-9
PRIMARY BREAST MALT LYMPHOMA – DIAGNOSED BY LYMPHOEPITHELIAL LESION- CASE REPORT

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Background: Primary breast lymphoma, particularly primary mucosa- associated lymphoid tissue (MALT) lymphoma is rare disease. Methods: An 78 year- old woman presented with large firm 10,5x7,8 cm mass into the central part of the right breast with extension to outer lateral quadrant. Ipsilateral axillary lymph nodes were enlarged. The rest of the physical examination findings were normal. Patient underwent mastectomy with axillary lymph node dissection. Results: Pathologically the tumor tissue was composed of small to large lymphoid cells. The large cells contained considerable number of centroblasts and immunoblasts. Small cleaved centrocites were predominant in small cell population. Predominant tumor cell populations in lymph nodes were centroblasts and imunoblasts with high mitotic activity. Using a panel of immunohistochemical stains, tumor cells expressed B cell phenotype CD20+, CD 79α +, CD3-, CD5-, CD23-, bcl 2+, bcl 6-, CD43-, CD10+/-, CD38+, CD 138+/-, Ki-67+. The tumor cells infiltrated the ductal epithelial cells, distording the duct structures, to form, lymphoepithelial lesions for the diagnosis of MALT lymphoma of the breast. Conclusions: Patient post- operatively was given chemotherapy. We emphasize the importance of lymphoepithelial lesions for the diagnosis of MALT lymphoma of the breast.

PP1-10
A PEROPERATIVE EXAMINATION OF A SENTINEL LYMPH NODE IN BREAST CANCER : ...TO BE OR NOT TO BE...

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A peroperative examination of a sentinel lymph node in breast cancer has not been considered yet as a standard in the surgical therapy and being de facto rejected by pathologists in the Czech Republic. It is an examination demanding for both a surgeon and pathologist. Methods: A sentinel lymph node is identified by a surgeon by the patent blue and radio colloid. Individual lymph nodes are prepared, measured and divided in halves in a longitudinal direction. From each half of the node, impression cytological preparations are made, and, simultaneously, a cryostat cut is made from one half of suspecting node. Cytological specimens are stained by May Grünwald-Giemsa in a shortened regime, histological sections by haematoxylin-eosin in a shortened regime, too. A time interval between the material supply and result announcement varies depending on a number of examined nodes in a range of 5 to 20 minutes. Then the material is fixed in a solution intended to fixation of the lymphatic tissue. The fixation is followed by a histological examination from a paraffin sections. From each half of the sentinel node, 10 cuts are made (haematoxylin eosin) in 30 μm levels, each third and eighth cut are stained immunohistochemicaly against pancytokeratins. We considered compliance between a result of the peroperation and definitive histological examination to be a completely negative result and occurrence of a macro and micro metastasis in case of a positive result of a peroperation examination. A presence of the isolated cell cluster was considered to be a negative result in both examinations. Results: In the period of January 2006 to February 2007, we at our workplace made peroperative examinations of 338 nodes from 113 patients in the age of 36 to 83 years (the age median being 60 years) with breast malignity followed by classical histopathological techniques, while the sensitiveness of the peroperation examination against paraffin techniques was 87.6 % (99 cases in compliance with the peroperation finding). False positive findings occurred in two cases (1.8 %). Conclusion: A peroperative examination of a sentinel node can be made while taking into account false negativity (in this file 12.4 %), or false positivity (in this file 1.8 %). In order to eliminate mistakes, it is suitable to use a combination of an impression cytology and cryostat section which, however, puts great demands for technical and personal equipment of a biopsy executing laboratory.

PP1-11
THE CORRELATION OF CYCLIN EXPRESSION TO HISTOLOGICAL GRADE IN BREAST CANCER

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Background Cyclins are proteins which control the cell cycle. They are associated with and activated by cyclin dependent kinases at different stages of cell cycle. A-type cyclins are expressed during late G1 and S phase. Cyclin B1 controls the G2-M transition of the cell cycle. The D-type and E-type cyclins control the G (1) to S phase transition during normal cell cycle progression. Loss of normal cell cycle control is a key event in tumorigenesis. The aim was to investigate the protein expression

and gene amplification of cyclins A, B1, D1 and E in breast carcinomas and to correlate the results with traditional prognostic factors. Design Immunohistochemistry and Tissue microarray (TMA) technique were used to analyse cyclin A, B1, D1 and E protein expression in 50 tumours. The results were compared with clinical variables. In addition, real time quantitative PCR (RT-PCR) was used to determine gene amplification of cyclins in 13 tumours. The results were compared with immunohistochemical cyclin protein expression. Statistical analyses Statistical analyses were carried out using Kruskal-Wallis -test. Non-parametric test was applied because of non-normal distribution of responses. In pair wise comparisons Bonferroni-adjustment was applied. P-value less than 0.05 were considered as significant. Results: The immunohistochemical expression of especially cyclin A and also cyclin E showed a significant relationship with histological grade. Cyclin A index seemed to present well the cyclin-associated prognostic power. The results estimated from TMA were not as clear as from tumour sections. RT-PCR revealed that cyclin protein expression occurred in both the presence and absence of gene amplification. Conclusion: The prognostic value of cyclin A and to a certain extent also cyclin E had significant relationship with histological grade. Cyclin A was the most suitable single cyclin to characterize the cell fraction in the proliferation cycle. The results indicate that strong Cyclin A expression correlates to high breast cancer grade and poor prognosis. In the literature there are contradictory results concerning the prognostic value of cyclin A. However in several studies Cyclin A is associated with poor prognosis in breast cancer patients. The finding that expression occurred in both the presence and absence of gene amplification supports previous studies that protein expression is not necessarily caused by gene amplification, and emphasizes the need for further studies with RT-PCR samples.

PP1-12
INTEGRIN LINKED KINASE EXPRESSION IN BREAST CANCER PATIENTS AND RELATION TO LYMPH NODE METASTASIS

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Background: Integrin linked kinase (ILK) over-expression results in anchorage-independent cell survival, oncogenic transformation, increased tumorigenicity and invasiveness. A correlation between ILK expression and tumor grade and survival has been shown in some tumor types. Previous molecular studies in breast tissue have demonstrated that ILK over-expression in mammary epithelial cells results in hyperplasia and tumor development. Method: This study was undertaken to investigate whether the expression of ILK correlates with the number of lymph nodes involved by metastasis. Seventy five cases with a diagnosis of infiltrative ductal carcinoma (not otherwise specified) were included in this study. 3-5 m thick sections were taken from tumor blocks of patients representing pN0, pN2 or pN3 in the TNM classification. ILK expression was evaluated immunohistochemically by a mouse monoclonal antibody raised against full-length recombinant ILK (Santa Cruz Biotechnology). ILK expression levels were graded according to the percentage of cells showing staining. The grades were as follows; no expression (0), less than 10% (1+), 11-50% (2+) and greater than 50% (3+) expression. Results and Conclusion: Seventy five patients with an age range of 32-78 were included. In 37 cases there was no lymph node involvement (pN0), in 13 cases there was 4-9 metastatic lymph nodes (pN2) and 25 cases had more than 10 lymph nodes involved (pN3). The number of tumor samples with strong (3+) ILK expression was significantly higher in the pN3 group (p= 0.03) compared to the other two groups.

PP1-13
MODIFICATION OF GRID REFERENCE DEVICE FOR BREAST SPECIMEN RADIOGRAPHY

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Specimen radiography is mandatory for image localized excision biopsies of mammographically detected impalpable breast lesions, usually focal stellate spiculations, or microcalcifications. It is essential that a radiologist report the specimen radiograph, to correlate radiological and pathological features; and to indicate to both surgeon and pathologist, whether or not, the lesion has been excised. Ideally, in-house radiographic examination, with an attendant radiologist,will examine and report radiographs of measured, thin serial slices of the specimen. In the absence of such facilities, use of a grid localization device,such as a Dubin TranSpec is acceptable. These devices can accurately detect the defined lesion. However, the downside is that they tend to distort the specimen. They retain the specimen, by restraining it with a ratcheted perforated compression plate marked with radio-opaque grid references, to identify the co-ordinates of the lesion.This compression causes attenuation of excision margins, the width of which is an important prognostic feature. In extreme cases, the specimen becomes as flat as a pancake.To overcome compression artefact; we have modified the Dubin system, by placing the specimen above, rather than below, the perforated plate. Which rests on a polystyrene base in the bottom of the container. The specimen is restrained by transfixing it with four or five 21 gauge needles, which can easily be manipulated through underlying perforations, and pinned into the polystyrene base. The polystyrene inserts are 10mm thick, and can be readily made in most workshops.They are attached to the bottom of the container with a thin rim of glue. Using a separate compression plate as a template, lightly held above the specimen, the lesion can be accurately located, by inserting a slender skewer into the appropriate co-ordinates

PP1-14
THE IMPORTANCE OF APPLICABILITY OF THE SENTINEL LYMPH NODE BIOPSY IN ADVANCED INVASIVE BREAST CANCER

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Introduction: Breast cancer is the most frequent malignant tumour in female patients. 4200 new breast tumors are diagnosed every year in Romania and from this, 2/3 in an advanced stage. In such cases, radical mastectomies followed by axillary lymph node resection are performed. The aim of this study is to establish whether it is necessary to perform axillary dissection in all patients with advances stage breast cancer. Material and results: We studied 250 cases of invasive breast cancer (2004-2005) in patients with mean age of 55 years. Maximum diameter of the tumor was 2-7 cm. Axillary's lymph node dissection done in 222 cases (89 %) and microscopic examination revealed that the metastases were absent in 88 cases (39,6%). In 15 of these cases, the maximum diameter of the tumor was 5 cm. Discussion: It was demonstrated that the axillary's lymph node status is the most important prognostic factor for breast cancer patients, TNM staging, therapeutic management, but also patient's survival, depending on the presence or absence of axillary's lymph node metastases. Systematic studies in breast cancer have shown that the tumour spreads to one or a few lymph nodes (the sentinel lymph node(s)), before it spreads to other axillary nodes and that these nodes can be identified by using vital blue dye, a radiolabeled colloid or both and the biopsy can be performed in selected patients avoiding the associated morbidity. Recent

recommendations indicate that sentinel lymph node biopsy should not be performed for large or locally advanced invasive breast cancer. We demonstrated in this study that even in advanced cases metastases could be absent in axillary lymph node. Thus, the indication of intraoperative lymphatic mapping and performing the selective biopsy of the sentinel lymph node could be applied also in advanced stages of the disease.

PP1-15
EXPRESSION OF P27 IN INVASIVE DUCTAL CARCINOMAS OF THE BREAST WITH TISSUE MICROARRAY

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Background: Breast carcinoma is the most common malignancy among women worldwide including Malaysia. Protein p27 is one of the cyclin-dependent kinase inhibitory proteins, and plays an important role in cell cycle regulation. The aim of this study was to investigate the relationship between the expressions of p27and MIB1 and their clinico-pathologic parameters in invasive breast carcinomas. Methods: The immunohistochemical expression of p27 protein was evaluated and correlated with MIB1 and clinico-pathological parameters by tissue microarray in 112 invasive ductal carcinomas of the breast. The patients consisted of 109 females and 3 males and the ages ranged from 26 to 85 years (median, 63: mean, 56) and the histological types were mainly invasive ductal carcinomas (84%; 94/112). Results: Over expression of p27 and MIB1 proteins was found in 56% (54/96) and 45% (41/91) of breast carcinomas respectively. P27 protein was statistically significant with ER whereas MIB1 was statistically significant with c-erbB-2 ($p<0.05$) and p53 proteins. Positive staining of both ER and p27 was exhibited significantly in patients who were than older than 50 years ($p<0.05$). The expressions of p27 and MIB1 in breast carcinomas were not correlated with race, size, histological types, grade and nodal metastases. Conclusion: These findings showed that this protein, p27 expressed at relatively high levels, was correlated with positive estrogen receptor compared to MIB1 expression, was associated with c-erbB-2 and p53 proteins.

PP1-16
VIMENTIN, P-CADHERIN AND BASAL TYPE CYTOKERATINS EXPRESSION IN INVASIVE DUCTAL BREAST CARCINOMA

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Background: Basal-type breast carcinomas defined by the CK5/6, CK14 and CK17 expression and ER and HER2 negativity are related with poorer outcome. There were suggestions that these tumours more often present vimentin and p-cadherin expression and these antigens characterize high grade and ER-negative tumours. Method: We investigated the expression of vimentin, p-cadherin, cytokeratins: 5/6, 14, 17 and ER, PGR, HER2, Cyclin E and Ki67 in 193 invasive ductal breast cancer by immunohistochemistry, to find out the relationship among vimentin and p-cadherin expression and basal cell phenotype. We check also association of analyzed antigens with clinical and morphological factors. Results: Forty one (21,2%) cases expressed vimentin, whereas 111 (57.5%) tumours were p-cadherin-positive. Expression of CK5/6 showed 66 (34.2%) tumours, 48 (24.9%) were CK17-positive and 48 (24.9%) CK14-positive. Expression of vimentin showed 32 (45.7%) cases from the group of 'CK 5/6 or CK 14 or CK17'- positive tumours and 9 (7.4%) remaining tumours ($p<0.001$). P-cadherin was positive in 73.2% and 48.4% ($p<0.001$) cases accordingly. Conclusion: P-cadherin and vimentin expression are more often present in the group of basal cytokeratin positive tumours, but can not be regarded as sensitive markers of basal-type tumours.

PP1-17
RADIOFREQUENCY ABLATION OF INVASIVE BREAST CARCINOMAS. RESULTS AND PROBLEMATIC OF HISTOLOGICAL STUDY

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Purpose: To evaluate the safety and accuracy of office-based radiofrequency ablation (RFA), under local anesthesia and US guidance, as local therapy for primary invasive breast carcinomas (PIBC). Problematic of histological study of tumour treated and assessment of residual enzymatic activity using the nicotine amide adenine dinucleotide-diaphorase (NADH-D) technique on frozen material. Methods and materials: Prospective study of 20 PIBC smaller 20 mms. previously diagnosed by needle core biopsy (NCB) and then, treated by RFA. All patients underwent conservative surgical therapy 2-4 weeks after RFA. Complete lymphadenectomy was done if axillary node metastasis were demonstrated. Sentinel biopsy in the remaining cases. Protocol for pathological study: Prior NCB to determine histological type, degree, hormonal receptors and Her-2. Study of the surgical specimen: Intraoperation study of surgical borders, photograph, freezing of the sample for NADH-D tecniche, conventional study with H-E, to assessment size, residual tumour, extension of coagulative necrosis and final state of surgical borders. Evaluation of NADH-D: positive: blue, negative: colourless. Results: Surgical specimen: Tumour size: 4-20 mm (X: 10). Complete coagulative necrosis: 19 cases, incomplete: 1 case. NADH-D: Negative: 12 cases, partial positivity: 2 cases, (one case showed slightly positive and, other, an intraductal component was found out side the effet ring), not done: 4, not assessed (the sample frozen didn't include a tumour): 2 cases. In 16 cases the surgical borders were negative. In all cases no significant complications were observed. Conclusions: RF ablation is a safe procedure which permits the oncologically correct treatment of malignant breast tumours. The histological study of the tumour treated should be similar to any malignant breast tumours. The specimen treated presents a concentric image: a central ablation, with destructured, necrotic tissue, with the residual tumour; a red halo 1 cm. beyond the tumour (the halo marks the limit of the necrotic effect) and tissue of normal appearance outside the hyperemic ring. The assessment of enzymatic activity with NADH-D technique is problematic. It is easy to carry out and assess: Positive (blue) and negative (colourless). The problems are technical: 1: To obtain good histological sections in frozen and bad tissue (frequently adipose, necrotic and friable). 2: how much material to freeze, as the tumours are very small, and 3: what zone to freeze as, at times, it is difficult to recognize the residual tumour.

PP1-18
IMPORTANT CLINICOPATHOLOGICAL PARAMETERS ON THE HER 2 POSITIVE BREAST CARCINOMA

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Introduction Treatment of breast carcinoma as a category of hormone-dependent tumors requires the evaluation of receptor status (ER, PR, and HER 2), which is fundamental for planning and application of adequate therapy. Information on HER2 status is particularly important because it selects the patients suitable for the application of Herceptin. Aim: The aim was to analyze receptor status in case of 200 breast carcinoma patients and to select all HER2 3+ findings. These findings were compared to main clinicopathological parameters: age of patients, histological type and grade of the carcinoma, TNM status, and receptor

status). Material and Methods: Samples for IH analysis of receptor status were examined by means of ER, PR, and HER2 antibodies N and M series. LSAB2 kit was used for visualization. Estrogen and progesterone were ranged with 1, 2, and 3 and HER2 was ranged according to reference ranges (DAKO) 1+, 2+, and 3+. Results HER2 3+ results were found in case of 16.58% of patients. Those were mainly patients younger than 54 years (40.55%) with ductal (53.57%) or lobular invasive carcinoma (17.86%), stage pT1c (18.18%) or pT2 (45.45%) with positive nodal status. The results showed that HER2 3+ were most frequently associated with ER-0 (57.57%) and PR-0 (65.62%). Conclusion: The analysis of obtained results showed that HER2 3+is correlates most often to loco regional advanced ductal invasive carcinoma of the breast with negative ER and PR, which suggests that HER 2 status predicts more aggressiveness of the tumor.

PP1-19

INTRAOPERATIVE HISTOLOGICAL EVALUATION OF SENTINEL NODES IN BREAST CANCER BY USING IMMUNOHISTOCHEMISTRY

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Background: Histological methods that are usually used for determination of metastatic status of SN in breast cancer have a postoperative character; that is why subsequent extirpation of fat tissue must be performed in some patients. Therefore, the authors suggest the application of intraoperative histological evaluation of SN by means of the method of serial ex tempore analysis with immunohistochemistry Purpose of the study: The specimen consisted of 100 patients with breast cancer pT1 classification (£ 2 cm) who underwent surgical treatment involving sentinel concept. Material and methods: Sentinel lymph nodes found in one half of investigated patients were analyzed by routine (postoperative) histological technique; the other half of the sentinel nodes was analyzed by the suggested intraoperative serial extempore analysis with immunohistochemistry. The control histological method identical for both groups of patients was used to record the cases with the conversion of initial histological finding (false negative), which made possible to establish the sensitivity of used methods and their mutual comparison. Results: The main result was to find metastatic status of sentinel lymph nodes in both investigated groups of patients, which the authors obtained by using the suggested and its competitive methods. After the verification of the results obtained in both groups of patients by means of control (extensive) histological method the number of false-negative sentinel lymph nodes was established and used to calculate the sensitivity of applied methods. Finally, the results were compared and it was noted that the most effective histological method was the suggested serial extempore analysis with immunohistochemistry. Conclusion: By the analysis, conclusions were made related to positive and negative performances of the suggested histological method; it was also noted that the control serial section of paraffin blocks with immunohistochemistry did not significantly increase the number (percent) of conversed histological findings in sentinel lymph nodes in relation to suggested method.

PP1-20

FIBROADENOMA ARISING IN ECTOPIC BREAST TISSUE OF THE PUBIC REGION. A CASE REPORT

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BACKGROUND: Ectopic breast tissue is usually found within the milk lineextendind from the axilla to the pubic region, and can develop any disease that affects the normal breast, including fibroadenoma. MATERIALS AND METHODS: We report a new case in 50-year-old woman occurring at the pubic evolving more than ten years without pain. A surgical resection was performed and the pathological study concludede at a fibroadenoma arising in ectopic breast tissue CONCLUSION: Through this observation we try to recall the clinical particalities, the diagnosis, differential diagnosis and treatment of this rare entity.

PP1-21

IMMUNOPHENOTYPE OF ESTROGEN RECEPTOR (ER), PROGESTERONE RECEPTOR (PR) AND HER-2 NEGATIVE BREAST CARCINOMAS

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Background: Gene expression analysis has identified several breast cancer subtypes that are associated with different clinical outcomes. The ER/PR/HER-2 negative (triple negative) phenotype represents an aggressive subtype of breast cancer that is unresponsive to therapeutic agents used to treat other type carcinomas. The aim of this study is to characterize the histological and immunohistochemical features and to identify potential prognostic markers of these tumours. Method: We retrospectively evaluated 70 triple negative breast tumours for different histological features, including tumour type, grade, architectural growth pattern, circumscription and presence of necrosis and inflammatory infiltrate. Formalin-fixed paraffin-embedded tissue sections were immunostained for CK5/6, EGFR, E-Cadherin and COX-2. Results: Morphologically the 70 triple negative tumours included 55 grade 3 ductal (NOS), 7 metaplastic, 6 medullary, 1 papillary and 1 lobular carcinomas, that frequently showed geographic necrosis, a pushing border of invasion and a stromal lymphoplasmatocytic infiltration. Marker of basal-like differentiation (CK5/6) was weakly to strongly positive in 21/70 (30%) cases. EGFR and COX-2 positivity was observed in 19/70 (26%) and 15/70 (21%) cases, respectively. Nine (47%) of EGFR and three (7%) of COX-2 positive tumours were also CK5/6 positive. COX-2 expression was observed in 6/19 (30%) of EGFR positive tumours. Loss of E-Cadherin immunoreactivity was seen in 32/70 (46%) tumours. Conclusion: Triple negative carcinomas of the breast are a distinct clinical and pathological entity, characterized by predominately high nuclear grade and lack of hormone receptor and HER-2 expression. Our study indicates that approximately 30% of these tumours express a basal-like phenotype and show positivity for EGFR and COX-2; about half cases display loss of E-Cadherin immunoreactivity. Therefore, the use of these markers in a standard immunohistochemical panel may assist in sub classification and search of new therapeutic approaches in triple negative cancer.

PP1-22

CLINICAL-PATHOLOGICAL FACTORS ASSOCIATED WITH NODAL INVOLVEMENT IN T1A-B BREAST CANCER. A RETROSPECTIVE ANALYSIS ON A MONO-INSTITUTIONAL SERIES

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BACKGROUND: The proportion of T1a and T1b breast cancers is progressively increasing due to early diagnosis and mammographic screening. In these patients the incidence of axillary node metastasis (ANM) is relatively low (6-31%). However it remains the most important prognostic factor. Few information are available on the correlation between clinical and histological features and the frequency of lymph node involvement in small invasive breast cancer (ibc). Our retrospective analysis was designed to determine if it is possible to identify any clinical and/or pathological predictor of ANM metastasis in T1a and T1b ibc. PATIENTS AND METHODs: A series of 818 patients with T1a and T1b ibc, treated at our institution from 1990 to 2005 with primary tumor resection and axillary lymph node clearance was investigated. Univariate and multivariate analyses were used to evaluate the relationship between axillary node status and clinical-pathological factors (age, tumor size, histologic type and grade, ER and PgR status and immunohistochemical expression of MIB-1, HER-2 and p53). RESULTS: Of 818 tumors, 222(27%) were T1a and 596 (73%) T1b. The incidence of ANM was 16,5% (135/818) with 30 detected in T1a (3,7%) and 105 (12,8%) in T1b tumors. In univariate analysis we found a significant correlation between ANM and younger people (OR= 1,85; p=0.01); further, poorer histologic grade was also associated with an increased risk of ANM (OR = 2,45; p=0.01). No statistically significant correlation was observed between node status and tumour size (T1a vs T1b), histologic type (ductal, lobular, medullar, mucinous), hormone receptor status, MIB-1, p53 and HER-2 expression. Then, we divided the positive node group in [LTEQ] 3 (109/135) and > 3 (26/135) positive nodes patients. In N [LTEQ]3 group 27 patients presented T1a and 82 presented T1b, while in other group (N > 3) 3 patients were T1a and 23 T1b. This subgroup analysis revealed a statistically significant relationship between more severe nodal involvement and younger age (p= 0.03), ductal histological type (p=0.02), high histological grade(p=0.003) and > 20% MIB-1 (p=0.02). In multivariate analysis, the risk of ANM was related to increasing histologic grade and to larger size, but was independent from age. CONCLUSIONS: In our series, the incidence of nodal metastases in pts with very small ([LTEQ]1cm) breast cancer is not negligible, particularly in pts with worse histological grade. Therefore, it remains important to investigate axillary nodes with sentinel node biopsy technique or radical dissection.

PP1-23
TELECONSULTATION OF BREAST PATHOLOGY DATA: THE BELGIAN-LUXEMBURG TRIAL

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Virtual slide generators offer new opportunities in teleconsultations of pathology cases. Nevertheless, the validity of virtual slides as a routine diagnostic tool is not yet confirmed. A clinically valuable diagnosis is not only requiering high quality virtual slide (VS) images but also a securized network between refering centers, the opportunity of sending reports and consultation of medical imaging. The one year Bel-Lux trial, started January 2007, proposes to evaluate the efficiency of virtual needle core and surgical breast specimens slides teleconsultation between the Laboratoire National de Santé of Luxemburg and pathology laboratory CMP in Brussel. VS are compared to standard glass slides sent by post. Delays in responding are monitored and compared in an economical model. VS are generated by Mirax Zeiss and information exchanged via the Kisano Euromedim platform. Early results confirms the quality of VS images allowing routine diagnosis in clinical settings. This trial is supported by IRSIB granting (Brussel).

PP1-24
MITOTIC INDEX OF EPITHELIAL CELLS IN FIBROEPITHELIAL BREAST TUMOURS – DIAGNOSTIC SIGNIFICANCE

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BACKGROUND: Human breast fibroadenomas (FA) and phyllodes tumours (PT) have almost identical morphology of epithelial components, but different kinetic potential of these cells. In the present study we investigate mitotic index of epithelial cells in different histological and biological types of these tumours with regard to diagnostic significance. METHOD: 100 FA and 102 PT were analyzed in the National Cancer Research Institute of Serbia. In 10 PT (1 border line and 9 malignant tumours) epithelial component were absent ("stromal overgrowth"). On H&E semi-thin histological slides of each tumour, epithelial mitotic figures were detected on 50 (malignant PT), 100 (benign PT) and 600 (FA) microscopic fields, with size 0.0962 mm2. Mitotic index was expressed as mitotic count per 100 epithelial cells. Statistical analyses were performed by ANOVA test. RESULTS: Epithelial mitotic index (mean±SD, 10-3%) in 100 FA and 92 PT was 1.45±4.02 vs. 2.92±3.72 ($p<0.001$), in 53 intracanalicular FA and 73 benign PT was 1.51±4.86 vs. 2.67±3.41 ($p<0.001$). Epithelial mitotic index 4.82±5.15 was the highest in 13 border line PT among all analyzed tumour types, and it was significantly higher in comparison to the intracanalicular FA ($p<0.01$). CONCLUSION: Epithelial component of the breast phyllodes tumours have significant higher growth potential than fibroadenomas. Benign intracanalicular fibroepithelial breast tumours (intracanalicular fibroadenomas versus benign phyllodes tumour) may be distinguished by mitotic count of epithelial tumour cells.

PP1-25
POTENTIAL DOUBLING TIME OF TUMOUR CELL NUMBER IN HUMAN FIBROEPITHELIAL BREAST TUMOURS – A MORPHOMETRIC APPROACH

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BACKGROUND: Kinetic cells proliferation parameters of human solid tumours are still poorly investigated. Potential doubling time of total tumour cell number (PDT) is the time for which the total number of tumour cells will be doubled, under the supposition that losing cell number is equal to zero. PURPOSE: Calculate PDT by mitotic and nuclear numerical density determination of mesenchymal and epithelial tumour cells in H&E semi-thin histological slides of human breast fibroepithelial tumours after tumourectomy. MATERIAL AND METHODS: 100 fibroadenomas (FA) and 102 phyllodes tumours (PT) were analyzed on the National Cancer Research Institute of Serbia. Epithelial and mesenchymal mitotic figures were detected in 50 (malignant PT), 100 (benign PT) and 600 (FA) microscopic fields with size 0.0962 mm2. Number of nuclei was determinated in Weibel testing system M42 by area 0.0576 mm2. Nuclear numerical density (Nv) was calculated by formula of Weibel and Gomez. PDT in hours was calculated by originally adapted formula $PDT=(Nv/Nvm)\cdot\ln 2\cdot 0.5$ in which Nvm is the numerical density of mitotic figures. RESULTS: PDT (mean±SD, years) was 60.47±97.42 in 100 fibroadenomas, 9.40±21.38 in 73 benign FT, 0.61±0.76 in 14 border line FT and 0.06±0.06 in 15 malignant FT (ANOVA, $p<0.001$). CONCLUSION: PDT in human fibroepithelial breast tumours largely varies from 22 days in malignant phylodes tumour to 60 years in fibroadenomas.

PP1-26

ASYMMETRY OF TUMOUR VOLUME LOGNORMAL DISTRIBUTION – AN INDICATOR OF MALIGNANCY

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BACKGROUND: Kinetic growth parameters of human solid tumours are still poorly investigated. Tumour volume is a prognostic indicator for many human tumours. PURPOSE: To calculate skew ness for the volume frequency distribution with regard to the biological behaviour of different intra-canalicular fibroepithelial human breast tumours. MATERIAL AND METHODS: Three diameters of first extirpated 1702 intracanalicular fibroadenomas (IFA) and 348 phyllodes tumours (PT) were measured in the National Cancer Research Institute of Serbia. Tumour volumes were calculated by ovoid volume formula. Skewness of logarithmic tumour volumes for different growing and non-growing tumour groups were calculated by StatPlus 2006 Professional software. RESULTS: Skew±SE were in 27 non-growing hyalinized IFA 0.209±0.431, in 1438 normocellular IFA 0.366±0.065, in 237 hypercellular IFA 0.438±0.157, in 264 benign TP 0.288±0.149, in 21 border malignant TP −0.176±0.477 and in 63 malignant TP −0.224±0.297. Normalized volume frequency distribution of analyzed benign tumours and benign non-growing tumours are skewed to the right (positive skewness), but of malignant ones are skewed to the left (negative skew ness). CONCLUSION: Negative asymmetry of tumour volume lognormal distribution may be an indicator of tumour malignancy.

PP1-27

NODULAR FASCIITIS OF THE MALE BREAST : A CASE REPORT

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Introduction: Nodular fasciitis is a rare benign soft tissue lesion, interesting a fibroblastic proliferative condition of unknown aetiology .It can clinically and radiologically mimic a malignant tumour. To our knowledge, there was only one case reported of a lesion of this type in the male breast. Methods and results: We describe a case of nodular fasciitis of the breast that involved the breast parenchyma of a 81-year-old man. The patient complained of a slowly growing mass in his left breast. There was no evidence of axillary lymphadenopathy. The patient underwent a total excision of a lesion measuring (7cm x 6cm) Histologically the appearances included short interweaving fascicles of spindle cells in a loosely textured mucoid matrix. There was also present an extravasated red blood cells and a focal lymphocytic infiltration. Discussion : Spindle cell lesions of the breast constitute a wide spectrum of benign and malignant proliferations. The differential diagnosis of nodular fasciitis in the male breast mainly includes fibromatosis and myofibroblastoma. The histologic features and a review of the literature are presented.

PP1-28

C-KIT EXPRESSION IN COLUMNAR CELL LESIONS OF THE BREAST ACCOMPANIED TO BENIGN AND MALIGNANT BREAST DISEASES

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Columnar cell lesions (CCLs) of the breast are being increasing frequency reported in pathology specimens. However the significance and treatment approach of these lesions are unknown. The proto-oncogene c-KIT is believed to play an important role in growth control and differentiation of normal breast epithelium, but it is shown to decreased expression in invasive carcinoma. In this study we aimed to evaluate c-KIT expression in CCLs accompanied to benign and malignant breast diseases. Of 65 patients with CCLs, 18 were coexisted benign breast diseases, 8 of them were coexisted DCI and 39 of them were coexisted invasive carcinomas. C-KIT was found to be expressed intensively in normal breast epithelium. Staining intensity (SI) in normal epithelium was 3±0.0, whereas SI in CCLs (All cases were CCLs without atypia) accompanied to benign diseases was 1.45 ±0.52. Whereas SI in CCLs with or without atypia accompanied to DCI were 1.25 ±0.50 and 1.38 ±0.52 respectively. Finally SI in CCLs with and without atypia accompanied to invasive carcinoma was 0.77 ±0.73 and 1.21 ±0.42 respectively. However c-KIT expression was detected in 10.4% of invasive carcinomas. No significant association was observed between c-KIT expression and tumour histological grade and nodal status. Reduction of c-KIT expression with malign transformation of breast epithelium is believed to be c-KIT has a role of breast carcinogenesis. Additionally similar c-KIT expression pattern in CCLs to malignant breast diseases suggest that at least some CCLs could be a premalign status of breast carcinoma. But implication of c-KIT in CCLs and relation to breast Carcinogenesis should be evaluated in follow up studies in larger series.

PP1-29

REPORT OF A CASE OF BREAST CARCINOMA WITH CARTILAGINOUS AND OSSEOUS METAPLASIA

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The reported frequency of invasive breast cancer with cartilaginous and/or osseous metaplasia (mixed epithelial / mesenchymal metaplasic carcinoma. WHO Classification) is 0’03 – 0’2 % of all breast cancer.There is a paucity of information on clinically relevant pathologic and clinical outcomes for these rare tumors. CASE REPORT A 72 year-old spanish woman presented to the external consultation of our hospital, with a tumor of the right breast, palpable, as numerous adenopaties in the right axillary area. After mammography and ultrasonography were performed two continuous nodular masses with greater tissue density than the adjacent parenchyma with irregular-shaped grouped calcification within the tumor area, were revealed. A core needle biopsy was done and a report of invasive ductal carcinoma provided. No distant metastasis or elevations of tumor markers were detected.With this diagnosis a modified radical mastectomy of the right breast and dissection of the axillary lymph nodes was practised.Gross examination showed two more or less well circumscribed tumors of 2´5 x 1´7 x 1 cms. and 2´6 x 2´4 x 0´75 cms., white with yellow areas. Necrosis and hemorrhage was seen.Histologically the tumor showed invasive ductal carcinoma with pushing margins, and moderate lymphocytic infiltrate and necrosis. Numerous areas of osseous and cartilaginous metaplasia,with osteoclastic giant cells, were observed. In some fields the bone trabeculae were found adjacent to the epithelial cancer cells, in others in necrosis foci, and in some places related to cartilaginous plates and a bland looking area of fusiform cells. The ductal cancer was classified as Grade II (Bloom- Richardson). Twenty one of the twenty six lymph nodes encountered, were found to be metastasic.Immunohistochemical studies were positive in the invasive ductal cancer for estrogen and progesterone receptors and negative for HER2 (CB11 clone) overexpression. The behaviour of metaplastic carcinoma seems to be more aggresive than of ordinary invasive ductal-type carcinoma, although some

authors stated that the presence of metaplasia in human breast cancer did not alter the behavior of the tumor in comparison with similar tumors.In a case reported recently by Kisima and cols the patient died of rapidly progressive pulmonary metastasis despite no lymph node involvement at the time of the diagnosis. Those authors recommended a large number of studies to examine the predictive value of known prognostic variables in patients this type of breast carcinoma, that justifies the report of our case.

PP1-30

METAPLASTIC CARCINOMA OF THE BREAST WITH OSSEOUS DIFFERENTIATION AND PRIMARY OSTEOGENIC SARCOMA OF THE BREAST - REPORT OF TWO UNUSUAL CASES OF BREAST NEOPLASMS

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AIM: The aim of this paper is to present two rare cases of breast neoplasms characterized with development of bony structures but with different etiology. MATERIALS AND METHODS: Samples from the breast lesions, formalin fixed and paraffin embedded were stained with Hematoxyllin Eosin, and additionally immunohistochemical analyses were performed using antibodies for Cytokeratin, Cytokeratin high molecular weight, Cytokeratin 18, Cytokeratin 19, Epithelial specific antigen (ESA), S-100 protein and Vimentin. RESULTS: Case1. Female with a clinical diagnosis of mammary cancer presented with a breast nodule confirmed by fine needle aspiration cytology as a malignant lesion. Partial resection of the breast with minimal axillary lymphadenectomy was performed. The nodule measured 18 mm, and was presented as a star shaped, hard grey-white lesion. Histology showed malignant infiltrative neoplastic lesion arranged in solid nests or sheaths and partly tubular structures. One focus showed a desmoplastic reaction with a differentiation of a hyaline collagenous stroma toward cartilage and bony structures with a calcium deposition. Case2. A 71 year old female presented a well defined breast mass that was clinically diagnosed as a benign breast lesion. Due to the large size (85 mm) quadrantectomy was performed. Morphological analysis revealed hard calcified well circumscribed nodule that was additionally treated with a decalcination procedure. Histology showed malignant lesion composed of spindle shaped cells placed in a collagenous stroma with foci of myxomatous differentiation. Toward the center of the lesion the cells differentiate in bony elements: bone trabeculae, calcium deposits and cartilaginous elements. Immunohistochemical analysis differentiated this lesion into primary osteogenic sarcoma. Eight months later a recidivant lesion emerged with identical histological characteristics. CONCLUSION: These rare breast neoplasms can often present a differential diagnostic challenge. First they are rarely present in everyday pathological practice, second they can have almost identical histology, and most importantly even though immunohistochemistry helps in their differentiation sometimes these results are inconclusive.

PP1-31

DISTRIBUTION AND SIGNIFICANCE OF CAVEOLIN 2 EXPRESSION IN NORMAL BREAST AND BREAST CANCER: AN IMMUNOFLUORESCENCE AND IMMUNOHISTOCHEMICAL ANALYSIS

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Backround: The distribution and significance of caveolin 2 (CAV2) in normal breast and breast cancers remain poorly understood. The aims of this study were to define the distribution of caveolin 2 in frozen and formalin fixed, paraffin embedded (FFPE) normal breast samples and the significance of CAV2 expression in breast cancer. Methods: Caveolin 2 distribution in frozen and paraffin-embedded whole tissue sections of normal breast was evaluated using immunohistochemistry and immunofluorescence, in conjunction with antibodies to define luminal epithelial cells (oestrogen receptor and cytokeratin 8/18) and myoepithelial/ basal cells (cytokeratins 14 and 5/6, p63 and smooth muscle actin). CAV2 expression was also immunohistochemically analyzed in a cohort of 245 invasive breast carcinomas from patients treated with surgery followed by anthracycline-based chemotherapy. Results: In normal breast, CAV2 was expressed in myoepithelial cells, endothelial cells, fibroblasts and adipocytes. Luminal epithelial cells showed no or only negligible staining. CAV2 expression was observed in 6.2% of all breast cancers and was strongly correlated with lack of oestrogen and progesterone receptor expression and cyclin D1, and positivity for epidermal growth factor receptor, cytokeratins 5/6, 14 and 17 and p53, and high proliferation index. Furthermore, CAV2 expression was significantly associated with basal-like immunophenotype and proved to be a prognostic factor for overall survival independent of tumour size and grade. Conclusion: Our results demonstrate that CAV2 is preferentially expressed in basal-like cancers and is associated with poor prognosis. Further in vitro studies are required to determine whether CAV2 has oncogenic properties or is only a surrogate marker of basal-like carcinomas.

PP1-32

EFFECT OF HORMONE RECEPTOR POSITIVITY IN c-erbB-2 POSITIVE BREAST CARCINOMAS

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AIM: C-erbB-2 overexpression occurs in 10-30 % of breast carcinomas. This oncogene encodes a transmembrane tyrosine kinase receptor belonging to the epidermal growth factor family. Its overexpression in breast carcinomas associates with poorer prognosis. Generally it's inversely correlated with estrogen and progesterone receptors (ER,PR). However some of the c-erbB-2 positive breast carcinomas also express estrogen and progesterone receptors. We investigate the difference between the clinicopathologic characteristics (menstrual status, histologic grade, lymph node metastasis, stage) of hormone receptor positive and hormone receptor negative but c-erbB-2 positive carcinomas and also compare the outcome of these patients. MATERIAL and METHODS: We analysed the tumors of 370 patients, operated at the General Surgery Department and diagnosed as invasive ductal carcinoma at the Pathology Department of Akdeniz University between 2003 and 2006. Immunohistochemical method was used to determine the hormonal status and c-erbB-2 overexpression. Flouresence in situ hybridization method (FISH) to confirm the Her2/neu overexpression was performed only in randomly selected 20 carcinomas. Statistical analysis was done by using Chi-square test and survival analysis by Kaplan Meier. RESULTS: Among 370 invasive ductal carcinomas 146 revealed c-erbB-2 immunopositivity. Seventyfour of 146 positive carcinomas showed both ER and PR positivity where 42 were ER (-) and PR (-). There were 22 (52 %) grade 3 carcinomas in the ER-/PR- and c-erbB-2 + group but only 18 out of 74 ER+/PR+/c-erbB-2+ group (p= 0.001). Lymph node positivity was seen in 69 % of ER-/PR-/c-erbB-2+ and 44.6 % of ER+/PR+/c-erbB-2+ carcinomas (p=0.009). Twentytwo of 42 (57.1 %) ER-/PR-/c-erbB-2+ carcinomas were stage II A, in the ER+/PR+/c-erbB-2+ group 44 (59.5 %) out of 74 were stage I (p= 0.063). ER-/PR-/c-erbB-2+ carcinomas were associated with a higher likelihood of recurrence. In 36 months, 7 of ER-/PR-/c-erbB-2+ carcinomas showed local recurrences but none of the hormone and c-erbB-2

positive carcinomas had recurrence (p=0.0012). CONCLUSION: There was a statistically significant difference between the histologic grade, lymph node metastasis of hormone receptor and c-erbB-2 positive and hormone receptor negative but c-erbB-2 positive carcinomas. Disease free survival of hormone receptor positive and c-erbB-2 positive carcinomas was also better so we concluded that even in the c-erbB-2 positive carcinomas, hormone receptor expression improve the prognosis.

PP1-33
IMMUNOEXPRESSION OF HER FAMILY IN INVASIVE DUCTAL CARSINOMAS OF THE BREAST
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Background: The purpose of the study was to investigate the frequency of expression of erbB/HER family of growth factor receptors, in invasive ductal carcinomas of the breast. The frequency of expression of the antibodies, their coexpressions and their correlations with the histopathological prognostic parameters as tumor stage, grade, lymph node status, hormonal status, and patients clinical follow-up has been searched. Material Method: 59 invasive ductal carcinomas of the breast has been studied for EGFR, c-erbB-2, c-erbB-3, c-erbB-4, by streptavidin-biotin horseradish method. EGFR, c-erbB-3, c-erbB-4 were all evelatuated as; ›20% of tumor cells with moderate 2+ and strong membranous 3+ staining were regarded as positive immunoexpression or overexpression and ›10% of the tumor cells strong, complete membranous staining for c-erbB-2. Statistical analysis was based on associations between categorical variables were assessed by means of ^{2} and coefficient of associations with Somers'd tests. P-value of less than 0.05 was considered to indicate a statistically significant difference. Results: Of the 59 tumours, 44 (75%) were ER+, 37 (63%) PR+, 4 (7%) EGFR+, 7 (12%), c-erbB-2+, 7 (12%) c-erbB-3+, 14 (24%) c-erbB-4+. Results of the immunohistochemistry revealed statistically significant correlation with PR negativity and EGFR overexpression (p‹0.05). There were also statistically significant correlation between c-erbB-2 and c-erbB-3 overexpression, c-erbB-3 and c-erbB-4 overexpression (p‹0.05). Although there were a tendency of coexpression found to be between c-erbB-2 and c-erbB-4 (p=0.062) and between c-erbB-2 and EGFR (p=0.066). According to the clinical follow-up results of the patients the only statistically significant correlation found to be between c-erbB-4 overexpression and good prognosis (p‹0.05). Conclusion: The results of our study demonstrates a subgroup of invasive ductal carcinomas of the breast which are PR negative. PR negative breast carcinomas show different molecular characteristics as, EGFR and c-erbB-2 overexpression. Expression and co-expression profile of receptor throsine kinase growth factors are usually not characterized in pathological diagnosis. However targeting multiple erbB-receptors may provide an exceptional strategy for an effective cancer therapy.

PP1-34
IMMUNOHISTOCHEMICAL EXPRESSION OF C-MET / HGF REGULATORY SYSTEM IN N0 BREAST CANCER
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Background: Breast cancer is on the top of tumor morbidity in women, with an estimated incidence of 1 from 8 in the next decade. C-met is a proto-oncogene encoding a protein tyrozin-kinase (HGFR) – the receptor for scatter factor / HGF, involved in invasion and metastasis of tumor cells. Material and method: 40 archived formalin fixed parrafin embedded samples with breast cancer (invasive ductal carcinoma – IDC), with negative lymph nodes have been assessed, using an ABC peroxidase immunohistochemical method, by mean of 5 monoclonal antibodies: c-met, HGF alpha, HGF beta, c-erbB2, EGFR. All cases were graded according to the revised Bloom Richardson system. Therefore, in the study batch have been recorded: 10 cases with G1 IDC, 25 cases with G2 IDC and 5 cases with G3 IDC. Results and discussions: The HGF alpha reaction was diffusely positive (99% of cases), with various intensity and the HGF beta stained focally in IDC, specially in solid pattern types or with high nuclear pleomorphism (67,5% of cases); 70% of cases were positive to c-met intracytoplasmic, while EGFR was positive in 10% of tumors; 37,5% of cases have shown a 2+ and 3+ positivity for c-erbB2. Between HGF alfa and HGF beta have been recorded the following data: a negative correlation (r = -0.47) in G1N0 IDC, a direct positive correlation, statistically significant (r = 0.36, p = 0.01) in G2N0 IDC, and no correlation in G3N0 IDC. There was no correlation between c-met or HGF alpha/beta (independent parameters) in our study. A slightly positive correlation statistically significant between HGF beta and c-erbB2 has been noticed (r = 0.31, p = 0.05) in all investigated cases. Also, a resonable correlation between HGF alpha and microvascular density (assessed with EGFR) was recorded in G2N0 cases (r = 0.35, p < 0.001). Conclusion: c-met expression is independent of HGF alpha and beta activation, but the similar high percentage of c-met and HGF beta suggests their potential as predicting factors for invasion and metastasis in N0 breast tumors; the receptor seems to be activated only when both HGF alpha / beta subunits are present, the IHC expression decreasing with the tumoral grade.

PP1-35
IMMUNOEXPRESSION OF HER-2, NEUREGULIN, MAPK, AKT IN BREAST CARCINOMAS
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Background: The purpose of this study was to investigate the frequency of expression of Her-2/neu, neuregulin, mitogen activated protein kinase (MAPK) and serine/threonine kinase (Akt), their co-expressions and their correlations with the histopathological prognostic parameters as tumor stage, grade, lymph node status, hormonal status, and patients clinical follow-up, in invasive ductal carcinomas of the breast. Material Method: 59 invasive ductal carcinomas of the breast has been studied for c-erbB-2, Neuregulin Ab-3, Phospho-Akt, and Phospho-p44/42 Map Kinase by streptavidin- biotin horseradish method. While strong and complete membranous staining of more than 10% of the tumor cells was scored as positive for c-erbB-2, strong cytoplasmic and/or nuclear staining pattern ›20% of tumor cells with moderate 2+ and strong 3+ staining were regarded as positive immunoexpression for Neuregulin Ab-3, Phospho-Akt. Moderate or strong (2 +and 3+) nuclear immunoexpression of ›20% of the tumor cells was scored as positive for phospho-p44/42 Map Kinase. Statistical analysis was based on associations between categorical variables were assessed by means of ^{2} and coefficient of associations with Somers'd tests. P-value of less than 0.05 was considered to indicate a statistically significant difference. Results: Of the 59 tumours , 44 (75%) were ER+, 37 (63%) PR+. Strong complete membranous staining revealed with 7 (12%) c-erbB-2 and cytoplasmic + nuclear immunoexpression with 17 (29%) neuregulin and 13 (22%) pAkt. Nuclear immunoexpression with pMAPK found to be 17 (29%) of the cases. While 4 (7%) of the cases were positive with both c-erbB-2 and neuregulin, there were only 3 (5%) cases co-expressing neuregulin+pMAPK+pAkt. Results of the immunohistochemistry revealed statistically significant correlation between PR negativity and both pAkt, and pMAPK overexpression (p‹0.05).There were a stastistically tendency of correlation between lymph node metastases and pAkt overexpression (p=0.067). According to the clinical follow-up results of the patients the only statistically tendency of correlation found to be between pAkt overexpression and patients poor

prognosis (p=0.059). Conclusion: PR negative breast carcinomas seems to be activated by receptor thyrosine kinase growth factors which leads to phosphorilation of the Akt and MAPK. This pathway known to be as inducing resistance to endocrine therapy. Information about tumorigenesis in the diagnostic pathology reports may lead an exceptional strategy for an effective cancer therapy.

PP1-36

EXPRESSION OF CYCLOOXYGENASE-2 AND ITS RELATION TO HISTOLOGICAL GRADE, INOS, MMP-2, CD-34, CASP-3 AND CD8 IN INVASIVE DUCTAL CARCINOMA OF BREAST

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Background: Breast cancer is the most common cancer in women and is second only to lung cancer as a cause of cancer related mortality. Interest in chemoprevention in oncology using suppressants of prostaglandin (PG) synthesis has been stimulated by epidemiological observations that the use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with reduced incidence of some cancers such as breast carcinoma. The main target of NSAID activity is the cyclooxygenase (COX) enzyme. The aim of our study was to assess by immunohistochemistry COX2 expression in invasive ductal carcinoma and its possible correlation with histological grade, iNOS, MMP-2 and other common immunohistochemical parameters (CD-34, CASP-3 and CD8). The mean value of positive stained cells were independently calculated by three pathologists and the results were averaged. Method: This study included 50 patients with invasive ductal carcinom with a mean age of 54.9±12.0 years. These cases were graded according to a modified form of the Bloom Richardson Histological Grading Index. The routine hematoxylin-eosin staining and COX2, iNOS, MMP-2, CD-34, CASP-3 and CD8 immunoperoxidase techniques were performed on paraffin–embedded tissues. Immunohistochemical antigen staining was performed with Ultra Vision Polyvalent, HRP-AEC kit (Lab Vision, Neomarker-Biogen USA). Results and Conclusion: The mean COX2 and MMP-2 values were found to be 274.02±54.4 and 263.42±54.30 respectively, whereas the mean iNOS values were found to be 258.10±56.05. CD-34 staining also yielded positive results as 26.18±8.00. The mean values of CASP-3 and CD8 were 284.06±41.2 and 164.17±69.5 respectively. This reveals an inverse correlation between CASP-3 reactivity and CD8 (Spearman correlation r = -0.33, p= 0.01). There was also an inverse correlation between iNOS reactivity and patients age (Spearman correlation r = -0.29, p= 0.03) There was a positive correlation with COX2 and MMP-2 (p=0.00) but there was not any relation with COX2 and other parameters. While there was a positive correlation with lymph node positivity and histological grade (p=0.007), a positive correlation was found between lymph node positivity and CD-34 reactivity and patients age (p=0.007, p=0.01 respectively)

PP1-37

DUCTAL CARCINOMA IN SITU (DCIS) OF THE MALE BREAST

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BACKGROUND: According to the World Health Organization the ductal carcinoma in situ (DCIS) of the male breast is a rare disease. Both DCIS and the invasive occur at a ratio of 1:25. The histological features are similar to those in the female breast, with the predominant architectural pattern being papillary and the comedo DCIS occurring rarely. METHODS AND RESULTS: We report a case of DCIS in a 74-year-old patient displaying the clinical features of gynaecomastia, with retroareolar enlagement of the breast tissue but without nipple discharge. The patient underwent excision of this mass, under local anaesthesia. The mass weighed 31 grs, its size was 4X3X2 cm and it was firm and grey white on the cut surface. The microscopic findings showed gynaecomastia lesions with multiple foci of prominent atypical epithelial intraductal hyperplasia, which, in rare locations, had the appearance of ductal carcinoma in situ of cribriform and solid type. The patient underwent simple mastectomy without axilla dissection four weeks following the original procedure. The DCIS expressis the estrogen (ER) and progesterone (PR) receptors and the oncogene c-erB-2 CONCLUSION: Although a review of literature suggests that most DCIS in men is of the papillary type, this case was of the rare cribriform and solid type. More cut sections should be taken from the macroscopically suspicious areas when we observe sites of atypical epitheliosis. It should be noted that there must be high clinical suspectability for DCIS of the male breast, especially in unilateral gynaecomastia in middle aged males.

PP1-38

RET FINGER PROTEIN EXPRESSION IN INVASIVE BREAST CARCINOMA: RFP CORRELATES WITH ErbB2 EXPRESSION

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Background: RET finger protein (RFP), which belongs to the large B-box RING finger protein family, has been reported to be expressed in breast carcinoma cell lines. To examine the role of RFP in breast cancer tumorigenesis, the expression of RFP was assessed by immunohistochemistry. Methods: Tissue microarrays were constructed from one hundred thirty three cases of breast carcinomas. Two punches, one from central and another from peripheral part of the tumor were put in the arrays. Sections obtained from tissue arrays and whole sections of ten nonneoplastic breast tissue were stained for ER, PR, ErbB2 and RFP by immunohistochemistry and ErbB2 gene status was evaluated by FISH. The correlation between various immunohistochemical markers were determined. Results: We found nuclear RFP expression in 41.4% of carcinomas and none of the nonneoplastic breast tissues. In statistical analysis, there was a significant positive association between RFP and ErbB2 status at protein level (P = 0.002) as well as at the gene level (P = 0.049). There was no statistically significant association between the expression of RFP and ER, PR and nuclear grade (p>0.05). Conclusion:The expression of RFP in breast carcinomas together with a highly significant positive association between RFP and ErbB2 suggested that RFP could play a role in breast carcinogenesis.

PP1-39
EXPRESSION LEVELS OF FHIT AND WWOX CORRELATE WITH HER-2 PROTEIN OVEREXPRESSION AND GENE AMPLIFICATION IN BREAST CANCER

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BACKGROUND: We noticed borderline association of Fhit and Wwox expression with Her-2 in ductal carcinoma in situ of the breast in our previous study. The purpose of this study was to compare expression levels of Fhit and Wwox with Her-2 in invasive breast cancer. MATERIALS AND METHODS: Tissue microarrays were constructed from 130 invasive breast cancer cases. Two punches, one from central and another from peripheral part of the tumor were placed in the arrays. The expression levels of Fhit, Wwox, ER, PR and Her-2 were determined by immunohistochemistry and Her-2 gene amplification status was assessed by FISH. Correlations between various immunohistochemical markers and their relation with Her-2 gene amplification status were determined. RESULTS: Her-2 gene amplification determined by FISH was observed in 2.1% of Her-2 negative, 59.5% of Her-2 2(+) and 86.2% of Her-2 3(+) cases. There was a significant association of loss of Fhit and Wwox expression with Her-2 protein expression ($p<0.001$, $p=0.001$; respectively) and gene amplification ($p=0.002$, $p=0.001$; respectively). We divided our cases into four groups according to their ER (ER(+) means >10% nuclear staining) , PR (PR(+) means >10% nuclear staining) and Her-2 status (Her-2 (+) means existence of gene amplification detected by FISH): (group1, ER and/or PR (+), Her-2 (-) ; group 2: ER and/or PR (+), Her-2 (+); group 3: ER (-) PR (-), Her-2 (-); group 4: ER (-) PR (-) Her-2 (+). Reduced or lost Fhit staining was seen in 53.7% of group 1, 79.2% of group 2, 75% of group 3 and 93.8% of group 4 cases. Reduced or lost Wwox was detected in 50% of group 1, 66.7% of group 2, 42.9% of group 3, 76.5% of group 4 cases. Reduced Fhit and Wwox expression in these four groups were significantly different from each other ($p=0.026$, $p=0.023$, respectively).
CONCLUSION: Our results show that reduced Fhit and/or Wwox expression is more common in Her-2-positive invasive breast tumors.

PP1-40
FASCIN EXPRESSION IN INVASIVE DUCTAL CARCINOMA OF BREAST

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BACKGROUND: Malignant transformation is usually characterised by decreased cell–cell and cell–matrix adhesion and invasion to surrounding tissue. Tumor cells have many phenotypical alterations that include changes in cell shape, cell rounding, increased cell motility, loss of anchorage dependency and loss of cell–cell contacts. These changes are due to the rearrangement of cytoskeletal actin microfilaments. Fascin is an actin-binding protein that regulates the rearrangement of cytoskeletal elements and their interactions with the cell membrane and induces cell membrane protrusions and increases the motility of cells. In fact fascin is normally expressed by cells characterized by different types of membrane protrusions, such as neurons, glial and dendritic cells. In addition it is markedly up regulated in several types of carcinomas.
MATERIAL AND METHOD: We examined the expression of fascin in the invasive ductal carcinomas (IDC) with lymph node negative (IDC+LN-)(n=16), lymph node positive (IDC+LN+)(n=16) and in their metastatic lymph nodes (n=16),microinvasive + infiltrating ductal carcinoma with extensive insitu component (IDC with EIC)(n=9) by immunohistochemical method using monoclonal antifascin antibody. RESULTS: Fascin immunoreactivity was detected in 4 (44.4 %) microinvasive+IDC with EIC, 7 (43.7 %) IDC+LN-, 13 (81 %) IDC+LN+ tumors and 10 (62.5 %) metastatic LN. There was a significant difference for fascin immunoreactivity in these groups (p=0,044). Though the expression of fascin was not different between IDC+LN- and microinvasive+IDC with EIC but fascin immunoreactivity were significantly higher in IDC+LN+ than IDC+LN- group (p=0,033). No statistically significant difference was seen between IDC+LN+ group and their metastasis in the lymph nodes. The tumors with high fascin immunoreacivity tended to show more frequently lymphovascular invasion (p= 0.002). Advanced stage and high grade correlated significantly with higher fascin immunostainining (p=.0.036, p= 0.01).There were no significant association between fascin expression and tumor size, ER/PR and cerb-B2 overexpression. CONCLUSION: We evaluated the expression of fascin by performing immunohistochemistry to determine its role in the progression of invasive ductal carcinomas, invasive phenotype and metastatic potential. Our findings suggest that fascin expression plays a role in metastatic potential of invasive ductal carcinomas.

PP1-41
OCCULT CARCINOMA OF THE BREAST - CASE REPORT

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BACKGROUND: Occult carcinoma is defined as an axillary lymph node metastasis as a first clinical manifestation of a breast carcinoma. AIM: To present an unusual case of metastatic carcinoma in the axillary lymph nodes thought to origin from the breast even though the primary lesion was not identified. MATERIAL AND METHODS: Tissue sections were standard formalin fixed, paraffin embedded, and stained with Hematoxyllin Eosin. Additionally sections were immunohistochemically processed using antibodies for Cytokeratin wide spectrum (CKHMW), Cytokeratin 18 (CK18), cytokeratin 19 (CK19), HMB45, LCA, CD3, CD20, CD43, CD30, CD79a and MAC387. RESULTS: Female patient age 60 presented with axillary lymphadenopathy. Fine needle aspiration cytology revealed malignant cell population in a punctate from a lymph node, suggestive of a ductal invasive metastatic cancer, but mammography did not reveal a suspected malignant lesion. However radical mastectomy was performed based on the cytology finding. After a thorough macroscopic examination of the breast tissue suspected lesion was not detected. From the axillary fat tissue 13 lymph nodes were extracted, and two of them measuring 7 and 3 cm in diameter, contained timorous infiltrates. Microscopic results, from random tissue samples of the breast parenchyma, did not reveal malignant lesion. Samples from lymph nodes showed presence of a metastatic adenocarcinoma in 3 of 13 lymph nodes. The tumor cells formed solid nests and tubular structures, partly with necrotic areas. This morphology was highly suggestive of ductal breast carcinoma. Immunohistochemical analysis gave the following results: CK18, CK19 positive staining, CKHMW negative staining, HMB45 negative staining, and negative staining for lymphoid markers (LCA, CD3, CD20, CD43, CD30, CD79a and MAC387). CONCLUSION: The lesion was diagnosed as metastatic adenocarcinoma. Morphology suggests metastasis from ductal invasive breast cancer. We recommended exclusion of subtotal

resection of the breast, contralateral breast carcinoma, carcinoma arising in extra mammary breast parenchyma or aberrant breast tissue. These possibilities were excluded, so in this case the literature suggests existence of occult carcinoma of the breast.

PP1-42
"COMPLEX APOCRINE METAPLASIA" AND "ATYPICAL COLUMNAR CELL LESION" ARE THEY REALLY SO DISTANT?
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BACKGROUND: Both complex apocrine metaplasia (CAM) and atypical columnar cell lesion (ACL) are considered as candidates of early premalignant breast lesions. However, because of their distinct immunophenotypical features, it is thought that these two lesions might be involved in different breast carcinogenesis pathways. As we noticed their histological coexistence and proximity in our daily practice, we decided to investigate their relation at the morphological level, systematically. METHODS: We selected cases who received both a diagnosis of CAM and ACL. We found 93 cases, meeting these criteria, 38 of which was non-neoplastic and the rest neoplastic, among 197 non-neoplastic and 280 neoplastic (a total of 477 cases) breast specimens. We reevaluated sections and measured the distance between these lesions. Five groups were described: "Zero" for lesions with direct continuity, "<1mm" for lesions in adjacent lobules, "1-2mm", "2-5mm" and ">5mm". In case of multiple lesions the closest one was chosen for analysis. All excision materials were examined totally and mastectomy materials were sampled using a topographical map. RESULTS: Ninety-three of 477 (19.4%) breast specimens contained both CAM and ACL and in 73.1% of them, the two lesions were in continuity or adjacent to each other. Two main groups were formed: "zero" and "<1mm" groups were considered together as "adherent lesions" and the rest was grouped as "distant lesions". When neoplastic and non-neoplastic specimens were compared, a significant difference (p=0.006) was found between adherent ("zero" + "<1mm") and distant (">1mm") lesions, that is the CAM and ACL were much closer in the neoplastic group. During the detailed investigation of continuing lesions, some hybrid lesions were also encountered. CONCLUSION: CAM and ACL, when found in the same breast tissue, tend to appear in close contact, especially in neoplastic breasts. This result implies, CAM and ACL may be included in the same pathogenetic pathway, despite of their distinct immunophenotypical properties.

PP1-43
IMMUNOHISTOCHEMICAL DETECTION OF BRCA1 MUTATION AND cerbB-2 AMPLIFICATION IN BREAST CANCER PATIENTS WITH POSITIVE HISTORY
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Aim: It is believed that BRCA1 mutations account for the majority of hereditary breast carcinomas. Women who carry BRCA1 mutations have a high probability for developing ovarian, colon and stomach cancer. On the other hand cerbB-2 amplification has been identified in 25%-30% of breast carcinomas. The aim of our study is to detect immunohistochemicaly the BRCA1 mutations and the cerbB-2 amplification in breast cancer patients with positive history. Materials and Method: Forty patients diagnosed with breast carcinoma (3/40 lobular and 37/40 ductal carcinomas) in University Hospital of Patras between January 2000 to December 2005. Five out of 40 had a family history, 15/40 had a second breast carcinoma and 20/40 had a second primary cancer in other organs (8/20 had endometrial cancer, 4/20 lymphoma/leukemia, 3/20 colon cancer, 1/20 vaginal cancer, 1/20 astocytoma, 1/20 renal cell carcinoma, 1/20 ovarian carcinoma and 1/20 skin carcinoma). Tissue sections from breast cancer were immunohistochemicaly examined by using polyclonal antibodies against BRCA1 (Biogenex) and cerbB-2 (Dako). Results: Reduced staining or loss of staining for BRCA1 was not detected in any case. Expression of cerbB-2 was evident in 26/40 (65%) cases (in 3/5 women with breast cancer and family history, 10/15 cases with second breast cancer and in 13/20 cases with second primary cancer in other organ). Conclusions: Our findings suggest that immunohistochemical detection of BRCA1 mutation is not an effective method. High percentage of cerbB-2 expression in patients with breast cancer and positive history indicate that this gene play a substantial role in carcinogenesis in these patients.

PP1-44
ANALYSIS OF THE LOSS OF HETEROZYGOSITY AT THE SHORT ARM OF CHROMOSOME 3 IN SPORADIC BREAST CANCER AMONG TUNISIANS
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Background: Studies of allelic imbalance and suppression of tumorigenicity have consistently suggested that several regions on the short arm of chromosome three (3p) are commonly affected in different epithelial tumors, including breast cancer. However, several discrepancies between the frequencies of loss of heterozygosity (LOH) have been reported. Further, molecular genetic events associated with sporadic breast cancer in North Africa, area with low incidence but early onset, are lacking. Method: Here we investigated 3p for allelic imbalances, including LOH, homozygous deletion and microsatellite instability in 45 sporadic breast tumors of Tunisian patients using six highly polymorphic microsatellite markers spanning the most frequently affected regions. Results: Allelic loss at one or more loci was observed in 17 (37.8%) of these tumors. The marker D3S1228 at 3p14.1-14.2 showed the highest frequency of LOH (28%), followed by D3S1568 at 3p21.3 (19.5%), D3S1038 at 3p25.2-26.1 (15%), D3S659 at 3p13 (13%), D3S1067 (11.5%) at 3p14.3-21.1, and D3S1076 at 3p21.1-21.2 (7.5%). A low frequency of microsatellite instability (6.6%) was identified. Conclusion: These finding suggest that LOH at 3p, in particular at the region of D3S1228, may play a role in sporadic breast tumorigenesis among Tunisian women.

PP1-45
EXPRESSION OF CD 10 IN 7 MAMMARIAN METAPLASTIC CARCINOMAS
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Background: Metaplastic carcinomas of the breast that have unknown histogenesis are biphasic neoplasms composed of carcinomatous component contiguous or admixed with a pleomorphic mesenchymal component. They are rare neoplasms and there is somewhat confusing terminology related with this group of tumors in the literature. In this study, 7 metaplastic carcinomas were evaluated for CD 10 expression which is a reliable marker for myoepithelial cells, with respect to different cellular components. Method: All of the metaplastic carcinomas were assessed for CD 10 and conventional epithelial and mesenchymal markers of pancytokeratin, vimentin, smooth muscle actin and steroid receptors by immunohistochemistry. Results: All different categories of metaplastic carcinomas showed similar clinico-pathological features (age, tumour size, nuclear grade, mitotic activity, lymph node metastasis and hormonal receptor status). Out of 7 case, 1 was adenosquamous carcinoma and the others were biphasic tumors with sarcomatous

and carcinomatous components. In metaplastic carcinoma with epitehelial component only, CD 10 was not expressed. Sarcomatoid component was positive for CD 10 in 3 of 6 cases. Smooth muscle actin was also positive in these 5 cases. Remaining 1 case did not expressed smooth muscle actin. Conclusion: CD 10 expression with smooth muscle actin may be used as an adjunct marker in the diagnosis of metaplastic carcinomas.

PP1-46

BLINDED RETROSPECTIVE STUDY EVALUATING THE DIAGNOSTIC ACCURACY IN THE INTERPRETATION OF BENIGN AND PRECANCEROUS LESIONS OF THE BREAST

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BACKGROUND-OBJECTIVE: Quality assurance of diagnostic accuracy in surgical pathology is an important part of a pathologist's total quality management program. Blinded retrospective review is a highly sensitive and effective method to detect errors and/or disagreements. Breast biopsies were reviewed in order to assess the rate of diagnostic discrepancy. MATERIAL–METHODS: Blinded retrospective review of 257 cases of benign, pre-cancerous and pre-invasive lesions of the breast was performed, by three independent observers, with special interest in breast pathology. The diagnostic discrepancies between the two observers were correlated with the diagnosis of a third observer, the opinion of which was estimated as the gold standard. The results of the pathology reports were also correlated with the gold standard diagnosis. RESULTS: A. Review of the three observers: • Agreement of all three reviewers on 231 cases (89,88%) • Disagreements on 26 cases (10,12%), as follows: a. ADH:atypical ductal hyperplasia(DIN1b)versus lowDCIS(DIN1c) 1(0,39%) (hypoestimation) b. UH: usual hyperplasia versus ADH(DIN1b): 21(8.17%) (19 hypo-& 2hypest) c. ALH: atypical lobular hyperplasia versus LCIS:1 (0,39%) (hyper-estimation) d. UH: usual hyperplasia versus ALH: 3 (1,17%) (2 hypo-& 1hyperest) In total, 23 hypo-estimations (8,95%) were detected, 21 of which (8,17%) of the b group and 3 hyper-estimations (1,17%). B. Correlation between the initial and the gold standard diagnosis : • Agreement on 221cases (86 %) • Diagnostic discrepancies on 36 cases (14%) subdivided as follows: 1 error (0,39%) concerning hypo-estimation (UH instead of DIN1c) and 35 disagreements (13,65%) (16 common among the three observers), as follows: α. ADH(DIN1b) versus DIN1c:2 (0,78%) (1 hypo-& 1hyper-estimation) b. UH versus ADH: 27(10,5 %) (18 hypo-& 9 hyper-estimations) c. ALH versus LCIS: 1 (0,39%) (1 hypo-estimation) d. UH versus ALH: 5 (1,95%) (4 hypo-& 1 hyper-estimations) In total, we detected 25 hypo-estimations (9,75%), 18 (7,02%) of which concerned the b group and 11 hyper-estimations (4,24%). CONCLUSION: A low rate of diagnostic discrepancy was detected between the initial and the gold standard diagnosis, as well as between the three reviewers (14% and 10,12%, respectively), with an error rate of 0,39%. The majority of the detected diagnostic discrepancies concerned hypo-estimations of the subgroup b, a group with diagnostic difficulties even for expert breast pathologists.

PP1-47

BLINDED RETROSPECTIVE EVALUATION STUDY OF THE DIAGNOSTIC ACCURACY IN THE INTERPRETATION OF CARCINOMAS OF THE BREAST

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BACKGROUND-OBJECTIVE: Medicine is currently challenged by society to substantially improve patient safety and to significantly reduce medical errors. Oncologists and radiotherapists still look to the pathologists for assistance in tumor diagnosis. It is of crucial importance that the pathologic diagnosis be accurate. 'Blinded' retrospective review is a highly sensitive and effective method to detect errors or/and disagreements in diagnosis of histopathologic material. MATERIAL-METHODS: 'Blinded' retrospective review of 283 cases of breast carcinomas was performed by three independent observers with a special interest in breast pathology. The diagnostic discrepancies between the two observers (A: T.O. & B: T.H.) were correlated with the diagnosis of a third observer (C: AD.P.), the opinion of which was estimated as the 'gold standard'. The result of pathology report was also assessed in regard to the 'gold standard' opinion. RESULTS: A. Review of the three observers: - Agreement between the three observers on 236 cases (83,39%) - Disagreements between A/B: 47 cases (16,6%) - Disagreements between A/C: 33 cases (11,6%) - Disagreements between B/C: 24 cases (8,48%) As to the observer A, the disagreements concerned 16 cases of histological grade, 8 cases of histological type and 9 cases of both. Of the total 25 cases (grade), 19 cases were hyper-estimated and 6 cases were hypo-estimated. As to the observer B, the disagreements concerned 13 cases of histological grade, 8 cases of histological type and 3 cases of both. Of the total 16 cases (grade), 14 cases were hyper-estimated and 2 cases were hypo-estimated. B. Correlation between the pathology report and the 'gold standard' diagnosis: - Agreement on 219 cases (77,38%) - Disagreement on 64 cases (22,61%) Of the total 64 cases, 34 concerned grading, 26 the histological type and 4 both. Of the total 38 cases (grade), 23 were hypo-estimated (10,5%) and 15 (6,8%) were hyper-estimated. CONCLUSION: a low rate of diagnostic discrepancy was detected between the pathology report result and the 'gold standard' diagnosis, as well as between the three reviewers (22,61% versus 8,48%/11,6% respectively). No errors were detected. The diagnostic disagreements concerned misinterpretation of tumor type or hyper- or hypo-estimation of tumor grading. The clinical impact of the diagnostic discrepancies is not easy to assess, although decisions on therapeutic management could have been adversely influenced.

PP1-48

BREAST LESIONS OF UNCERTAIN MALIGNANT POTENTIAL IN THE SCREENING PROGRAM. AN AUDIT FOR QUALITY ASSURANCE

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Background: The low ratio for benign to malignant open biopsy is an audit for the quality of breast cancer screening programs. Screen-detected benign breast lesions are a wide spectrum that also includes lesions at risk of cancer. Nevertheless, the open biopsies carried out because of these lesions are included in the rate of useless benign breast surgery. We evaluated the impact of screen-detected benign lesions at risk of cancer in the open biopsy rate. Methods: We subdivided open biopsies into 6 groups: Histo1-normal histology; Histo2-"pure" benign lesions; Histo3-low risk proliferative lesions; Histo4-high risk proliferative lesions; Histo5- breast carcinoma; Histo6-other, non epithelial malignant neoplasms. Results: In the Verona Breast Cancer Screening Program the benign to malignant ratio was 1:5 and standards for cancer detection rate were high. Of the 510 open biopsies performed, 83 were benign. Among the benign lesions, Histo1 were 4.8% of the benign biopsies. Histo2 made up

37.7%. Histo3 made up 31.3% (10 radial scars, 1 mucocele-like lesion, 10 papillomas, 4 multiple papillomatosis, 1 phyllodes). Histo4 were 26.5% (1 atypical columnar cell hyperplasia, 22 atypical ductal hyperplasia, 1 atypical lobular hyperplasia). The benign to malignant ratio changed from 1:5 to 1:12 when biopsies due to lesions at risk of cancer were excluded. Conclusions: In screening programs lesions at risk should not be included in the benign rate. Only Histo1 and Histo2 open biopsies should be considered the real quota of false positive results. A detection rate for lesions at risk of cancer should represent an audit for high screening performance. This work was supported by: Fondazione Cassa di Risparmio di Verona Vicenza Belluno e Ancona. "Carcinoma della mammella: marcatori fenotipici e molecolari indicatori di prognosi e risposta terapeutica. Bando di ricerca scientifica 2004, indirizzo biomedico.

PP1-49

EVALUATION OF EXPRESSION OF PHOSPHORYLATED AKT AT THREONINE 308 [PAKT(THR308)] AND MEMBRANE TYROSINE KINASE RECEPTORS (HER-2 AND FLT-4) IN INVASIVE BREAST CANCER

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BACKGROUND: The PI3K-Akt signaling pathway plays a pivotal role in tumorigenesis. Akt is a serine/threonine kinase which, when full activated [phosphorylated Akt (pAkt)], promotes cell survival by inhibition of apoptosis. In addition, pAkt promotes angiogenesis and contributes to tumor invasion and metastasis. Previous studies regarding PI3K-Akt pathway in breast cancer, have mainly investigated the role of the phosphorylated Akt at Serine 473 [pAkt (Ser473)]. Our purpose was to investigate the expression pattern of the phosphorylated Akt at Threonine 308 [pAkt (Thr308)] and its possible role in invasive breast carcinomas. METHOD: Immunohistochemistry was performed on paraffin-embedded tissue specimens from 152 invasive breast carcinomas to detect the pAkt (Thr308) protein. pAkt was assessed in combination with clinicopathological parameters, ER and PR receptor status, p53, Ki67, c-erbB-2, caspase-3, Flt-4, VEGF-C, VEGF-D and MMP-14. RESULTS: pAkt (Thr308) protein was immunodetected in the cytoplasm and the nuclei of the malignant cells, as well as in in situ carcinoma and normal epithelium. Of the classical clinicopathologic parameters, cytoplasmic pAkt was found to positively correlate with the lobular histologic type, while it was found to exert no impact on patients survival. Cytoplasmic pAkt (Thr308) immunopositivity was inversely correlated with Ki-67 and p53 ($p=0.013$ and $p=0.020$ respectively), while positive was found to be the association between pAkt (Thr308) and c-erbB-2 ($p=0.005$), caspase-3 ($p=0.031$), Flt-4 ($p=0.023$) and MMP-14 ($p=0.014$). Nuclear expression of pAkt (Thr308) was positively correlated with VEGF-C ($p=0.025$) and VEGF-D ($p=0.009$). CONCLUSION: This is the first study to mention a more frequent detection of pAkt(Thr308) in the lobular breast carcinomas and to correlate pAkt(Thr308) with some of the membrane tyrosine kinase receptors in invasive breast carcinomas. Membrane tyrosine kinase receptors, like HER-2 and Flt-4, activation enhances Akt activity and contributes to the transduction of mitogenic and angiogenetic signals, a fact that is reflected in the positive correlations with MMPs and VEGFs.

PP1-50

THE DIFFERENCES BETWEEN CENTRAL AND LOCAL LABORATORIES IN HER2 TESTING: IHC AND FISH STUDY WITHIN 2+ IHC GROUP

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Background: Proper HER2 testing is an essential issue in breast cancer management. There are two established methods of HER2 status evaluation: immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH). According to the wide-accepted algorithm of diagnostic procedure, all cases immunohistochemically described as "2+" should be re-tested with more reliable method – FISH. Although IHC has got clear interpretation criteria, the reproducibility of this method differs significantly between local and central laboratories, especially in "borderline" group (2+ according to DAKO). Aim: The aim of the study was the comparison of differences in HER2 assessment between central and local laboratories within 2+ group. Materials and methods: The material consisted of 944 breast cancer cases from 2+ group. In all cases HER2 status was determined by IHC in central (n=732 cases) and local (n=212) laboratories. There were four local laboratories: A, B, C and D, which differ with the experience and the quantity of IHC tests performed yearly (A is the most experienced laboratory, D – the less one). All FISH tests were performed in central laboratory. Results: In the analyzed 2+ cases, the lowest percentage of HER2-positive tumours (FISH) was observed in the central laboratory material (15%). In the material obtained from A laboratory, the percentage of HER2-positive cases was similar to the result of the central lab (15,1%). Lab B had 26%, lab C – 34,9% and lab D – 36,8% of HER2-positive cases within 2+ group. The less experienced hospital, the more HER2-positive cases in 2+ group. According to the statistical analysis, the dependence was linear ($p<0,001$). Conclusion: The experience of the pathologist plays an important role in HER2 assessment in breast cancer. The IHC borderline group (2+) should be re-tested by FISH not only in the central laboratory, but especially in the small local labs.

PP1-51

USE OF LASER SCANNING CYTOMETRY (LSC) IN DETERMINING S-PHASE FRACTION AND DNA CONTENT IN BREAST CANCER PATIENTS

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Introduction: High proliferation rate in surgicaly removed breast tumours is an unfavourable prognostic factor. In node negative cases it can help distinguish patients with higher risk for distant metastases from those with a lower risk. Patients and Methods: In a prospective study we investigated 97 breast tumours, of which 81 were invasive ductal carcinomas, 8 were invasive lobular carcinomas, and 9 tumours were of other histological types. Cells for LSC investigations were prepared from fresh, surgicaly removed tumours by mechanical disintegration and the suspensions were cytocentrifuged onto miscroscopical slides. After fixation the cells were stained with FITC conujgated anti-cytokeratin (CK-FITC) to distinguish tumour CK positive tumour cells from CK negative stroma, and with propidium iodide to stain DNA. Results: In our group of patients, we identified three S-phase fraction (SPF) groups (<5 %, 5 to 10 %, and >10 %). As to aneuploidy, 53.8 % of the tumours were aneuploid, 36.3 % were diploid, and 9.9 % had a bimodal distribution of DNA content. Aneuploid DNA content and higher SPF values tended to be associated with larger tumour size, lymph node involvement, and higher histological grade. An attempt was made to correlate DNA content and SPF to tumour marker values (CEA, TPS, CA 15-3). Conclusions: Our results showed that the measurement of DNA content and S-phase fraction of tumours by laser scanning cytometry completed by and correlated with the

investigation of tumour markers and other biological properties of the tumour cells may be a useful tool in assessing prognosis and clinical outcome of patients with breast cancer.

PP1-52
EVALUATION OF HER2 STATUS IN BREAST CARCINOMA BY CISH AND FISH; COMPARISON OF THE RESULTS IN IHC 2+ GROUP OF 257 CASES

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Determining the HER2 status in breast carcinoma management has become crucial for introducing proper therapy (chemo- and hormonotherapy). Therefore reliable methods for HER2 status evaluation, even at time of primary diagnosis are necessary. In Poland IHC is employed as a basic method for HER2 status evaluation. Interpretation of IHC stains according to four-tier DAKO system generates three groups HER2 positive, HER2 negative and "borderline" (2+ cases according to DAKO method). The cases from the last category need to be additionally evaluated. In Poland FISH (Fluorescents in situ Hybridization) method is used in these cases. Costs of the method and necessity of fluorescent microscopy make this method "unfriendly" for smaller laboratories. The alternative for FISH method is CISH (Chromogenic in situ Hybridization). The CISH method is cheaper and there is no need for fluorescent microscopy. Still, FISH method is considered as golden standard in HER2 status evaluation. Aim: The aim of the study was comparison of FISH and CISH results in the group of 257 cases labeled as "borderline" by IHC method (2+ according to DAKO). Narrowing the study group on this category only in our opinion should had result in more practical answer to question of value of CISH method in determine HER2 status. Methods: Two methods of HER2 status evaluation were applied to all 257 cases: CISH and FISH. Results: The overall concordance between these two methods was 78,6% (202 out of 257). In 21,4% of cases (55 out of 257) the discordant results were found. In the discordant group of 55 cases, 40% (22 cases) of them presented with chromosome 17 aneuploidy (by FISH). In this specific group alone, as many as 55% tumors (22 out of 40) presented discordance between CISH and FISH results. Conclusions: In the group selected for the study (IHC 2+) a significant (21,4%) discordance between CISH and FISH results was observed. The discordance is the highest (55%) in the subgroup with chromosome 17 aneuploidy.

PP1-53
USE OF LASER SCANNING CYTOMETRY (LSC) IN DETERMINING S-PHASE FRACTION AND DNA CONTENT IN BREAST CANCER PATIENTS

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Introduction: High proliferation rate in surgicaly removed breast tumours is an unfavourable prognostic factor. In node negative cases it can help distinguish patients with higher risk for distant metastases from those with a lower risk.
Patients and Methods: In a prospective study we investigated 97 breast tumours, of which 81 were invasive ductal carcinomas, 8 were invasive lobular carcinomas, and 9 tumours were of other histological types. Cells for LSC investigations were prepared from fresh, surgicaly removed tumours by mechanical disintegration and the suspensions were cytocentrifuged onto miscroscopical slides. After fixation the cells were stained with FITC conjugated anti-cytokeratin (CK-FITC) to distinguish tumour CK positive tumour cells from CK negative stroma, and with propidium iodide to stain DNA.
Results: In our group of patients, we identified three S-phase fraction (SPF) groups (<5 %, 5 to 10 %, and >10 %). As to aneuploidy, 53.8 % of the tumours were aneuploid, 36.3 % were diploid, and 9.9 % had a bimodal distribution of DNA content. Aneuploid DNA content and higher SPF values tended to be associated with larger tumour size, lymph node involvement, and higher histological grade. An attempt was made to correlate DNA content and SPF to tumour marker values (CEA, TPS, CA 15-3).
Conclusions: Our results showed that the measurement of DNA content and S-phase fraction of tumours by laser scanning cytometry completed by and correlated with the investigation of tumour markers and other biological properties of the tumour cells may be a useful tool in assessing prognosis and clinical outcome of patients with breast cancer.

PP1-54
CYTOLOGIC NUCLEAR GRADE OF MALIGNANT BREAST ASPIRATES AS A PREDICTOR OF NOTTINGHAM PROGNOSTIC INDEX, ESTROGEN RECEPTOR, PROGESTERON RECEPTOR, cerb B2 AND p53 EXPRESSIONS

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BACKGROUND: Nuclear grade is one of the key prognostic factors of breast carcinomas since 1990's. The aim is to compare the nuclear Grade (NG) in cytological material (cNG) obtained from breast fine needle aspiration cytologies (FNACs) with the Nottingham Prognostic Index (NPI), Estrogen Receptor (ER), Progesteron Receptor (PR),cerb B2, and p53 expressions, observed in surgical materials(SM) of the same tumors. METHOD: From Jan.2000 to Dec.2006, 835 consecutive cytologic breast FNACs were performed at Pathology Dept. Of these cases 702 were benign, 133 were malignant, on MGG stain. The study group consisted of 63 carcinomas (21 low NG (LcNG) -42 high NG (HcNG) cases, according to simplified Black; F=60, M=3; age range=23-85) with both FNAC and SM which were reviewed by two pathologists. Most of them (95 %) were invasive ductal carcinomas (IDC); 2 were mucinous and 1 was lobular ca. Corresponding tissues of IDC were independently evaluated with modified Bloom-Richardson and NPI systemsNPI scores were grouped into good (score 1-3.3, n=12), moderate (score 3.4-5.4, n=26) and bad prognostic groups (score 5.4, n=18). Then was modified (mNPI) into low (good & moderate) and high (bad) prognostic groups. All of the SM were stained with ER, PR, cerbB2 and p53 and the immunohistochemical staining was categorized as negative and positive . Chi-square test is used for statistical evaluation. RESULTS: There was overall aggrement between cNG and mNPI in 91.7% of tumors with LcNG and low prognostic group. The percentage coincidence was greater (100%) for HcNG and MBR Grade 3 and MBR Grade 3 cases.Most of the LcNG group was ER(+), cerbB2(-) and p53(-). However in HcNG group, most were cerbB2(+), p53(+) and ER (-). In patients younger than 50 year-old, ER was lower, cerbB2 was higher and p53 was higher expressed than the older patients. PR expressions were not statistically significant ($p>0,05$). All of the results, (except PR) were statistically significant ($p<0,05$). Therefore it seems to be important and significant to distinguish LcNG from HcNG in breast aspirates of ductal carcinomas. CONCLUSION: Assigning a NG in breast carcinoma aspirates is done with little effort, is reproducible and correlates with NPI, ER, cerbB2, and p53 expressions, while grading, nuclear pleomorphism, presence of nucleoli in aspiration material retained their statistical significance. The lack of correlation may have been due to sample limitations, tumor heterogenity and observer subjectivity. The cNG should appear in FNAC reports of breast carcinomas.

PP1-55
EGF AND HGF/SF REGULATE THE DIFFERENT STAGES OF BREAST CANCER PROGRESSION

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BACKGROUND: Numerous studies have shown an indirect regulation of steroid action by growth factors. Growth factor signaling could selectively be activated in different stages of cancer progression and stimulate the steroid responsiveness as well as become a cause for hormone-independent growth. In our study we evaluated the relationship between epidermal growth factor (EGF) and hepatocyte growth factor/scatter factor (HGF/SF) production and steroid receptor status, oncoprotein expression and the basic clinicopathological factors in breast cancer. METHODS: Estrogen receptor (ER), progesterone receptor (PR) and Her2/neu expression in paraffin sections and in primary cultures of breast cancer from the same tissue specimens of 40 patients was demonstrated by immunohistochemistry. Production of HGF/SF and EGF in culture media was examined by enzyme-linked immunosorbent assay. RESULTS: EGF content in supernatant of breast cancer primary cultures demonstrated an association with ER expression (p=0,05) and a tendency for correlation with Her2/neu oncoprotein expression (p=0,07). The median value was higher in ER-positive tumors (527,44±132,16 pg/ml) when compared to those without ER protein expression (283,09±119,40 pg/ml). Correlation analysis between HGF/SF concentration and clinicopathological factors showed that the HGF/SF level exhibited an independence of ER status but it was positively correlated with nodal status (p = 0,01). The average of HGF concentration in primary culture media from the patient with node metastasis was 1369,47±594,87 pg/ml (range, 756,9 – 3009 pg/ml) versus 293,48±169,08 (range 102,0 – 674,1) in patients without lymph node involvement. On the contrary the association of EGF concentrations with nodal status had no statistical significance. Neither HGF/SF nor EGF content correlated with histological type, histological grade and PR expression. CONCLUSION: The present findings suggest that up-regulation of several growth factors and the appearance of autocrine pathways in malignant breast epithelial cells could occur during alternating periods as cancer progresses. Enhanced EGF having a close connection with ER expression might trigger Her2/neu and the growth of mammary cells expressing ER at the beginning of cancer development. Such critical steps as adhesion and migration are elicited by increasing of HGF/SF but not by EGF in advanced cancer stage. An independence of HGF/SF of steroid receptor status could be a principal factor of hormone resistance development in breast cancer.

PP1-56
HER-1 (EGFR) IN INFLAMMATORY BREAST CANCER: A NEW PROGNOSTIC FACTOR AND A POTENTIAL PREDICTIVE MARKER

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Background: Inflammatory Breast Cancer (IBC) is an highly invasive manifestation of Breast Carcinoma characterized by clinical inflammatory signs and/or dermal lymphatic carcinomatosis. HER-1 (EGFR) in breast carcinoma has been reported with different expression rate ranging from 14% to 91%. Few papers described EGFR expression in IBC with a rate ranging from 19% to 31%. Methods: We collected 65 cases of IBC referred to Department of Pathology at "G.B. Rossi" Hospital of Verona (Italy) between January 1989 and December 2005. We studied, by tissue micro array technique, EGFR immunohistochemical expression as prognostic factor on overall (OS) and disease free (DFS) survival time. Results: In our series, HER-1 positive cases were 13% (8/65 cases) and were associated with poor prognosis (OS: P=0,00025), with a mean overall survival time of 29 months. This result confirm the published data on the influence of HER-1 as a prognostic factor on overall survival time. Nevertheless, this marker cannot predict a neoplastic recurrence of IBC (DFS: P=0,0543). In our experience HER-1 positive carcinomas didn't show HER-2 expression. The publication of clinical trials of patients suffering from breast carcinoma treated with HER-1 inhibitor candidates this marker as a predictive factor and the patients who can't benefit of the anti-HER-2 neoadjuvant therapy, could be treated with an HER-1 inhibitor. Conclusion: EGFR is a good prognostic factor of overall survival time in IBC and it could represent an important predictive marker for therapy. This work was supported by: Fondazione Cassa di Risparmio di Verona Vicenza Belluno e Ancona. "Carcinoma della mammella: marcatori fenotipici e molecolari indicatori di prognosi e risposta terapeutica. Bando di ricerca scientifica 2004, indirizzo biomedico.

PP1-57
HER-2/neu STATUS IN BREAST CARCINOMA: CORRELATION OF GENE AMPLIFICATION BY FISH WITH IMMUNOHISTOCHEMISTRY

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Background: Numerous biologic markers have been proposed to assist in deciding treatment modality in breast carcinoma. Assessment of HER2/neu protein expression by immunohistochemistry (IHC) or HER2/neu gene amplification by fluorescence in situ hybridization (FISH) have been suggested to provide valuable prognostic information, particularly in selection of patients for treatment with trastuzumab. Patients with gene amplification by FISH are expected to have poor prognosis but respond better to trastuzumab therapy. We evaluate HER2/neu status in mammary ductal carcinomas by using both methods. Method: Formalin-fixed paraffin embedded archival tissues of mastectomy specimens from 44 patients with invasive ductal carcinoma between February 2002 and October 2006 were evaluated. Immunohistochemistry for HER2/neu expression and FISH for HER2/neu gene amplification were performed for all cases. Immunohistochemically, only membrane staining was considered and scored on a scale of 0+ to 3+. Cases with negative or 1+ immunostaining were considered negative for HER2/neu, while cases with 2+ and 3+ were classified as positive. Results: Overall, 12 of 44 (27.3%) cases showed amplification by FISH, while 35 cases (79.6%) were either 2+ or 3+ by immunohistochemistry. Two of nine cases (22.2%) with negative IHC staining (either 0+ or 1+) were amplified; the both amplifications were from seven cases (28.6%) with 1+ immunostaining; two cases with clear-cut negative IHC staining (0+) were also negative by FISH. Four of 23 cases (17.4%) with 2+ positivity showed amplification. Amplification was observed in six of 12 cases (50%) with 3+ staining. Conclusion: The unexpected finding of 50% discordance rate between 3+ IHC and FISH may be associated with high overall IHC positivity rate in the current study exceeding recommended IHC rate. However, compared to previous studies, relatively higher gene amplification in 1+ (28.6%) and 2+ (17.4%) cases seems more conflicting, particularly if these expressions are misleading due to high IHC rate and may in fact represent lower (ie. 0+) scores.

Gastrointestinal Pathology

PP1-58
PROTECTIVE EFFECT OF ISCHEMIC PRECONDITIONING AGAINST HEPATIC ISCHEMIC INJURY DURING THE COURSE OF LIVER RESECTION IN A RAT MODEL

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Background: Ischemic preconditioning (IP) is a process by which a brief ischemic episode confers a state of protection against subsequent sustained long-term ischemia-reperfusion injury. The purpose of this study was to evaluate the protective effect of ischemic preconditioning on ischemic injury of the liver during the course of hepatic resection in a rat model. Material and Method: Three groups of 6 Wistar rats were examined; Group A: subjected to sham-operation, Group B (ischemia/reperfusion group I/R): animals subjected to left-lobe hepatic ischemia for 30 min followed by reperfusion and right lobe resection and Group C (ischemic preconditioning group IPC): subjected to 10 minutes to left-lobe hepatic ischemia followed by 10 minutes of reperfusion followed by the prolonged ischemia for 30 min followed by reperfusion and right lobe resection. After 10 days the animals were killed and the livers were excised for histopathological assessment of the degree of liver damage. Serum concentrations of lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), were measured as markers of hepatocyte injury at completion of the first laparotomy and at relaparotomy after 10 days. Results: There were significantly higher levels of AST ,ALT and LDH in ischemia/reperfusion group as compared to ischemic preconditioning group ,($p < 0.05$). Furthermore, histopathological evidence of liver damage was more pronounced in ischemia/reperfusion group as compared to ischemic preconditioning group, ($p < 0.05$). Conclusion: Ischemic preconditioning exerts a protective effect on hepatic ischemia-reperfusion injury in rat model. Such a manoeuvre may be useful for hepatic resection in the clinical setting.

PP1-59
OBSTRUCTIVE JAUNDICE PROMOTES INTESTINAL BARRIER DYSFUNCTION AND BACTERIAL TRANSLOCATION: EXPERIMENTAL STUDY

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Background: Clinical and experimental studies have demonstrated a correlation between obstructive jaundice and sepsis.The aim of this study was to investigate the influence of biliary obstruction on bacterial translocation as a possible source of infection in cases of obstructive jaundice. Material and Methods: Two groups of 12 Wistar rats were examined: rats subjected to common bile duct (CBD) ligation (group A) and rats subjected to a sham operation (group B). After 7 days blood samples were taken and liver, spleen, and mesenteric lymph nodes (MLN) from the ileocaecal area were removed, divided into small pieces and cultured. Quantitative culture results were determined by the number of colony-forming units (CFU) per ml homogenate. Bacterial translocation was defined as the presence of a positive culture of mesenteric lymph nodes, blood, liver and/or spleen. Samples for histopathological examination were taken from the mucosa of the ileum and the colon and evaluated for inflammatory and destructive changes. Results: There was no evidence of bacterial translocation to MLN, blood, spleen or liver detected in any of the 12 sham-operated control rats. In contrast, bacterial translocation was demonstrated in 8 of the 12 CBD ligated rats ($P < 0.01$). In all 8 cases in which translocation occurred, Escherichia coli was cultured from the mesenteric lymph nodes. There were no histological changes in the mucosal samples of the control animals. In the CBD ligated rats hyperemia, vacuolization, reduction of goblet cells, decreased mitotic activity and infiltration by lymphocytes and PMNLs were detected. Cases in which translocation occurred were significantly associated with decreased mitotic activity in the colon ($r = -0.5$, $p < 0.01$) and higher infiltration by PMNLs in the ileum ($r = -0.62$, $p < 0.05$). Conclusion: Obstructive jaundice predisposes to bacterial to bacterial translocation.

PP1-60
MUTANT p53 PROTEIN AS A BIOMARKER OF HISTOPATHOLOGICAL FEATURES AND PROGNOSIS OF HEPATOCELLULAR CARCINOMA AFTER SURGICAL RESECTION

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Background and Aim: Prognosis after hepatectomy for hepatocellular carcinoma (HCC) has been improved by early diagnosis, progress in the surgical techniques and perioperative management. However, even when curative resection is performed at a relatively early stage, a considerable number of patients develop early intrahepatic and/or extrahepatic recurrence postoperatively. .This study analysed the correlation between the p53 mutations and the clinical, pathological features, tumor recurrence and patient survival after surgical resection of HCC. Patients and methods: Specimens from 20 HCCs and surrounding liver tissues from patients who underwent surgical resection were examined histopathologically and by immunohistochemisty for detection of p53 gene mutation. The association between p53 mutation and histopathological features of HCCs, as well as tumor recurrences and patient survival were evaluated. Results: p53 mutation was found in 9 out of 20 HCCs. p53 mutations were frequent in large, and poorly differentiated HCCs. Five out of 9 with p53 mutation showed microvascular invasions in contrast to none out of 11 without mutation. Hepatocellular carcinoma recurred in 6 out of 9 with p53 mutation, in contrast to only 2 out of 11 without mutation. The 1-year survival rate in patients with p53 mutation was significantly lower than those without. Conclusion: The detection of p53 mutation in HCC may provide additional and independent prognostic information for HCC and may serve as an indicator of high-risk patients for whom closer follow-up and aggressive adjuvant therapy may be required.

PP1-61
ANGIOGENESIS IN COLON CANCER

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Abstract Introduction: The basic pathogenic step in the process of tumor growth, invasion and metastasis, is tumor – induced angiogenesis, the aim of this study was to evaluate the angiogenesis in colorectal carcinoma by microvascular density (MVD) determination with IHC method and to determine if and how angiogenesis correlates with clinicopathologic parameters.
Material and Methods : Seventy cases, archival, paraffin embedded tissue of colorectal carcinoma of Omid hospital (Mashad, Iran) were selected. Microvesseles were identified immunohistochemically, using monoclanal CD34 antibodies. CD34 positive staining was obtained in 62 out of 70 cases, and observed mainly in the cytoplasm of endothelial alls. Counting was performed at the invasive foci of tumor and not in granulation tissue or necrotic area. Two investigators examined the microvessel density by counting immunoreactive cells in the three hot spot area of tumor using a 400 X field in a blind fashion, then the median and mean value of MVD was determine and correlate with clinicopathologic parameters.

Results : Tumor – induced angiogenesis of colorectal carcinoma statistically correlated with histologic tumor grade (P=0.000). there was no significant correlation between intratumoral microvessel density and sex and age of patients, localization, stage and histologic tumor type. (P>0.1) Conclusions: Intratumoral microvessel density quantification in histologic specimens of colorectal carcinoma reflects the biological malignant potential of tumors and may be a useful additional prognostic factor. Microvessel density in tumor specimens may be valuable in stratifying patients in planning appropriate adjuvant and antiangiogenic therapy after surgery.

PP1-62
TUMORS OF THE DIGESTIVE SYSTEM BY FINDINGS OF POST-MORTEM EXAMINATIONS
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The current study was designed to retrospectively determine the incidence rate of neoplasms of the digestive system on pathoanatomical material of the Municipal Institution "Taldom Central Regional Hospital" over the period from 1995 to 2004. Of 495 postmortem examinations of adults, we revealed a total of 36 cases (21 males and 15 females, mean age 68 years) with stage 4 tumours diseases of the digestive system, which turned out to have been the primary cause of death, with the majority of cases accounting for cancer of various portions of the colon (8 cases), sigmoid colon cancer (6 subjects)and pancreatic cancer (5 cases) with the histological form, as a rule, of adenocarcinoma of various degree of differentiation. Diffuse peritonitis was registered as the most frequent cause of death (10 cases). Mention should be made that in 13 cases we revealed discrepancies between the clinical and pathoanatomical diagnoses by the primary disease, these discrepancies were primarily caused by insufficient examination of the patient (neither diagnostic laparotomy, nor ultrasound examination of the abdominal organs performed), the severity of the state and a short hospital stay of the patient, overestimation of the diagnosis and erroneous interpretation of the obtained findings by the surgeon, and difficulty of making the accurate diagnosis due to comissural process during to operative intervention. Further analysis showed that amongst the adult patients having died of neoplasms, first came tumours of the digestive system, to be followed by tumours of the genitourinary system (8 cases), ranking third were tumours of the respiratory system (4 cases), and place 4 being shared by tumours of the central nervous system and haemopoietic and lymphoid tissues - 2 cases each, respectively. Hence the incidence rate of neoplasms of the digestive system amounts to 7.27% amongst the 495 postmortem examinations of adult patients of our general somatic hospital over the decade under investigation. In the majority of cases, the immediate cause of death was diffuse peritonitic resulting from ingrowing of the tumour of all the membranes with decomposition, to be followed by perforation of the wall of the intestine, or stomach.

PP1-63
CYCLOOXYGENASE-2 AND TRANSFORMING GROWTH FACTOR-B1 EXPRESSION IN HEPATOCELLULAR CARCINOMA FOLLOWING HEPATITIS C VIRUS INFECTION AND CIRRHOTIC LIVER DISEASE
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It is unknown whether COX-2 or TGF-β1 contribute to the malignant growth in HCC. Their expression were investigated in 30 cases of post-HCV, HCC whose corresponding non-tumorous tissues were hepatitis C virus-related chronic hepatitis (n=50) and post-hepatitis C liver cirrhosis (LC) (n=30). Samples of ten histologically normal livers were also analyzed. Serum alpha fetoprotein level (α-FP), histopathological diagnosis and immunohistochemistry were done for all cases. Histologically, normal livers were weakly positive for COX-2 (<10%) & TGF-β1 (<50%). The intensity of tissue expression of COX-2 increased from normal liver to chronic hepatitis to cirrhosis. In HCC, 36.7% (11/30) of samples displayed a high COX-2 expression with a significant correlation with differentiated carcinomas. No significant relation was found with the level of α-FP. TGF-β1 was expressed in all cases of LC& HCH at different staining intensities; which was mostly marked being in 93.3% & 52% of cases respectively. It was also expressed in 60% of HCC cases because it serves as either an epithelial cell growth inhibitor or a tumor promoter, depending on the cellular context. However, no correlation could be detected between TGF-β1 expression and grade of HCC differentiation or serum level of α-FP. ver-expression of COX-2 in HCV, LC and well-differentiated HCC suggest that it is a potential marker for hepatocarcinogenesis particularly in its early stages. Meanwhile, HCV & LC regulate TGF-β1-induced signal transduction pathway. Interruption of TGF-β1 signaling improves liver fibrosis and stimulates liver regeneration.

PP1-64
Bcl-2 POSITIVITY IN B AND C HEPATITIS AND HEPATOCELLULAR CARCINOMAS
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Background Hepatocellular carcinoma (HCC) is a widespread disease and constitutes up to 1/4th of all adult malignancies in Asia and Saharan Africa. Worldwide HCC is the 7th and 6th most common cancer in males and females, respectively. Predictive value of bcl-2 expression in the development of HCC is unknown and results of the studies so far have been contradictory. In the present study bcl-2 expression in chronic hepatitis B and C cases, as well as in neoplastic and non-neoplastic peritumoral tissue in HCC have been immunohistochemically investigated and bcl-2's possible role in the development of hepatocellular malignancy has been discussed. Method In this study, 23 chronic hepatitis C, 17 chronic hepatitis B and 29 hepatocellular carcinoma cases, which were obtained from the archives of the Department of Pathology, Faculty of Medicine, Ondokuz Mayis University, between years 1992 and 2004 in the were examined. They have been immunohistochemically stained for the expression of bcl-2. Positive staining was semi-quantitatively graded from + to +++. Results Two out of 23 hepatitis C cases and 3 out of 17 hepatitis B cases were found to express bcl-2, staining was more intense in areas adjacent to active inflammatory process. Of the 29 hepatocellular carcinoma cases 6 stained positively for bcl-2; 9 biopsies in this group had also non-neoplastic liver tissue, and, of these 6 stained positively for bcl-2. Of the latter 6 bcl-2 positive biopsies 3 also showed tumoral staining while in the remaining 3, neoplastic elements were bcl-2 negative. Conclusion As the present study is a cross-sectional study, no causative relation between bcl-2 positivity and hepatocellular carcinoma can be implied, however high incidence of bcl-2 activity in the non-neoplastic liver parenchyma of the HCC cases suggest that bcl-2 activation may be involved in the development of at least some cases of hepatocellular carcinoma. Case control and/or prospective studies are needed to show whether bcl-2 positivity in a chronic hepatitis case has a predictive value for the development of hepatocellular carcinoma.

PP1-65
GASTROINTESTINAL STROMAL TUMORS RECOGNIZED AS OVARIAN NEOPLASMS BEFORE SURGICAL OPERATION

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Introduction: Gastrointestinal stromal tumors (GISTs) are the most common neoplasms of mesenchymal origin of stomach and small bowel. They derive from precursors of Cajal's cells and express positive reaction with CD117 antibody. This immunohistochemical examination is currently a base of recognition them and allows to treat with imatinib. Aim: The histopathologic analysis of tumors diagnosed preoperatively as ovarian neoplams in women, whereas postoperative pathologic recognition revealed, that they were gastrointestinal stromal tumors (GISTs). Materials and methods: Among 240 women with GIST , 12% were operated due to ovarian neoplasm. These tumors were diagnosed as GISTs in Department of Pathology at Cancer Center in Warsaw. A part of postoperative material came from Cancer Center, whereas a large amount were provided as consult examinations. Results: Grossly the tumor's diameter ranged from 10 to 15cm, cream - coloured, sometimes with many haemorrhages. Most of them were attached to small bowel. A few of them were connected to large intestine. In two cases they looked like big leiomyomas of uterus. Microscopically: the spindle cells were seen mostly mixed with epitheliod and signet – ring ones. There were no big atypia.. There were skenoid fibers in a few tumors. Mitotic figures were counted in 50 high power fields. They ranged from 5 to 20. Most of neoplams expressed a positive reaction with CD 117 antibody. The most of them were no resected within healthy limits, so they demanded postoperative treatment. Conclusions: GISTs in females, very often are clinically similar to ovarian neoplams. The fundamental aim is good histopathologic recognition and finally – treatment with imatinib.

PP1-66
HEPATIC STELLATE CELL/MYOFIBROBLAST SUBPOPULATIONS IN CHRONIC HEPATITIS C AND NONALCOHOLIC STEATOHEPATITIS

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Background: In human fibrotic liver, activated HSC, interface myofibroblasts (MF) and portal/septal MF, which represent the source of collagen production, differentially express mesenchymal (α-smooth muscle actin-SMA) and neural/neuroendocrine markers (glial fibrillary acidic protein, GFAP; neural cell adhesion molecule, N-CAM; synaptophysin, SYN). We evaluated the distribution and quantity of HSC/MF subpopulations in chronic hepatitis C (CHC) and nonalcoholic steatohepatitis (NASH) and correlated these with disease grade and stage. Methods: Needle liver biopsies from 21 CHC and 14 NASH patients were immunostained for αSMA, GFAP, N-CAM, SYN and semi-quantitatively evaluated in zones 1,2,3 and portal/septal areas using a 12-point scoring system (Feldstein et al, 2005). Histological lesions of CHC and NASH were evaluated according to Ishak's and Brunt's classifications respectively. Results: In CHC and NASH, portal/septal and interface MF were positive for αSMA, GFAP and N-CAM but not SYN, while activated HSC were positive for all markers. Immunohistochemistry for αSMA identified the highest number of activated HSC, while SYN the lowest. In CHC, αSMA and SYN HSC scores correlated with grade (p=0.036 and p=0.035), but not with stage or steatosis' severity. In NASH, portal/septal MF αSMA score was related to stage (p=0.049), while there was no correlation of any other marker's score with global grade, stage or steatosis' grade. The total scores for all markers were no significantly different between CHC and NASH. However, in zone 2 the quantity of GFAP+, N-CAM+ and SYN+ HSC was higher in NASH compared to CHC (p=0.036, p=0.012 and p=0.003). Furthermore, in the subgroup of cases with minimal/mild fibrosis, αSMA and GFAP HSC scores and aSMA and N-CAM portal MF scores were higher in CHC (p=0.05, p=0.007, p=0.026 and p=0.05). Conclusions: a) Portal/septal MF, in contrast to previous reports, express N-CAM in addition to αSMA and GFAP; b) αSMA is the best marker for identification of activated HSC, while GFAP, N-CAM and SYN identify only a subpopulation of HSC; c) In CHC, αSMA and SYN HSC scores are related to necroinflammation grade, while in NASH, αSMA portal/septal MF score is related to fibrosis stage; d) There are no significant quantitative differences in total activated HSC between CHC and NASH. However, there may be quantitative differences in the zonal distribution of neural/neuroendocrine marker-expressing HSC; in the subgroup of cases with minimal/mild fibrosis the number of HSC and portal MF is higher in CHC.

PP1-67
EFFECT OF SPLENECTOMY ON LIVER FIBROGENICITY IN PORTAL HYPERTENSION PATIENTS

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This study was designed to assess the role of the spleen on the presence and localization of liver fibronectin, to measure the amount of hepatic collagen and serum level of fibronectin and procollagen III in cases of portal hypertension. The study was performed on 40 patients divided into two groups: Portal hypertension group (20 patients) included one pure schistosomal hepatic fibrosis, three mixed schistsomal and virus C cirrhosis, ten virus C hepatic cirrhosis, two virus B hepatic cirrhosis and four mixed B&C infections. Control group (20 patients) presented with different splenic insults without hepatic affection. In the first group, through clinical examinations and sonographic findings revealed hepatosplenomegaly and grade III & IV varices with history of injection sclerotherapy in 65% of cases. Preoperatively, marked hepatic fibronectin deposition in portal tracts and around blood vessels raised the stage of fibrosis. This was accompanied by decrease in plasma level of fibronectin and increase of serum level of procollagen III in relation to the control group. Six months post-operatively, tissue fibronectin, collagen content and serum procollagen III were insignificantly decreased. However, plasma level of fibronectin was increased significantly. These results denoted that fibronectin is one of the predominant early fibrogenic components in chronic liver diseases, stimulating further collagen deposition. It is concluded that the role of the spleen in the development of hepatic fibrosis seems ancillary. Multifactorial influences, including etiology, timing of splenectomy, hepatic vascular adjustment, and factors associated with the biology of extracellular matrix of the liver, probably play a more significant role. Further studies were recommended to assess the role of splenectomy on liver fibrogensis at different stages of fibrosis. Intervention with splenectomy could improve or reverse fibrogenic activity at early stages. Key words: Splenectomy, fibrogenicity, fibronectin, procollagen III, liver fibrosis, portal hypertension.

PP1-68

EVALUATION OF THE ANTI-SCHISTOSOMAL ACTIVITY OF THYMOQUINONE IN MURINE SCHISTOSOMIASIS MANSONI: INFLUENCE ON NITRIC OXIDE, ICAM-1 AND COLLAGEN

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We have proved previously that Nigella Sativa oil reduced Schistosoma mansoni (S. mansoni) induced hepatic fibrosis, the total worm burden, egg load and granuloma formation. Therefore, we aimed here to study the possible antischistosomal effect of one of its active constituent's thymoquinone (TQ). The drug was given to S. mansoni infected mice in two dose levels (5 and 10 mg/kg, p.o.) for two weeks, and its effect was compared to that of the standard antischistosomal drug, praziquantel (PZQ). Two weeks later, the following parameters were estimated, viz., worm load and distribution, oogram changes, egg load in liver and intestine, granuloma diameter, collagen deposition and intercellular adhesion molecule-1 (ICAM-1) expression. Moreover, serum levels of L-alanine aminotransferase (ALT) and nitric oxide (NO), as well as NO hepatic level were estimated. Mice treated with TQ showed a significant reduction in total number of worm burden, both in liver and portomesentric blood vessels. The oogram pattern recorded a decrease in immature and mature ova, and an elevation in dead ova. Tissue egg load was reduced in both liver and intestine. In infected mice treated with TQ, the elevated serum ALT activity leveled off. However, neither serum NO level was altered, nor in the infected group nor in those treated with TQ or PZQ. In liver homogenate, infection decreased NO level, which was not corrected with PZQ, but in-groups treated with TQ, a further reduction was reported. TQ caused shrinkage in granuloma diameter, and a diminution in collagen deposition. On the other hand, PZQ failed to reduce collagen deposition. Both TQ and PZQ decreased ICAM-1 expression in Kupffer and inflammatory cells. However, neither TQ nor PZQ affected its expression in endothelial cells. These results point to a possible antischistosomal role of TQ, which may be correlated with modulation in the immune system. Key words: schistosomiasis mansoni; nitric oxide; granuloma; intercellular adhesion molecule-l (ICAM-l), collagen; thymoquinone; praziquantel.

PP1-69

OSTEOPONTIN AND CD44 PROTEIN AND mRNA EXPRESSION IN COLORECTAL ADENOCARCINOMA

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PURPOSE: B (NFkB) and mediatesκOsteopontin (OPN) binds to CD44 and nuclear factor kappa tumorigenesis, invasion, and metastasis. This study evaluated the relationship between the expressions of OPN, CD44, and NFkB in untreated colorectal adenocarcinoma. METHODS: One hundred and two patients with colorectal adenocarcinoma were included between 2001 and 2003. We constructed tissue microarray blocks that were immunostained with primary OPN, CD44, CD44v6, CD44v3, and NFkB monoclonal antibodies. Real-time RT-PCR with OPN was performed with fresh frozen tissue. RESULTS: OPN and CD44 protein expressions were noted in 76.5% and 34.0% of the adenocarcinoma tissue compared to 23.2% and 5.9% of the paired normal colonic mucosa, respectively, and the protein expressions were correlated in the adenocarcinoma tissue ($p<0.05$). The expressions of OPN and CD44 proteins, however, were not correlated with any of the clinicopathological factors including age, sex, location, stage, lymph node metastasis, lymphatic, angioinvasion, and perineural invasion separately. The CD44v3, CD44v6, and NFkB protein expression rates were 0.9%, 8.9%, and 97.0% in tumorous tissue. No correlation was detected between the OPN, CD44, CD44v3, CD44v6, and NFkB protein expressions. The OPN mRNA expression was higher in tumor tissue than in paired normal mucosa, but the difference was not statistically significant. The lymph node status was the only independent prognostic factor of survival in Cox's regression analysis. CONCLUSIONS: OPN and CD44 may participate in colorectal tumorigenesis, but their roles are relatively minor in the progression of human colorectal adenocarcinoma.

PP1-70

CORRELATION BETWEEN LIVER BIOPSY FINDINGS AND DIFFERENT SERUM BIOCHEMICAL TESTS IN STAGING FIBROSIS IN EGYPTIAN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION

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Hepatitis C virus (HCV) infection is characterized by silent onset in most infected individuals, a high rate of viral persistence, and the potential for the development of chronic hepatitis, liver fibrosis that eventually may progress to cirrhosis or hepatocellular carcinoma. Liver biopsy is still the gold standard for liver fibrosis assessment. Recent studies strongly suggest that due to limitations and risk of biopsy, as well as the improvement of the diagnostic accuracy of biochemical markers, the requirement for liver biopsy in clinical practice should be reduced. Fibro test, several simple laboratory tests, scores and indices have been proposed for non invasive prediction of hepatic fibrosis in patients with chronic hepatitis. Therefore, the aim of this study was to assess the value of different non invasive routine serum biochemical tests of hepatic fibrosis in the prediction of advanced fibrosis in patients with HCV infection. One hundred and sixteen patients with chronic HCV infection selected from the outpatient clinic of the National Liver Institute, Menoufiya University, Egypt, were subjected to the following tests: serum ALT, AST, albumin and total bilirubin; prothrombin time and concentration; complete blood count; Hepatitis B surface antigen (HBsAg); Hepatitis C virus antibody (anti-HCV); HCV- RNA by quantitative PCR and liver biopsy. The following ratios, scores and indices were calculated and compared with the grade of necroinflammation activity and the stage of fibrosis. AST/ALT ratio (AAR), Cirrhosis discriminating score (CDS), Age platelet index, AST to platelet ratio index (APRI), Pohl score, Goteborg University Cirrhosis Index (GUCI) and Hepatitis C Antiviral Long -term Treatment against Cirrhosis (HALT-C). Results from our study revealed that CDS, APRI, GUCI and HALT-C had good diagnostic accuracy, moderate sensitivity, high specificity and significant correlation between both the grade of activity and the stage of fibrosis. Whereas, AAR and Pohl score demonstrated significant correlation with advanced stages of fibrosis. In conclusion, the combination of simple biochemical serum tests may replace the requirement to liver biopsy in staging advanced fibrosis and cirrhosis in chronic hepatitis C patients.

PP1-71

CARDIAC MUCOSA: CONGENITAL OR ACQUIRED? A MORPHOLOGICAL STUDY IN CHILDREN AND ADULTS

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BACKGROUND & AIM The incidence of gastric cardiac adenocarcinoma has increased dramatically. Adequate prevention is hampered by limited knowledge on precursor lesions and controversy about the origin of cardiac mucosa (CM). Some investigators consider CM as a normal structure present from birth, while others propose that it is always acquired by metaplasia of oesophageal squamous epithelium in a setting of reflux. To solve this question, we investigated the presence and structure of CM in children and adults. METHODS We studied fixed endoscopic gastro-oesophageal junction biopsies of 46 children and 56 adults. Detailed clinical information was available for all subjects. The following stains were applied: haematoxylin-eosin (HE), mucin stains (AB/PAS, AB/HID) and immunohistochemical stains for Ki-67, CK7 and CK20, MUCs 2, 5AC and 6 and CDX2. CM was defined by the presence of mucous glands without parietal cells. RESULTS Cardiac mucosa was present immediately distal to the Z-line in 79% of children and 87% of adults. Its length varied between 0,15 and 0,90 mm, being only slightly larger in patients with reflux signs. Acid mucins were predominantly present in the foveolae of CM. The epithelial proliferative compartment was more often superficially localized and discontinuous in CM than in the more distal mucosae. CK7 was expressed both superficial and deep in CM's foveolae, while CK20 was present only superficially. MUC 5AC was present in the foveolar and MUC 6 in the glandular epithelium of CM. We observed several transformations in CM related to patient age and the presence of reflux signs or active inflammation. Increasing age correlated with CDX2 and MUC2 positivity, indicating a trend to develop intestinal metaplasia. Deep foveolar CK7 expression was seen more commonly in patients with reflux signs. In case of active inflammation, more acid mucins (especially sulfomucins) were produced in CM. CONCLUSIONS The near universal presence of CM and the absence of conspicuous lengthening in case of reflux argue against the hypothesis that CM is always acquired by metaplasia. Native CM undergoes age-related changes which may be accelerated in a setting of reflux with active inflammation.

PP1-72

USE OF ALPHA METHYLACYL-COA RACEMASE FOR DISTINCTION OF REACTIVE VERSUS DYSPLASTIC LESIONS IN COLONIC EPITHELIUM

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Background: Alpha methylacyl-CoA racemase (AMACR) catalyses the racemization of alpha-methyl branched carboxylic coenzyme A thioesters and is overexpressed in a variety of neoplasms, such as prostate and colon cancer. The aim of this study was to evaluate AMACR expression in adenoma of the colon and regenerative lesion in inflammatory bowel disease (IBD) and to determine if its expression can be used to distinguish regenerative changes from dysplastic epithelium in these conditions. Design: 34 routinely processed biopsy and/or resection specimens from 34 patients with regenerative lesion in IBD [11with crohn s disease (CD);15 with RCH ; 8 with adenomas] were immunostained with a monoclonal AMACR antibody (p504S). Results: there were highly significant differences between the percentages of AMACR immunopositive cells in adenomas compared with regenerative epithelium. AMACR staining was uniformly positive in the adenomas group (75%).only 2 (25%) of 8 specimens with adenomas showed negative. Of 26 specimens, 21(80%) showed negative staining for AMCAR in regenerative group [crohn's disease and RCH] The p values were fisher bilateral test was considered to be significant 0.007<0.05 Conclusions: The high degree of specificity is of AMACR but moderately sensitive for distinguishing dysplasia from regeneration in IBD suggests that it may be useful to distinguish reactive from neoplastic epithelium.

PP1-73

EPSTEIN-BARR VIRUS ASSOCIATED GASTRIC CARCINOMA: A STUDY IN ONE REFERRAL CENTER IN IRAN IN THE LAST FOUR DECADES

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Background: Epstein-Barr virus has been proved to be associated with many of the human malignancy including gastric carcinoma, one of the most important human malignancies in the world. There has been no study about the presence of EBV in gastric adenocarcinoma in Iran. Method: We examined the presence of EBV in 273 formalin fixed paraffin-embedded cases of gastric carcinoma from Cancer institutes of Tehran University, from 1962 to 2004. In situ hybridization of EBV-encoded small RNA-1 (EBER-1) was conducted. The strain of positive cases was examined by means of polymerase chain reaction and/or restriction fragment length polymorphism analysis. Results: We found 9 (3%; 95% CI=1-5%) EBV positive cases. The gender difference was not statistically significant. The proportion of EBV-GC cases in diffuse type was higher than intestinal type (OR=0.08; 95% CI=0.002-0.64). EBV-GC cases had no relation with age, location and invasion. Six out of 9 EBV-GC cases were born during the period between 1928 and 1930. All 9 cases were Type A. Prototype F was seen in 6 out of 8 cases. Type "i" was found in 8 cases and type I in 1 case. XhoI+ and XhoI- polymorphism accounted 6 and 3 of the cases, respectively. Conclusion: Our study is the first to describe the frequency of EBV-GC in Iran and the Middle East, highlighting a very low prevalence with specific clinicopathologic features. The predominance of EBV-GC birth year in a fixed period, suggests that EBV infection or other events at early childhood may be related to the development of EBV-GC later in the life. The predominance of the type "i" and XhoI+ cases are contradictory to other studies in Asia and is similar to what is reported from Latin American countries.

PP1-74

ROLE OF bcl2 ONCOPROTEIN IN CARCINOGENESIS OF COLORECTAL NEOPLASIA AND CORRELATION WITH HISTOPATHOLOGIC AND PROGNOSTIC PARAMETERS

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Background: Tumor progression is a multistep process and consist, loss of regulation and balance between cell death and cell proliferation. The bcl2 oncoprotein plays an important role in carcinogenesis by inhibiting cell death (apoptosis). It was initially discovered in follicular B cell Lymphoma with t (14, 18) and subsequently in other malignant lesion. The aim of this study was to (1) investigate role of bcl2 immunostaining expression in colonic adenoma and carcinoma. (2) Identify any relationship between bcl2 marker and several histopathologic parameters. Materials and Methods – surgical specimen colorectal carcinoma (50) and adenoma (50) have been studied. Sections of paraffin block are used for Immunostaining according to the avidine - biotin - peroxidase complex method. Then correlation of bcl2 expression in colon adenoma and carcinoma and relationship between bcl2 and several histopathologic parameters is identified. Results: bcl2 was consistently higher in adenoma than in carcinoma. There were 92% in adenoma and 40% in carcinoma. (P values = 0 .001) bcl2 oncoprotein expression in carcinoma was significantly higher in well differentiated than moderately differentiated carcinoma. But there is not any correlation between

bcl2 expressions with others histopathologic parameters. There was only statistically significant relation between bcl2 expressions with size of adenoma. Discussion: Correlation of bcl2 expression in colon adenoma and carcinoma and with Tumor differentiation is similar to the results obtained in most previous study in this field. This result confirms that expression of bcl2 is valuable in diagnosis and predicting the clinical course of the patients.

PP1-75
IMMUNOHISTOCHEMICAL CHARACTERIZATION OF HUMAN FETAL PANCREAS AND PANCREATIC DUCTAL ADENOCARCINOMA. A STUDY OF SOMATOSTATIN ANTIBODY NCL-SOMATOP

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Purpose: Somatostatin is a gastrointestinal peptide hormone that inhibits growth of pancreatic cancer as reported by an increasing body of evidence. Yet this is not always the case. To clarify the controversy we aimed to identify the expression of somatostatin in developing human embryonic pancreatic tissue and pancreatic adenocarcinoma given that somatostatin positive cells were shown either into primitive pancreatic ductal epithelium or into pancreatic carcinoma. Methods: Tissue sections representing pancreatic fetal specimens (n=15) and ductal pancreatic adenocarcinoma specimens (n=15) were assessed using immunohistochemical methods for somatostatin expression. Results: Normal primitive exocrine ductal epithelium and endocrine epithelium showed a definite, statistically significant, higher expression of somatostatin over neoplastic pancreatic tissue of mixed (ductalendocrine) and pure ductal type ($p1=0.021$, $p2=0.001$, $p3<0.0001$, and $p4=0.003$ respectively) during the 8th to the 10th week. No statistically significant differential expression of somatostatin in the mantle zone of the islets over neoplastic tissue of mixed ($p5=0.16$) and pure ductal type ($p6=0.65$), from the 13th to the 24th week was demonstrated. Conclusion: Pancreatic cancer cells can express somatostatin in a model that reproduces the normal expression of the peptide by δ-cells during embryonal organogenesis. Therapy aimed at pancreatic cancer must be targeted to somatostatin and analogues as potential adjuvant novel option.

PP1-76
GASTROINTESTINAL MESENCHYMAL TUMOURS ARE NOT ALL STROMAL: REPORT OF TWO RECTAL LEIOMYOSARCOMAS

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INTRODUCTION: Leiomyosarcoma (LS) is considered as a malignant gastrointestinal smooth muscle tumour that occurs most frequently in oesophagus and rectum. Rectal leiomyosarcoma is an uncommon malignancy; it accounts for less than 0,5% for all malignancies of the colon and rectum. This rarity makes the choice of suitable associated treatment after surgery difficult and requires long-term follow-up to determine their behaviour. We report two cases of rectal LS. We will discuss clinical and histological findings allowing us to distinguish this entity from gastrointestinal stromal tumour (GISTs). OBSERVATIONS: We report tow new cases of rectal leiomyosarcoma. A 77-year-old and a 65-year-old men, were admitted for gastrointestinal bleeding, abdominal pain, and the first patient had a loss of weight. Rectal examination revealed for the first, a firm polypoid mass at 2 cm from anal sphincter. The latter, presented an indurate circumferential ulcerative mass in the rectal ampullae. On abdominal ultra-sonography and CT scan appeared a circumferential inhomogeneous mass in continuity with rectal wall, showing intramural necrotic area in one case; in the other case, there was a second little mass having the same characteristics. No metastases were disclosed. Abdomino-perineal resection was performed for the two patients. Grossly, the tumours were fungated and polypoid and measured 3 cm for the first patient and 9cm for the second with surface ulcerations. On the cut surface, necrotic and hemorrhagic foci were occasionally recognized. Microscopically, in the rectal tumour, there were areas of broad necrosis and haemorrhage, and viable tumour cells occupied about 50% of the tumour. There were no metastases in the resected lymph nodes. The tumour consisted on a proliferation of spindle cells arranged in fascicles. with epithelioid features. The cytoplasm of the tumour cells was eosinophilic or clear. The cellular and nuclear atypia was high, characterized by marked mitotic activity (more than 20 mitosis/10HPF), supporting the diagnosis of high grade LS. Mucosal involvement occurred in only one patient. Surgical margins were microscopically negative. For confirmation, an immunohistochemical study was performed. The tumour cells were negative for cytokeratin and epithelial membrane antigen, but positive for vimentin, indicating that the tumour was a sarcoma. The tumour cells were positive for á-smooth muscle actin, desmin and PS100. However, they were negative to CD 117 (C-kit). The tumour cells showed high proliferate activity as evidenced by Ki-67 immunostaining. The disease free interval for the two patients was 1 and 4 months. No regional recurrences were detected; however in one case, there was a pulmonary metastasis. The evolution was fatal in both cases. CONCLUSION: Leiomyosarcoma (LS) of the rectum is a rare tumour associated to a poor prognosis. GISTs are the main differential diagnosis. Morphological and immunohistochemical findings allows to separate theses two entities, so long time confused. The treatment of LS is primarily surgical, where possible, and should guarantee complete clearance of the tumour, which often requires an aggressive approach. Histopathological characteristics predictive of poor prognosis include high mitotic activity, intra-tumoral necrosis and tumour differentiation. Non-curative resection, high tumour grade and size > 10 cm, are considered unfavourable prognostic factors. Further trials are needed to establish the exact role of adjuvant therapy.

PP1-77
PROGNOSIS OF THE HIRSCHSPRUNG'S DISEASE ALLIED DISORDER (HAD) OR NEURONAL DYSPLASIA: THE VALUE OF GANGLIA/NERVE FIBERS RATIO

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BACKGROUND: The Hirschsprung's disease allied disorder (HAD) pathohistological findings of abnormal myenteric nervous plexus vary depending of the quantity of ganglia, therefore, the complete absence of ganglia (the real Hirschprung), hypoganglionosis, and hyperganglionosis which is considered as the colonic neuronal dysplasia.1 The lack of ganglia cells and increased and abnormal proliferation of mucosal nerve fibers follow the congenital dismotility problems of the GIT. The aim of our study was to determine a possible connection between ganglia/nerve fibers ratio and the clinical outcome in HAD predicting possible recovery of patients even before treatment. METHOD: We have followed the recovery of 15 patients treated and compared the clinical outcome with the histopathological

findings of ganglia and nerve fibers. Sections of the full thickness bowel specimens, obtained from 15 patients with HAD, were treated with antibodies against neurofilament protein (NF), marking ganglia, S-100, marking nerve fibers. RESULTS: 5 patients undergoing surgery were found to have low ganglia/nerve fibers ratio while 9 out of 10 patients not showing the need for surgery had higher ganglia/nerve fibers ratio. CONCLUSION: We propose that the ganglia/nerve fibers ratio can be used to predict the clinical outcome and long term prognosis as well as the need for invasive procedures in patients diagnosed with HAD. High ganglia/nerve fibers ratio correlates with good clinical outcome and decreased need for surgery, while lower ganglia/nerve fibers ratio shows poorer prognosis and increases the need for surgical treatment in our patients.

PP1-78
PROGNOSTIC FACTORS OF GASTROINTESTINAL STROMAL TUMORS

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BACKGROUND: Gastrointestinal stromal tumors (GIST) are rare neoplasm of unknown etiology and pathogenesis. Clinical behaviour is very unpredictable and a reliable prognostic factor is lacking. The aim of this study is to analyze some prognostic factors and estimate which one is the most reliable. METHOD: 38 biopsy specimens of GIST were immunolabeled with PC –10 for PCNA, for CD-34, vimentin, NSE and actin. Greatest diameter, histological grading, mitotic count, DNA-indeks and S-phase were estimated for each case. All patients were followed-up for at least 24 months or to death. Data were analysed by univariate and multivariate statistical analysis using computer program. RESULTS AND CONCLUSION: Results showed that greatest diameter, tumor grade, mitotic count and PCNA-index are prognostic factors in univariate analysis. In multivariate analysis only greatest diameter are useful prognostic factor for planning further therapy. Tumors with diameter less than 6.3 cm have better prognosis and they are benign, without reccurence and distant metastasis. Prognostic parameters (PCNA-index, mitotic count and tumor grade) could be useful as an auxiliary methods in cases of GIST with greatest diameter within a range 5 – 9 cm.

PP1-79
LIVER PATHOLOGY IN CHRONIC HEPATITIS C AND NORMAL ALT LEVELS

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Introduction: Hepatitis C virus (HCV) is a major cause of chronic liver disease, affecting 175 million people globally. Over 80% of acutely infected patients go on to develop chronicity. To date, there are no accurate noninvasive markers of hepatitis C activity and fibrosis. Liver biopsy is indicated to establish the stage and character of liver disease. In this work the role of liver biopsy in the patients with chronic hepatitis C (CHC) and persistently normal serum alanine aminotransferase (ALT) levels was evaluated (PNALT) Methods: Sections of formalin fixed, paraffin-embedded liver biopsies from 20 patients with CHC and PNALT were stained by Haemotoxylin and Eosin. Diagnosis was made on the basis of histological findings, clinical and virological information. Results: As showed results of our investigation, in the liver of the patients with CHC and PNALT histologically was observed chronic mild hepatitis. It was characterized by appearance of lymphoid follicules and/or aggregates and liver fat infiltration in 3 cases. Conclusion: There is no absolute correlation between serum ALT levels and liver disease activity in the patients with CHC and PNALT. Liver biopsy is an important implication on staging of the liver diseases, prognosis and management of therapy. Treatment decisions of these patients should be based on global evaluation of the patients rather than the results of laboratory test. (This work is supported by Grant A – 9-400 RUz).

PP1-80
EXPRESSION OF PHOSPHORYLATED (ACTIVATED) RECEPTORS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGFR2/KDR) IN COLORECTAL CANCER

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Background: Phosphorylation of specific tyrosine kinase receptors of the VEGF on endothelial cell membrane promotes angiogenesis. Using a specific monoclonal antibody recognizing the phosphorylated form of VEGFR2/KDR receptor (pVEGFR2/KDR), we investigated the activation status of VEGF pathway in normal colon and in colorectal adenocarcinomas. Materials and Methods: Immunohistochemistry was performed on 128 paraffin-embedded sections of colorectal adenocarcinomas and 30 normal colonic specimens. The 34a monoclonal antibody recognizing the p VEGFR2/KDR (University of Oxford) was used. Results: Normal colon showed a weak expression of pVEGFR2/KDR. In contrast, pVEGFR2/KDR was strongly expressed in the cytoplasm and nuclei of cancer cells and the tumor-associated vasculature, mainly at the invading tumor edge. pVEGFR2/KDR expression in cancer cells was significantly associated with tumor size >6cm ($p=0.04$) and poor histological differentiation ($p=0.004$). Tumor-associated vessels expressing high pVEGFR2/KDR density was significantly related with high VEGF expression in cancer cells ($p=0.02$). This was also related significantly to high pVEGFR2/KDR expression in cancer cells. Conclusion: VEGFR2/KDR is not simply a vasculature restricted receptor, but has an additional role in cancer cell biology itself. It is suggested that VEGF released by cancer cells induce auto-activation of cancer cell VEGF receptors. This autocrine loop may function as a stimulus for cancer cell proliferation or resistance to apoptotic stimuli. Novel drugs inhibiting VEGF receptor phosphorylation may, therefore, have a direct activity on colorectal cancer cells, in addition to their function as inhibitors of neo-vascularization.

PP1-81
PRIMARY SQUAMOUS CELL CARCINOMA OF THE STOMACH: A CASE REPORT

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Primary squamous cell carcinoma (SCC) of the stomach is a very rare tumor, probably arising from ectopic or metaplastic squamous epithelium. We report a case of SCC of the stomach in

a 73-year-old woman, who was admitted to our hospital because of weight loss, anemia, epigastric pain and melena. Gastroscopy and CT of abdomen showed a big exophytic tumor of the anterior wall between corpus and antrum. A biopsy taken from the tumor revealed a SCC. The patient underwent a total gastrectomy. A 9cm in diameter cauliflower-like tumor was observed macroscopically. A total of 25 tissue blocks from the tumor showed a pure moderately differentiated keratinizing SCC. No foci of squamous metaplasia or component of adenocarcinoma were present. Immunohistochemically, the tumor cells were positive for HMW cytokeratin and negative for cytokeratin 7,20 and CEA. The tumor infiltrated the whole thickness of the wall without penetrating the serosa. All regional lymph nodes (31 in number) and the resection margins were free of tumor involvement. The TNM – staging was pT2pNoG2. 18 months after the diagnosis was established the patient is free of recurrence. SCC and adenosquamous carcinoma constitute less than 1% of all gastric carcinomas. The majority of them is localized in the distal half of the stomach. 80% of the reported patients were male with a peak incidence in the sixth decade of life. Our case fulfills the criteria proposed by Parks et al. Prognosis of SCC cannot be clearly assessed since the number of reported cases is low and many patients present with locally advanced disease.

PP1-82
RUSSELL BODY POLYP OF STOMACH

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A plasma cell tumor of the stomach with many Russell bodies is reported. A gastric polyp of 57 year-old woman was found by annual health check. Due to the low level of pepsinogen, she was recommended to take an examination of upper gastrointestinal endoscope. A 7mm in diameter sized polyp of gastric antrum was found and biopsy revealed Russell body gastritis. Polypectomy was done and the tumor of the stomach was diagnosed as Russell body polyp of the stomach. Russell bodies were stained positive for CD21, CD79α, and right-chain kappa. She is infected by Helicobacter pylori as well. CD21 is reported to be an activator of B-cell and CD21 proliferates B-cells, resulting in a round polyp of gastric mucosa. Seven cases of Russell body gastritis were reported in literatures, however, no report is about the Russell body polyp of stomach. This is the first case of Russell body polyp of stomach in the world.

PP1-83
MOLECULAR EVIDENCE OF INVOLVEMENT OF COX-2 AND NF- κB IN TRIGGERING CELL CYCLE ALTERATION IN BARRETT'S ESOPHAGUS

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Background: The reflux of gastric content, bile and acid , in esophagus , is able to induce first Esophagitis (ES) and then Barrett's Esophagus (BE). Nuclear factor kappa B (NF-kB) is a ubiquitous transcription factor that regulates the activation of genes involved in pro-inflammatory response. NF-kB and Cyclooxygenase (Cox-2) activation are increased in BE. p27 expression increases in accord to inflammation. CDX2, a transcription factor associated with intestinal differentiation, is related to cell cycle regulators cdk-2 and p27Kip1. The targeted degradation of cdk-2 is the mechanism through which CDX2 activity can be regulated in coordination with the cell cycle machinery.. Aim: To investigate the molecular pattern of inflammation metaplasia sequence in ES and EB. Matherials and methods: In order to investigate the molecular pattern of inflammation -metaplasia sequence in ES and EB, immunohistochemestry (IHC) with antiCDX-2, anti-p27Kip1, anti-cdk-2, anti NF-kB, and anti COX-2 monoclonal commercial antibodies was performed on paraffin embedded tissue sections of 60 BE , of 64 endoscopic (A,B,C los Angeles scale) and histologic esophagitis (ES), and of 30 non inflammed gastro-esophageal junction (GEJ) as control. Multiple (four) biopsies for each patient were obtained during endoscopy. Western blot (WB) was performed on proteins extracted from samples from each group Results: WB and IHC showed super imposable data. In GEJ is preferentially expressed cdk-2, instead NF-kB, COX-2, p27 and , CDX2 are absent. ES is associated with an high rate of NF-kB, COX-2 and p27, and with an iportant decrease of cdk-2 respect to the GEJ value.EB presented obviously high level of CDX2, NF- kB, and COX-2 , but a decrease of p27. Conclusions: Normal esophageal mucosa is characterized by the expression of cdk-2 and no inflammatory proteins, when both COX-2 and NF-kB increase esophagitis is present that fact may be able to bring an increase of p27. p27 determine selective inhibition of cdk-2. Low levels of cdk-2 are associated with BE and are the molecular prerequisite for the CDX2 expression. Then the high rate of inflammation regulators such as NF-kB and COX-2 are the trigger for CDX2 activation. But the coexistence on BE of high level CDX2, NF- kB, and COX-2 suggest that CDX-2 isn't directly influenced by inflammation.

PP1-84
WHICH APPENDICEAL NEOPLASMS LEAD TO PSEUDOMYXOMA PERITONEI AND HOW OFTEN: A POPULATION BASED STUDY

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Background: Pseudomyxoma peritonei (PMP) is a rare disease with an estimated incidence of 1 per million per year, and is thought to originate usually from an appendiceal mucinous epithelial neoplasm. However it is not known exactly how often these neoplasms lead to PMP. The aim of this study is to investigate the incidence of both lesions and their relation. Methods: The nationwide pathology database of the Netherlands (PALGA) was searched for the incidence of all appendectomies, the incidence of primary epithelial appendiceal neoplasms and the incidence and pathology history of patients with PMP. All regarded the 10-year period of 1995-2005. Results: In the 10-year period 167,744 appendectomies were performed in the Netherlands. An appendiceal lesion was found in 1482 appendiceal specimens (0.9%). Nine percent of these patients developed PMP. Coincidentally, an additional epithelial colonic lesion was found in 13% of patients with an appendiceal epithelial neoplasm. A mucinous epithelial neoplasm was identified in 0.3% (73% benign, 27% malignant) of appendiceal specimens and 20% of these patients developed PMP. For mucocele and non-mucinous neoplasm the association with PMP was only 2% and 3%, respectively. From the nationwide database 267 patients (62 men and 205 women) with PMP were identified, which demonstrates an incidence of PMP in the Netherlands approaching 2 per million per year. The primary site was identified in 68% and dominated by the appendix (82%). Conclusions: Primary epithelial neoplasms of the appendix occur in 0,9% of appendectomies. One third of these lesions is mucinous. In 20% of patients with a mucinous lesion of the appendix PMP develops. The incidence of PMP seems to be higher than thought before. Furthermore there is a considerable risk of an additional colonic epithelial neoplasm in patients with an epithelial neoplasm at appendectomy

PP1-85
HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY OF EPITHELIAL MUCINS IN CAP POLYPOSIS
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Background - Cap polyposis is rare disease characterised by mucoid and bloody diarrhea, with polyps that contain an overlying 'cap' of necroinflammatory debris and granulation tissue.The pathogenesis is not known. Aims – To pour some light on cap polypsis pathogenesis, we have examined the epithelial mucins of 30 polyps, taken from a patient with cap polyposis in whom has been done right hemicolectomy. Material and methods – The study was performed on biopsy specimens taken from 30 polyps of various size, sessile or with stalk.Fixation has been done in 10%formaldehyde.Paraffine sections were stained with:H&E, HID-AB at pH 2.5 with exclusive expression of sialo- and sulfomucins and LSAB2 immunohistochemical method, by using antibodies to MUC2, MUC3, MUC4 and MUC5A.The intensity of the expression was scored semiquantitatively by two independent observer as: -, absent, + weak, ++ moderate, and +++ strong; normal sygmorectal mucosa servs as control. Results – The diagnosis of cap polyposis was established on the basis of the appearance of the polyps, which formed from hypertrophic colonic mucosa with dilated glands containing mucus and granulocytes, covered by a cap formed from mucus,fibrin and leucocytes. In contrast to numerous small sessile inflammatory polyps found 15 cm from the anal margin, 24 filiform villous polyps('giant inflammatory polyps') with stalks, were discovered 6cm from the proximal colon margin. Comparative histochemical and immunohistochemical examination showed that the mucus of cap polyposis differed in two aspects from that of normal colon: predominance of non-sulfated mucins in the goblet cells and abnormal expression of the MUC4, MUC3 and MUC5AC genes. The intensity of the MUC5AC expression was strong (+++) in filiform polyps, moderate (++) in small sessile polyps, but it was not detected in normal colonic mucosa.We suggest that the mucin abnormality detected, which reflect deregulation of the expression of the apomucin genes, abnormal glycosylation and abnormalities of the secretion process, are also probably involved in the clinical manifestations of cap polyposis.

PP1-86
STATISTICAL CORRELATION BETWEEN SOME MOLECULAR MARKERS IN COLORECTAL CANCER
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Background Colorectal cancer (CRC) is one of the major cancer diseases in the world. For surgical resected malignancies, the pathologic stage is widely recognized as the most accurate predictor of survival and it determines the appropriateness of adjuvant treatment as well. In the same time, molecular tumor markers are often studied in CRC using immunohistochemistry to determine their prognostic or predictive value. Protein expression is typically assigned a positive score based on a predetermined cutoff. The aim of our study was to find out the statistical correlations between different molecular markers and tumor grade and stage. Methods. We investigate histopathological and immunohistochemical 40 patients who undergone surgery for colorectal cancer. The study included 19 female and 21 males. We analyzed using ABC method for immunohistochemistry the following markers for 10 selected cases: vascular endothelial growth factor (VEGF), CD44, carcinoembrionic antigen (CEA), transforming growth factor beta (TGFβ), cytokeratin 20 (CK20), epidermal growth factor receptor (EGFR) and oncoprotein p53. Then, we analyzed statistical the results using Student-t test. Results The 40 patients with CRC presented in 6 cases perforation (15%), lymph nodes invasion (85%). 23 cases (57.5%) were moderate differentiated, 6 cases had distant metastasis, and only 9 cases were mucinous cancers. We noticed positive correlation between VEGF1 and CK20 (r=0.4, p=0.05), and between VEGF1 and CEA (r=0.88, p=0.001). Also, our results demonstrated a positive correlation between tumor grade and CEA (r=0.43, p=0.009), and no relation among the other markers. Conclusions. Our present study show no correlation between EGFR protein status and the grade and stage of CRC, but direct proportional relation in VEGF expression and CEA, and CEA and tumor grade.

PP1-87
PNEUMATOSIS INTESTINALIS IN A CASE OF SIGMOID VOLVULUS
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BACKGROUND Pneumatosis intestinalis (PI) is a rare condition characterized by the presence of multiple gas filled cysts within the bowel wall. PI might occur as a primary idiopathic disorder, but a secondary form of the disease has been described in association with a variety of underlying conditions including traumatic or mechanical disorders, inflammatory, autoimmune, infectious and pulmonary pathologies. We report a case of PI associated to sigmoid volvulus. METHOD A 70-year-old man presented with a 48-hour history of abdominal pain and constipation. Clinical examination showed generalized gaseous distention of the abdomen and tympanism without signs of peritoneal irritation. Abdominal X-ray and computed tomography showed a grossly dilated large bowel segment consistent with sigmoid volvulus. After failured of non-operative management, the sigmoid colon was resected. Pathological examination of the resected loop showed numerous subserosal and submucosal gas cysts which histologically were lined by flattened cuboidal cells, macrophages and multinucleated giant cells. RESULTS AND CONCLUSION PI is a secondary finding caused by an underlying disease. The significance and the prognosis of PI depends on the nature and severity of the underlying condition. The pathogenesis of PI has been debated for decades and multiple explanations have been suggested. The breath of pathologic conditions associated with PI formation suggests that its development is a multifaced phenomenon in which mucosal integrity, intraluminal pressure, bacterial flora and intraluminal gas have an interactive role in formation of the pneumocysts.

PP1-88
ADENOCARCINOMA (AC) AND INTRAEPITHELIAL NEOPLASIA OF THE ESOPHAGUS (EIN), BARRETT'S ESOPHAGUS (BE): MOLECULAR AND MORPHOLOGICAL MARKERS p53, ki67, CK8, CK10/13 AND APOPTOSIS
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Risk of AC development increases from 30 to 125 times in patients with BE. Thus, it is of the essence to diagnose EIN in BE at early stages with the help of molecular markers. The aim of our study was to estimate malignancy potential in BE with the help of several immunohistochemical (IHC) markers of differentiation, proliferation and apoptosis. Materials and Methods. The study was performed on 4-quadrant biopsy of the lower esophagus. The specimens were embedded in formalin and fixed in paraffin. From each specimen several slices were made and fixed on the slides. 362 slides were made and selected into 6 groups: 1 - squamous epithelium, control group, 2 - squamous epithelium in BE, 3 - gastric metaplasia (GM), 4 - intestinal metaplasia (IM), 5 - EIN in BE (1 –low grade, 4 – high grade), 6 - AC in patients with BE. Slides were treated with standard IHC with following monoclonal antibodies: P-53, Ki-67, CK10/13, CK8 (Dako

Cytomation). Apoptosis was studied with the help of Tunnel test in situ labeling (Enzo Diagnostic). Reaction results were evaluated with the help of quantative and semi quantitative methods and statistic analysis. Results. Tunnel test showed apoptosis level increasing from GM to IM and EIN, where it was maximum, and comparative decreasing in AC. P-53 expression was moderate in GM, increased in IM and EIN and maximum in AC. The study revealed expression of Ki-67 to be moderate in IM, increased in EIN and maximum in AC. CK8 - glandular differentiation marker - showed moderate expression in each epithelial type. However, CK10/13 - squamous differentiation marker - showed high expression in squamous epithelium, it was detected in GM, IM, EIN and AC as well. Conclusion. GM in BE is characterized by moderate level of apoptosis, ki-67, P-53. IM is characterized by increased apoptosis and P-53 and Ki-67 expression. EIN develops only in BE with large IM and is characterized by high levels of apoptosis, P-53 and Ki-67 and AC has maximum P-53 and Ki-67 expression and decreased level of apoptosis comparing with EIN and IM. Obtained results showed that BE is a precancer condition and its malignancy potential depends on the prevalence of apoptosis and proliferation and altered differentiation.

PP1-89

PARTICULARITIES OF ANAL CANAL CARCINOMAS COMPARED TO COLORECTAL CARCINOMAS

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Background: Carcinomas of the anal canal represent 4% of all carcinomas of the digestive tract. According to data from the literature, predominates squamous carcinoma followed by adenocarcinoma. Method: We studied 507 colorectal carcinoma (CRC) specimens, following their classical clinical and pathologic parameters (sex, age, localization, gross and microscopic aspect, stadialisation and their association with polyps) as well as immunohistochemical parameters related to prognosis and angiogenesis. We studied the anal carcinomas and compared them with the colorectal carcinomas. For the immunohistochemical assays we used antibodies provided by DAKO and LabVision, with the immunoperoxidase method. We used the following antibodies: CEA, Chromogranin A, p53, Cathepsin D, Cadherin E, oncoproteins c-erb-B2 and bcl-2, CD31, CD105 and VGEF. For statistical analysis we used GraphPad InStat 3 software. We considered the association significant when $p<0.05$. Results: From the 507 cases with CRC, 12 (2.37%) were anal canal carcinomas, 2 of these were synchronous tumours. The average age was 63.17±10.87 and in the anal canal carcinoma group was 57.9±10.05 ($p<0.0001$). Compared with CRC the anal canal carcinomas occur more frequently at the women (58%). The polypoid-ulcerated tumours predominated. In our cases the incidence of adenocarcinoma is higher (83%) then of squamous carcinomas (17%). 70% of the cases were diagnosed in the T3N0 stage. Polyps were associated in 8% of the cases, whereas in the CRC group an association of 24% was observed ($p=0.003$). The expression of CEA, chromogranin A, Cathepsin D and Cadherin E was simmilar in the two groups. Oncoproteins (c-erb-B2 and bcl-2) and p53 were observed more rarely than in CRC. Angiogenesis (marked with CD31, CD105) was expressed in all the cases, although VEGF does not correlate with the expression of the former markers. In the anal canal the incidence of synchronous tumours was higher (16.66%) than in the other segments of the colon (1.58%). Conclusions: The performed assessments have showed a higher incidence of anal canal carcinomas at women and at younger ages, the predominance of polypoid-ulcerated forms and the higher proportion of synchronous tumours, compared to CRC. Immunohistochemically we observed differences between the expression of p53, c-erb-B2, bcl-2. The angiogenesis was marked in all cases, and it seems that the role of tumour cells is not significant in this process.

PP1-90

MISMATCH REPAIR PROTEINS AND CLINICOPATHOLOGIC FACTORS IN COLORECTAL CANCER

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Mismatch repair proteins and clinicopathologic factors in colorectal cancer Background: Microsatellite instability due to defective mismatch repair proteins(MMRP) is one of the major pathways for carcinogenesis in colorectal cancer (CRC).The impact of these proteins in prognosis is not well defined.The aim of this study were the evaluation of abnormal MMRP prevalence and its relationship with some clinical and pathologic factors. Methods: In our study 350 patients with CRC were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2.Patients with at least one abnormal above factors considered in abnormal MMRP group. Clinical factors such as sex, tumor site(colon or rectum),family history of CRC and vital status(alive or dead) is considerd.Pathologic factors including grade, T and N stage in tumor specimen were examined. Results: Totally 350 patients with median age of 51(20 to 94) were evaluated. One hundred ninety five patients were male and 151 were female. The site of tumor in 270 patients was colon and in 68 were rectum. Among 329 patients with known family history, 132(40.1%) had positive FH.The overall rate of abnormal immunostaining for hMLH1, hMSH2, hMSH6 and PMS2 were 4.4%, 7.2%, 3.5%and 4.9% respectively. Overall 44 patients (12.7%) had at least one abnormal MMRP staining. Abnormal MMRP were not significantly associated with histopathologic factors including T stage, N stage and grade. There was no difference in MMRP staining as a result of whether patients were male or female except PMS2 that was significantly more abnormal in male ($p=0.083$).Abnormal staining of MMRP were seen further in colon than in rectum that was significant for hMLH1 ($p=0.044$).Patients with family history of CRC had more abnormal staining that was significant for hMSH2 ($p=0.061$). There was no difference in MMRP staining according to vital status. Conclusion: Our results suggest that abnormal MMRP is associated with clinical factors such as family history of CRC but not with pathologic factors. Abnormal MMRP is more important pathway for carcinogenesis in colon than rectal cancer.

PP1-91

PLEOMORPHIC LIPOSARCOMA OF THE STOMACH

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Background: Liposarcoma is the most common soft tissue sarcoma. It may develop anywhere, but predominantly in the retroperitoneum and the soft tissues of the thigh. Liposarcoma of the stomach is very rare and only 7 cases have been described in the English literature. Case report: A 66 years old woman with hemorrhage of the upper GI tract was admitted to hospital. From laboratory exams anemia was found (Ht:36).Radiology showed a large ecogenic neoplasm in the frontal wall of the stomach. The patient was led to surgery and had a partial gastrectomy. MACROSCOPY: In the anterior wall of the stomach a neoplasm measured up to 8cm was detected. It was elastic with solid, lipomatous and necrotic areas. It also caused a central ulceration in the mucosa. MICROSCOPY: The solid areas had the morphology of vascular spindle cell sarcoma of high malignancy with multiple mitosis. In the lipomatous areas multinucleated cells were seen and lipoblasts were detected. It was positive for Vimentin ,CD34 and S-100 and negative for a-SMA, Desmin, CD117 and HHF35. The diagnosis of pleomorphic liposarcoma was made. Discussion: Liposarcomas of the GI appear at any age with a male to female ratio of 3:1.Clinical signs and symptoms

are not specific and include an increasing growth palpable mass, anemia, hemorrhage, obstruction and intessuception or symptoms related to increased intra-abdominal pressure. They are neoplasms that rise from the adipose tissue of the submucosa and rarely from the adipose tissue of the serosa. One of the most characteristic findings is the presence of adipose cells.The main histological subtypes are: Adipocytic (lipoma like), Sclerosing, Inflammatory and Spindle cell. The terms "atypical lipoma" and "atypical lipomatous tumor" have been suggested by different authors for tumors that may reccur but are uncapable of distant metastasis. Conclusion: Gastric liposarcomas are very rare neoplasms that affect both sexes at any age. Their histology is similar to this of the soft tissue tumors and the diagnosis is based on the presence of lipoblasts. The differential diagnosis is from inflammated lipomas of the gastrointestinal tract.

PP1-92

DLK: A POTENTIAL NEW IMMUNOHISTOCHEMICAL MARKER FOR HEPATOBLASTOMA

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Background: Delta-like protein (DLK) also referred to as pG2 (human adrenal specific mRNA), Pref-1 (preadipocyte factor 1), SCP-1, ZOG, as well as the soluble product FA1 (fetal antigen 1) is encoded by the gene DLK1. It belongs to the EGF-like homeotic protein family. The function of DLK is not known but the protein is highly expressed in human and rodent fetal hepatocytes and in transit-amplifying oval cells in stem cell-mediated liver regeneration in rat. We investigated in the present study if this protein is expressed in human hepatoblastomas. Method: Paraffin blocks of 30 hepatoblastomas were selected from our archives and DLK expression was studied by standard ABC based immunohistochemistry using DAB as chromophobe. The primary antibody was purchased from R& D (cat.: AF 1144). DLK expression was also investigated in other alpha-fetoprotein producing tumors: hepatocellular carcinomas (10), germ cell tumors (6) and in undifferentiated childhood tumors: Wilms tumor (5), neuroblastoma (7), meduloblastoma (5), kidney rhabdoid tumor (1). Results: All the hepatoblastomas were positive for DLK. The staining was mostly focal covering 5-30% of the areas of tumors, the surrounding liver tissue remained negative. The reaction was present always in the epithelial component of the tumors but there was no preferential staining over the fetal or embryonic parts. The staining pattern was membranous and cytoplasmic. The differentiating cells of two ganglioneuroblastomas stained positively, no reaction was observed in any other tumors. Conclusion: DLK immunostaining seems to be a highly sensitive and specific marker for hepatoblastomas. If this primary observation can be confirmed DLK can be used as an immunohistochemical marker for hepatoblastomas. The biological function of DLK in hepatoblastomas requires further studies. However since DLK is a membrane receptor it can be considered a molecular target for therapeutical approaches.

PP1-93

HEMORRHAGIC GIST WITH NEURAL DIFFERENTIATION IN AN INFLAMED MECKEL DIVERTICULUM – CASE REPORT

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Background: The Meckel's diverticulum tumors are observed in approx. 2% of cases and about 12% of these tumors are GIST; according to Pubmed / Medline database there are 5 such tumors described in the last 5 years. Material and methods: We report a 67 years old women admitted with abdominal pain at the hospital for surgical intervention, for a bleeding tumor developed in an inflamed Meckel's diverticulum, discovered at CT scan. A six centimeters, 150 gr., yellowish – white, firmly, encapsulated tumor was removed. Standard HE stain and the immunohistochemical indirect tristadial ABC-peroxidase technique for 9 antibodies (vimentin, CD117, PDGFR, CD34, S-100, NSE, actin, Ki-67, p53) have been done. DNA extraction was performed using the Qiagen kit- QiaAmp (Austria). PCR for mutations in exons 9 and 11 of c-kit proto-oncogene, with agarose gel electrophoresis has been carried out. Results and discussions: Microscopic, a proliferation with elongated eosinophilic tumor cells with fibril cytoplasm and with nuclear atypia, growing in palisades, dissecting the muscularis propria has been noticed; no mitotic activity was recorded. IHC reactions showed CD117 focally positive, PDGFR diffusely positive, CD34 positive in scattered tumoral cells, vimentin diffusely positive, S-100 focally positive, NSE diffusely positive, actin weakly positive in scattered cells, p53 positive in ~ 5-10% of tumor cells nuclei, Ki-67 negative. PCR has not revealed mutations such as deletions or insertions bigger than 6bp in the exons 9 and 11 of c-kit gene, thus the study requires investigations for point mutations. Conclusion: We considered this case as a GIST with neural differentiation developed in a Meckel's diverticulum, being the 3rd case recorded in the last 5 years in our institution. Further molecular investigations for point mutatins in exons 9 and 11 will be performed by SSCP (Single Stranded Conformation Polymorphism).

PP1-94

GASTROINTESTINAL DYSMOTILITY IN MITOCHONDRIAL NEURO-GASTROINTESTINAL ENCEPHALOMYOPATHY IS CAUSED BY MITOCHONDRIAL DNA DEPLETION

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Objective: To investigate the pathophysiology of gastrointestinal (GI) dysmotility in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Background: MNGIE, an autosomal recessive neuromuscular disorder clinically dominated by severe GI dysmotility, is due to loss-of-function mutations in the thymidine phosphorylase (TP) gene, which induce pathologic accumulations of thymidine and deoxyuridine, and generate mtDNA defects (depletion, multiple deletions and point mutations). Methods: Using laser capture microdissection and quantitative Real Time PCR techniques, we correlated histologic features with mtDNA abnormalities in different tissue components of the GI wall in 5 patients (4 M, 1 F; mean age 32, range 34-41) with genetically defined MNGIE and 10 age-

matched controls. Results: The external longitudinal layer of muscularis propria from stomach, small intestine and colon showed marked atrophy and cytoplasmic vacuolization of smooth muscle cells. These features were more prominent in the small intestine where ultrastructural analyses revealed marked mitochondrial proliferation. In contrast, the internal layer of muscularis propria and the myenteric plexus were unremarkable. Microdissection analysis revealed marked mtDNA depletion confined to the longitudinal layer of muscularis propria from stomach, small intestine and colon. Intriguingly, analyses from ten controls showed that the longitudinal layer of muscularis propria had a lower amount of mtDNA compared to the internal layer and the myenteric plexus (1:2). Multiple deletions were detected only in skeletal muscle cells from cricopharyngeal muscle. Site-specific somatic point mutations were detected at very low abundance both in the muscle and nervous tissue of the GI tract. Conclusions: Our results showed that GI dysmotility in MNGIE is associated with marked mtDNA depletion limited to the longitudinal layer of muscularis propria of stomach, small intestine and colon. The constitutive low abundance of mtDNA at this site may significantly predispose MNGIE patients to mtDNA depletion and visceral myopathy. Multiple mtDNA deletions accumulate only in skeletal muscle cells from cricopharyngeal muscle. The selective occurrence of mtDNA deletions in the cricopharyngeal muscle suggests post-mitotic skeletal muscle cells accumulation, whereas negative selection may decrease the load of deleted mtDNA molecule during turnover of smooth muscle cells.

PP1-95
GENDER AND LIVER FIBROSIS IN CHRONIC HEPATITIS C: ROLE OF THE TGF-BETA1

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Background. It is known that during chronic hepatitis C liver fibrosis progression is faster in males than in females. Experimental data also suggest that estrogens may have an antifibrotic effect. TGF-β1 is a key cytokine that is involved in the pathogenesis of hepatic fibrosis by activating of epithelial-mesenchymal transition (EMT). In this study we investigated whether gender affects the progression of TGF-β1 dependent EMT in the liver of patients with chronic active hepatitis C. Methods. Liver biopsies performed before antiviral therapy was analyzed using METAVIR scoring system. Ten men (age range 21-46 years) and nine women (age range 31-45 years) were enrolled in the study. Immunohistochemical methods were used to analyze TGF-β1, α smooth muscle actin (SMA), pan cytokeratin, desmin and CD8 expression in liver tissue. Results. Hepatic fibrosis (stages 2-4) was identified in 18.4 % of men and 5.9 % of women. TGF-β1 and desmin could be immunolocalized in the majority of limiting plate hepatocytes. A variable pattern of TGF-β1 expression in intralobular hepatocytes (e.g. TGF-β1 positive and negative hepatocytes) was observed. TGF-β1 immunoreactivity was inversely proportional to the size of the hepatocyte. About 57.8% of TGF-β1 positive hepatocytes was found in male patients and only 42.2% in female patients. Cellular morphology of some limiting plate hepatocytes changes to a more fibroblastic appearance with desintegration of cell-cell contacts. At the same time these cells remain pan cytokeratin and desmin positive. Meanwhile there was an increase in the CD8 positive T cells and α SMA-positive myofibroblasts in the close vicinity of a limiting plate with the appearance of the piecemeal necroses. These events take place more frequently in HCV-infected men, than in women. It is likely that during the disruption of limiting plate some hepatocytes of this plate under the influence of TGF-β1 undergo EMT-like phenotypic changes with the subsequent overproduction of matrix components of fibrotic liver. Conclusion. In conclusion, our results suggest that fibrosis progression is slower in females as compared to males with hepatitis C. It may be due to estrogen action on liver cells which preserve hepatocytes from TGF- β1 overexpression.

PP1-96
EXPRESSIONS OF C-erbB-2 AND p53 PROTEINS IN GASTRIC CARCINOMA: CORRELATION WITH PROGNOSTIC PARAMETERS

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Background: Expressions of c-erbB-2 and mutant p53 proteins have been observed in some gastric carcinomas but reports of their prognostic value are conflictual. The aim of this study was to investigate c-erbB-2 and p53 protein expressions and their relation to the clinicopathological factors in gastric carcinomas. Materials and Methods: Fifty-four cases with gastric carcinomas who had undergone gastrectomy at Haseki Hospital between the years of 2005 and 2006 were reevaluated. Formalin-fixed, parafin-embedded tissue sections were analyzed immunhistochemically for the expression of c-erbB-2 and p53. Correlation between c-erbB-2, p53 expressions and histopathologic parameters was evaluated. Results: Positive membranous staining for c-erbB-2 was detected in 6 of 54 gastric carcinomas (11,1%) and no positive reaction was evident in noncancerous tissue. All of c-erbB-2 positive tumors were intestinal type according to Lauren classification. There was no statistically significant association between c-erbB-2 expression and age, sex, histopathologic type, depth of invasion, the tumor size, growth pattern, lymph node involvement, lymphatic vessel invasion or perineural invasion. p53 expression was detected in 40 of 54 gastric carcinomas (74,1%), with strong inter- and intratumor heterogenity. Statistically, no significant correlation between p53 overexpression and age, sex, the tumor size, histopathologic type, growth pattern, depth of invasion, lymphatic vessel invasion, perineural invasion or lymph node involvement. Additionally, no correlation was found between c-erbB-2 and p53 expressions. Conclusions: The results suggest that expression of c-erbB-2 protein may occur selectively in intestinal type of gastric carcinomas. However, c-erbB-2 and p53 expressions are not related to the pathological characteristics of gastric carcinomas.

PP1-97
TRANSFORMING GROWTH FACTOR-BETA RECEPTOR-1 (TGFßR-1) EXPRESSION IN LIVER INJURY AND HEPATOCELLULAR CARCINOMA

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BACKGROUND: Hepatocellular carcinoma (HCC) is a tumor with fatal outcome and resistant to therapy. Leading etiologic factors of HCC include chronic hepatitis and cirrhosis with any etiology. Hepatic stellate cells (HSC) is the key fibrogenic cell type in liver. TGFß generated by the activated HSC is the most significant fibrogenic molecule of cirrhosis. The inflammatory, portal cells and hepatocytes of liver express different kinds of TGFß receptors (TGFßRs). We planned this study to observe the expressions of TGFßR-1 in different steps of liver injury. METHOD: A study group composed of 65 viral hepatitis, 58 cirrhosis and 21 HCC cases was chosen. Immunohistochemical technique was used for TGFßR-1 antibody and the slides were scored for the intensity and the distribution of staining. The correlations between TGFßR-1 expressions and the clinical and histological features including age, sex, etiology, histologic activity index, fibrosis score, tumor diameter and differentiation, vascular invasion, multifocality and presence of cirrhosis were analyzed. RESULTS: We observed staining with TGFßR-1 in

endothelial cells of central veins, sinusoids and portal veins, portal and sinusoidal macrophages, portal fibroblasts, few bile duct epithelial cells and lymphocytes. In hepatitis group, the only significant correlation was the one between fibrosis score and the intensity of TGFßR-1 staining. The intensity was positively correlated with the increasing fibrosis score ($p<0,05$). TGFßR-1 expression was sustained in active but decreased in inactive cirrhosis. The only significant finding in HCC group was the inverse correlation between tumor diameter and TGFßR-1 expression. The expression was significant in the smaller tumors (<3 cm), whereas it is less noticeable in the greater ones (>3 cm). But it was stable over 3 cm, even if the diameter gets bigger. CONCLUSION: TGFßR-1 expression increases in inflammation in correlation with fibrosis, sustains in active cirrhosis, decreases in inactive cirrhosis and during carcinogenesis, and almost disappears in HCC which is greater than 3 cm. These results support the idea that receptor down-regulation or mutation occur in the late stages of hepatocarcinogenesis.

PP1-98

COMPARING THE CLINICAL HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROPERTIES OF 40 GASTROINTESTINAL STROMAL TUMOR CASES WITH EACH OTHER AND ACCORDING TO PROGNOSTIC FACTORS

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AIMS: Gastrointestinal stromal tumors (GIST), which are originated from intestinal pacemaker cells (interstitial cells of Cajal , ICC) , which are normally found in bowell wall or originated from the neoplastic transformation of these cells' precursors, are the most common mesenchymal tumors of the gastrointestinal tract. In this study our purpose is to evaluate GISTs morphologically and immunohistochemically and compare their features with each other and according to the GIST risk table of Fletcher et. al. (20) and find the parameters that are important in GIST diagnosis and prognosis. MATERIAL AND METHODS: 40 GIST and extragastrointestinal GIST cases , which were diagnosed in Ministry of Health Istanbul Education and Training Hospital Pathology Department between 2000-2006 were retrospectively studied. Cases were gouped acording to location of the tumor, gender and age of the patient. Paraphine blocks of the cases were sectioned again as 4mm thicknes, stained with hematoxylen-Eosin and evaluated with light microscopy according to their mitosis, necrosis, tumor number, tumor diameter, cell type, cellularity, nuclear pleomorphism, ulceration, hemorrhage, lymphocytic cell infiltration, myxoid stroma, signet ring cell, skenoid fiber, mucosal invasion, collusion tumor. Immunohistochemicaly CD117, CD34, vimentin, smooth muscle actin (SMA), S100, desmin and ki-67 were studied. RESULTS: GıST cases were equally seen in both sexes and the median age was between 5.-6. decades. They were located in stomach (18 cases), small bowell (11 cases) , large bowell (4 cases) and extra-gastrointestinal tract location (7 cases). 100% of cases were positive for CD117, 90% were positive for CD34, 100% were positive for vimentin, 50% were positive for SMA, 20% were positive for S100 and 7,5% were positive for desmin. Ki-67 ratio was found >10% only in high risk group GIST cases. CONCLUSION: GISTs were mostly located in stomach and high risk GISTs were found in extra-gastric location in high proportion. (+++) cellularity, and (+++) nuclear pleomorphism were mostly found in extragastrointestinal system location GISTs; mucosal invasion, tumor necrosis, hemorrhage, signet ring cell, myxoid stroma, ulceration were mostly found in high risk GISTs. Lymphocytic cell infiltration parameter was not different between risk groups. In 4 cases which were operated for adenocarcinoma, GIST were found insidentally and these tumors were named as collusion tumors.

PP1-99

CLINICOPATHOLOGICAL VARIABLES AND THEIR CORRELATION WITH COX-2 EXPRESSION IN GASTROINTESTINAL STROMAL TUMORS

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BACKGROUND: Tissue cyclooxygenase-2 (COX-2) plays important roles in carcinogenesis and tumor progression. Evaluation of COX-2 expression in malignincies has been performed mostly on tumors of epithelial origin and little is known about its presence in mesenchymal tumors, including gastrointestinal stromal tumors (GIST). COX-2 expression has been reported in GISTs and suggested as a potential diagnostic marker in a few articles. METHODS: We reexamined surgically resected 41 cases of GISTs in our hospital in last nine years. We evaluated COX-2 protein expression and immunohistochemical markers like CD34, CD117, desmin, smooth muscle actin and S-100 in GISTs and analyzed correlation of COX-2 expression with other clinicopathological markers. RESULTS: Mean age of the patients was 57, tumor size were between 1 and 26 cm. All cases were positive for CD117 and/or CD 34. COX-2 was expressed in 19 (%46) of all 41 tumors. Extent of expression was greater in tumors with higher mitotic index, coagulative necrosis and infiltrative pattern of growth. CONCLUSIONS: Overall COX-2 expression was not so high to make it a diagnostic tool in GISTs. Observation of greater COX-2 expression in GISTs with unfavorable histopathologic factors is against previous reports and consistent with reported roles of COX-2 in carcinogenesis and tumor progression of epithelial malignancies

PP1-100

EVALUATION OF BARRETT'S MUCOSA IN TERMS OF CK7, CK20, Ki67, p53, AND COX2 EXPRESSIONS USING CHROMOENDOSCOPIC OESOPHAGEAL BIOPSIES

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Background: Barrett's mucosa (BM) is a complication of chronic gastroesophageal reflux disease and can be diagnosed when there is an endoscopically irregular Z line and intestinal metaplasia (IM) in a lower oesophageal biopsy. It is still not clear whether IM in the gastric cardia or columnar mucosa without IM in the lower oesophagus have any significance as BM which is considered as preneoplastic. Aims: To determine the immunohistochemical features of BM and columnar mucosa in the distal oesophagus and also to evaluate the value of chromoendoscopy in the diagnosis of BM in a prospective manner. Material & Method: A total of 18 chromoendoscopic biopsies (6 from normal looking unstained oesophagus, 6 from oesophageal mucosa stained with methyl blue-suspicious of BM, 2 from fundic mucosa, 2 from corpus mucosa and 2 from antral mucosa) were taken from 111 cases who underwent endoscopy because of a variety of upper gastrointestinal symptoms. Immunohistochemical analysis was performed using CK7, CK20, p53, Ki67, and COX2. Results: Of the 111 cases 19 cases with carcinoma (9 adeno, 5 squamous, 4 undifferentiated carcinomas) and 17 cases with normal squamous epithelium were excluded while 75 cases showing columnar epithelium including 46 (% 61.3) with IM and 29 (%38,7) without IM were further evaluated immunohistochemically. Chromoendoscopy yielded a sensitivity and specificity of 76% and 69%, respectively, while conventional endoscopy had a sensitivity and specificity of 54% and 77%, respectively, for the detection of BM. CK7 was observed in surface, crypt, and glandular epithelium whereas CK20 was expressed in surface and superficial crypt epithelium. No

significant difference was observed between the Barrett and non-Barrett type of CK7/20 staining pattern (p>0,05). Expression of p53 did not show any difference between BM and columnar mucosa without IM whereas Cox2 expression was significantly increased in BM (p<0.05) in comparison to columnar mucosa without IM. Ki67 expression was significiantly higher both in upper and lower crypts in BM (p<0.05). No significant difference was observed for the presence of gastric IM and H. pylori infection between BM (47.8%, 4.3%, respectively) and non-BM (24.1%, 13.8%, respectively). Conclusions: The present study showed that a Barrett pattern does not seem to exist however, there seems to be a CK pattern for the lower oesophageal mucosa different form gastric IM. Expression of Ki67 in the upper half of the crypts together with COX2 seems to be additional features of BM.

PP1-101

CLINICOPATHOLOGIC FEATURES OF TUBERCULOUS COLITIS DIAGNOSED IN ENDOSCOPIC BIOPSIES

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Background: Gastrointestinal tuberculosis (TB) currently represents an uncommon disease in Western world; however it remains as an important cause of morbidity and mortality in developing countries and immunodeficiency patients. As the diagnosis in endoscopic biopsies is often very difficult, the aim of this study is to pay attention to the possibility of TB colitis and to avoid more invasive diagnostic procedures. Design: Clinical manifestations, pathological findings and diagnostic methods in endoscopically biopsied colonic tuberculosis patients were evaluated. Gastroenterology archives of the last five years were reviewed to find cases with tuberculous colitis. 10 cases which were diagnosed as tuberculosis according to clinical and microbiologic findings were found and their colonoscopic biopsy materials were reexamined in Pathology Department. Clinical and histopathologic features of these cases were analyzed and compared with the results of other diagnostic methods. Results: All of the 10 cases had chronic diarrhea and investigated by colonoscopy and microbiologic analysis to differentiate especially inflammatory bowel disease and infectious colitis. Ulcers and erosions were common endoscopic findings. In the colonoscopic biopsies, granulomas were observed in colonic mucosa or submucosa of all cases, with suppuration of granulomas in 9, caseous necrosis in 4, and ulceration in 8 cases. Ziehl-Neelsen (ZN) stain revealed presence of acid fast bacilli in 5 cases however tissue culture and PCR studies for mycobacterium were found positive in all cases. Conclusion: The results of this study demonstrated that in colonoscopic biopsies of tuberculous patients, granulomatous colitis is the common diagnosis mostly with suppuration and ulceration; however EZN stain is less sensitive than PCR results.

PP1-102

TARGETED THERAPY WITH TRASTUZUMAB IN BARRETT'S DYSPLASIA AND ADENOCARCINOMA: A TRANSLATIONAL APPROACH

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Background: HER2/neu protooncogene results to be overexpressed/amplified in preneoplastic lesions and in adenocarcinoma (ADC) of the esophagus. Thus, HER2 could be considered a target for both chemoprevention and treatment of esophagus dysplasia/ADC. Our aim is to evaluate the therapeutic role of the anti-HER2 monoclonal antibody, trastuzumab, in the management of ADC derived from HER2-positive Barrett's esophagus (BE). Methods: We selected two patients after esophageal mucosectomy with a histological diagnosis of HGD and ADC of the esophagus without distant metastases. Patients were considered not eligible for radical surgery or chemo-radiotherapy because of age and comorbidities. The treatment was administered after ethical institutional approvation and patients' written informed consent was obtained, and consisted of four cycles of monotherapy with trastuzumab every 3 weeks. Patient #1 received four cycles at a dose of 6 mg/kg, and patient #2 received trastuzumab at a loading dose of 4 mg/kg and 2 mg/kg as maintenance dose. The different doses were chosen on the basis of cardio-vascular conditions of the patients. Trastuzumab was administered as an iv infusion according to the manifacturer's instructions. HER2 status was studied by IHC and FISH. Additional immunohistochemical analyses were performed. The whole panel was analysed at baseline, after treatment and then at scheduled follow-up controls. Results: At baseline, the two patients showed HER-2 overexpression/amplification in all areas of dysplasia and ADC but not in BE. Trastuzumab treatment was well tolerated without any acute or delayed toxicity. Early evaluation after treatment revealed no significant differences in terms of endoscopical and immunohistochemical patterns of the disease. However, in the patient who received the higher doses of trastuzumab, a downregulation of HER2 overexpression and an increase of the apoptotic index was observed six months after treatment. Conclusion: This study demonstrates that this treatment is feasible. No clear evidence of dysplasia regression was observed. However, the observation that HER2 downregulation and induction of apoptosis occurred 6 months after treatment, suggests that a dose-intensified regimen with trastuzumab alone should be investigated in a phase I-II dose-finding study.

PP1-103

EXPRESSION OF BONE MORPHOGENETIC PROTEINS AND THEIR RECEPTORS IN GASTRIC ADENOCARCINOMAS AND THEIR NODAL METASTASES

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Background: Bone morphogenetic proteins (BMPs) are multifunctional cytokines, which belong to the transforming growth factor-β (TGF-β) superfamily. So far, more than 20 mammalian BMPs have been identified and they are divided into several subtypes based on their structure. BMPs play an important role in the differentiation, proliferation, apoptosis and survival of different cell types. They are also involved in a variety of biological effects which are related to homeostasis and chemotaxis, like the migration of cells, or their activation in emergency situations. There is evidence that the BMPs are implicated in several aspects of embryonic development, from the establishment of the basic embryonic body plan to the morphogenesis of several organs, but also in the regular functions of normal cells during adulthood. Recent studies have demonstrated the involvement of BMPs and their receptors in the development of a variety of pathologic conditions, including several human tumors, such as carcinomas. Method: In this study, we have investigated the expression of BMPs and their receptors in gastric adenocarcinomas (12 gastric adenocarcinomas were of the diffuse, and 17 of the intestinal type). Immunohistochemical stains were performed by a standard streptavidin-biotin method

and we used commercially available goat polyclonal antibodies for BMPs (-2, -4, -6 and -7) and for their receptors (BMPRIa, BMPRIb). Results: BMPs and their receptors were expressed both in diffuse and in the intestinal type of gastric adenocarcinomas. BMP-6 was expressed in larger quantities in the intestinal type of gastric adenocarcinoma than in the diffuse type. In addition, there were statistically significant differences in the expression of BMP-6 between the neoplastic cells of the primary tumors and those of the nodal metastases of gastric adenocarcinomas.

PP1-104
HISTOLOGICAL EVALUATION OF LIVER OF CHILDREN WITH AUTOIMMUNE HEPATITIS AT INITIAL DIAGNOSIS AND FOLLOW-UP MANAGEMENT BIOPSY

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Autoimmune hepatitis (AIH) is a chronic liver disease characterized histologically by interface hepatitis, portal plasmalymphocytic infiltrate and parenchymal inflammation. Hepatic fibrosis and cirrhosis occur in many types of chronic liver injury including AIH. Recent studies have reported that hepatic fibrosis and cirrhosis may be reversible in some patients with AIH that respond to treatment. Therefore the objectives of this study were to compare between histological assessment of liver tissues of children with AIH at initial diagnosis and follow-up management biopsies regarding histological activity index (HAI), nature of inflammatory infiltrate and the stage of liver fibrosis. Nineteen patients with AIH who responded to medical therapy and had follow-up liver biopsy were included in this study. Paraffin embedded liver tissues were used for assessment of histological activity and stage of fibrosis according to Ishak et al (1996). Results revealed that the median HAI decreased from 11.8 to 3.2 ($p=0.003$) and the median fibrosis score decreased from 3.6 to 1.9 ($p=0.09$). The nature of the inflammatory infiltrate was predominantly plasma cell with few eosinophils at initial diagnosis biopsy whereas it was predominantly eosinophils with occasional plasma cells at follow-up biopsies. A significant number of biopsies at initial diagnosis revealed sclerosing cholangitis that was not present at follow-up biopsies. Data from this study demonstrate a significant improvement in follow-up management liver biopsies from initial diagnosis biopsies, in patients who responded to medical treatment, regarding histological activity and stage of fibrosis.

PP1-105
RETINOL BINDING PROTEIN 4 (RBP4) EXPRESSION IN NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Background: RBP4 is a novel adipokine mediating systemic insulin resistance. Insulin resistance plays a central role in the pathogenesis of NAFLD. We aimed to evaluate for the first time RBP4 liver tissue expression in NAFLD. Method: We used immunohistochemistry with a polyclonal antiserum to RBP4 (Phoenix Pharmaceuticals, Belmont, CA) on needle liver biopsies from 30 NAFLD patients (mean age 51 y, range 18-76, M/F: 3/2, mean BMI 29 range 22.1-40.9, normal oral glucose tolerance test-OGTT 17, impaired OGTT 13, mean serum ALT 80 IU/L range 22-308, simple steatosis 13, NASH 17: global grade 1 n=7, 2 n=8, 3 n=2, fibrosis stage 1 n=10, 2 n=4, 3 n=1, 4 n=2) and on normal liver tissue from 10 adults. NAFLD biopsies were assessed histologically according to Brunt et al, 1999 and Kleiner et al, 2005. RBP4 immunohistochemical expression was semiquantitatively evaluated (0-3) in each acinar zone and a summative RBP4 total score (RBP4 score, range 0-9) was issued for each case. Results: Anti-RBP4 produced mild cytoplasmic hepatocellular immunostaining, which in normal liver was mostly evident in zone 3 (RBP4 score range 1-3). In most NAFLD cases, RBP4 immunopositivity was more intense in zone 3 and milder in zones 2 and 1 or periseptal areas (RBP4 score 4-8). Inverse or no zonation was observed in high grade or high stage NASH. RBP4 score was significantly higher in NAFLD compared to normal liver and was correlated with male sex ($p<0.01$) and grade of steatosis ($p<0.01$). In the subgroup of NASH, RBP4 score was positively correlated with grade of activity ($p<0.01$) and stage of fibrosis ($p<0.05$). There was no correlation of RBP4 score with age, serum ALT, BMI or OGTT. Conclusions: In normal human liver, RBP4 is mildly expressed by zone 3 hepatocytes. In NAFLD, zonal RBP4 hepatocellular expression is increased. In NASH, elevated RBP4 expression is related to increased disease severity and high fibrosis stage.

PP1-106
CLINICOPATHOLOGICAL STUDY ON CHOLANGIOLOCELLULAR CARCINOMA SUGGESTING HEPATIC PROGENITOR CELL ORIGIN

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BACKGROUND AND AIMS: Cholangiolocellular carcinoma (CLC), a subtype of cholangiocarcinoma (CC), is thought to originate from Hering's canal, where hepatic progenitor cells (HPCs) are located. Since HPCs can differentiate into both cholangiocellular and hepatocellular direction, we studied these phenotypes in CLC. MATERIALS AND METHODS: We clinicopathologically studied 30 CLC cases. Immunohistochemical analysis was performed using hepatocytic markers (Hepar, canalicular polyclonal carcinoembryonic antigen (pCEA), CD10), biliary/hepatic progenitor cell markers (cytokeratin (CK) 7, CK19 and Neural cell adhesion molecule (NCAM)) and the ATP binding cassette (ABC) superfamily of membrane transporters, (MDR1, MRP1, MRP3, and BCRP). RESULTS: 29/30 (96.7%) CLC were associated with chronic hepatitis and/or cirrhosis. Gross findings were similar to CC: whitish color, solid, not encapsulated. CLCs formed small monotonous anastomosing glands and cords and sometimes small trabeculae with abundant fibrous stroma. The tumor cells varied from small cuboidal in the glandular areas to larger, with larger eosinophilic cytoplasm in the trabeculae areas. At the tumour boundary, all cases had replacing growth pattern like HCC. CLC, HCC-like and CC areas coexisted in various degrees in the tumors and these three different histological pattern showed transitional formation with each other. The tumor mainly consisted of CLC area, and the HCC like and CC like-areas occupied less than 10% of tumour area. On CK7 and CK 19 stains, surrounding liver showed HPCs activation. In the glandular areas tumour cells were reactive for CK19 and CK7 in a strong cytoplasmic pattern, 19/30 (63.3%) cases had focal mucin production and pCEA showed cytoplasmic reactivity. 100% of tumours showed hepatocellular differentiation in the trabecular area, characterized by submembranous CK7 expression and canalicular staining patterns for CD10 and pCEA. In CLC area, 26/30 cases (86.7%) were positive for NCAM. MDR1 showed a diffuse apical staining pattern and the expression IN CLC areas was up-regulated in comparison with

surrounding normal cholangiocytes. MRP1, MRP3 and BCRP showed a diffuse basolateral staining pattern. CONCLUSIONS: 100% of CLC show hepatocellular differentiation in part of the tumour and more than 80% of cases showed expression of HPCs markers. These findings highly suggests a progenitor cell origin of this subtype of CC. The MRP1, MRP3, MDR1 and BCRP up-regulation in CLC area is similar to what is seen in non-tumoral HPCs and provides these cells with a multidrug resistant phenotype.

PP1-107
MEDULLARY CARCINOMA OF THE STOMACH . CASE REPORT WITH IMMUNOPHENOTYPE COMPARED TO CONVENTIONAL TYPES

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Medullary carcinoma or carcinoma with lymphoid stroma is an unusual gastric neoplasm, subclassified as a separate entity and consisting of 3.5-4% of gastric carcinomas, according to the literature. We report the case of a 79 year old woman, who presented with dyspepsia and anaemia. CT examination revealed a polypoid mass measuring 5x6 cm, at the greater curve of the gastric body and ulcerated according to endoscopy. Cytological examination of imprints was suggestive of carcinoma with a heavy inflammatory component. Biopsy material consisted of necrobiotic and malignant neoplastic tissue. The cells of the latter where of medium or large size, with scanty cytoplasm and hyperchromatic nuclei, showing slightly increased mitotic activity. They formed irregular, mostly small nests or lined pseudoglandular and slit-like spaces. Immunohistochemistry substantiated the epithelial nature of the neoplasm (strongly positive for pankeratin and focal positivity for CEA). The stroma was desmoplastic and highly vascularized, with an extensive lymphocytic infiltration mainly composed of T-cells (CD3+). Histological diagnosis was strongly suggestive of medullary carcinoma and the patient underwent surgery (partial gastrectomy). Microscopical findings of the surgical specimen were similar to bioptic ones. It was a moderately differentiated carcinoma with lymphoid stroma and early infiltration of the muscularis mucosa (less than 1/3), while lymph node metastases were not detected. The special stain for Helicobacter pylori was negative.The immunophenotype of the carcinoma was: pankeratin (+), CEA (+focally), CA19-9 (+), CA15-3 (+), CK8 (+), CK18(+), CK19(+), CK4,7,10/13,14,20(-), and the majority of the T-population were CD8+. The patient received no adjuvant chemotherapy. She presented with reccurrence and fatal haemorrhage six months later.

PP1-108
ALTERED EXPRESSION OF E-CADHERIN IN PRECANCEROUS LESIONS AND GASTRIC CANCER CAN SERVE AS A DIAGNOSTIC MARKER

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BACKGROUND: E-cadherin is important for cell-cell adhesion of epithelial cells. Decreased expression has been observed in gastric cancer by immunohistochemical method. Studies also along the Correa's cascade showed that the membranous pattern alters gradually in normal gastric epithelium, in chronic atrophic gastritis, intestinal metaplasia, and dysplasia and eventually in adenocarcinoma. AIM: We study the expression of E-cadherin in gastric Dysplasia/Intraepithelial Neoplasia (IEN), indefinite dysplasia, Reactive hyperplasia and Gastric Cancer to search for a marker which could be used in guiding the pathological diagnosis of these lesions. METHODS: The E-cadherin expression (DAKO: 1/10) evaluated in gastric tissues of 52 patients (34 men and 18 women), by immunochemistry with a standard Envision technique in formalin-fixed and paraffin-embedded specimens. E-cadherin expression was evaluated as Membranous, Cytoplasmic, Cytoplasmic/Membranous in samples from 18 Indefinite dysplasia/Reactive hyperplasia (Vienna Group 2) (34, 6%), 17 gastric Dysplasia/IEN (Vienna Group 3 & 4) (32, 7%) and 17 Gastric Cancers (Vienna Group 5) (32, 7%) In addition Pearson's Chi-square test with continuity correction and t-test was used to investigate the correlation of E-cadherin expression type with Vienna groups (G2, G3/4, and G5) RESULTS: The membranous type identified in 16 cases: 14 RH/IDys(G 2) and 2 cases of IEN (G 3/ 4). Cytoplasmic / membranous type diagnosed in 12 cases: 4 RH/IDys(G2), 7 IEN(G3/4) and 1 GC.(G5).Cytoplasmic staining was found in 24 cases: 16 GC(G5) and 8 IEN(G3/4) In G2 membranous type was found in 14(77.8%) cytoplasmic/membranous in 4 In G3/4 membranous type was found in 2 (11.7%), cytoplasmic / membranous in 7(41.1%) and cytoplasmic in 8 (47.05) .In G5 cytoplasmic staining was found in 16 (94.1%) and cytoplasmic / membranous in 1 Normal cytoplasmic staining decreased gradually between Vienna Groups (G2: 77, 8%, G3/4:11, 7%, G5:0%) Abnormal cytoplasmic expression of E-cadherin increased gradually between Vienna Groups (G2:0%, G3 /G4:47.05%, G5:94.1%) had a significant difference, ($p<0.05$) and ($p<0.01$) CONCLUSION: There is altered expression of E-cadherin in gastric IEN and gastric carcinoma. Cytoplasmic expression of E-cadherin can be used as a marker to differentiate gastric IEN from reactive epithelial lesions.

PP1-109
THE RELATIONS BETWEEN CLINICOPATHOLOGIC/PROGNOSTIC FACTORS AND CD44S EXPRESSIONS OF OESOPHAGEAL SQUAMOUS CELL CARCINOMA

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AIM: CD44 is an adhesion molecule which is a member of the cell adhesion molecules family hyaladherins. CD44 has some effects including tumor-endothel interaction, cell motility and migration, cell adhesion and tumor invasion, tumor progression, and metastasing. In this study, we aimed to evaluate the relationship between survival rates and CD44 expressions in patients with ESCC. MATERIAL AND METHODS: Between 1999 to 2004, pathologic specimens belonging to 35 patients were examined by the Yuzuncu Yil University (YYU) Medical Faculty's Department of Pathology. In addition, clinical and laboratory findings were collected from medical records. The relations between clinical, laboratuary, histopathologic findings and survival rate, prognosis, and the status of CD44s expressions were examined. RESULTS: 13 patients were well-differentiated, 18 were moderately differentiated, and 4 were poorly differentiated. Inflamatory reactions were observed in 23 cases. The ratios of stained CD44 were positive in 32 patients and negative in 3 patients. The concentration of stained CD44 was positive in 30 and negative in 5 patients. The median survival periods were 5.2 months and the one year and two year survival rates were %34.4 and %11.3, respectively. We did not detect any correlation between CD44 expressions to survival and prognosis. The treatment options, response to chemotherapy, clinical stage and tumor size influenced the survival outcome in univariant analysis. In contrast, only tumor size at time of diagnosis was significant in multivariant analysis. ($P=0.045$). CONCLUSION: Tumor size at the time of diagnosis was found to be a valuable

factor on the survival and evaluation of the prognosis. CD44 expressions and its variants were absent as prognostic factors. We concluded that CD44 may act as a valuable parameter on metastatic and local advanced tumour cases because most of our patients have positive CD44 expressions.

PP1-110
ENDOSCOPIC ULTRASOUND GUIDED BIOPSY IN ROUTINE-DIAGNOSTICS

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Background: Endosonography will be used more and more as a diagnostic tool for solid tumors of the upper gastrointestinal tract. Among a random sample of patients the efficiency of the method according to gained tissue samples for histological and cytological examination and their clinical validity was examined prospektive. So far little data is available on the efficiency of endosonographical punctions outside specialized centres. There are no standards for the processing of endosonographically gained tissue samples. There is only little data available on the gained tissue samples for histological serial sections with the 22-G-needle at endosonographical punctions. Patients and methods: On 40 patients (median 67 years, 17 female) endosonographical punctions of the upper gastro-intestinal-system (22 mediastine, 7 esophagus- and stomach-wall, 6 pancreas and 5 retroperitoneum) were conducted with a longitudinal ultrasoundendoscope (HITACHI EG-3830UT) and a 22-G-needle (Sono Tip II, HITACHI Medical Systems GmbH). The acquired material was completely fixed in formaline 4% and only in the pathology separated for histology (embedded in paraffine for a minimum of 8 serial sections and the zytology (cell-block-cytology using the cyto-zentrifuge at 1500 rpm (rounds per minute) universal16A zentrifuge). The reference procedures to assess the clinical validity were an independent histological diagnosis through surgery or biopsy (n=10) or the results of other clinical imaging procedures and further clinical observations. Results: With 24 out of 40 patients a histological examination of the tissue sample could be conducted (17 as immuno-histochemical test) and in 21 cases a histological diagnosis was stated. Among the remaining 16 cases a diagnosis was found via cytological examination for 14 patients. Using histological and cytological examination the sensitivity was 78%, the specificity was 100%, the accuracy was 82% and a positive predictive value of 100% and a negative predictive value of 50% was reached. Conclusion: The embedding of the whole tissue sample acquired through endosonographical punction in formaline simplifies its postinterventional processing and allows both histological and cytological testing. Using both histological and cytological examinations on each tissue sample probably guarantees the most accurate diagnosis.

PP1-111
THE EFFECT OF MMP-26 GENE EXPRESSION IN TUMORIGENESIS AND INVASIVENESS OF GASTRIC CARCINOMA

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Background: The matrix metalloproteinases (MMPs) contribute to the invasive and metastatic abilities of a variety of malignant tumors. Among these MMPs, MMP-26 has been described to take role in invasion and metastasis of cancers of epithelial origin including choriocarcinoma, breast and prostate carcinoma. Moreover, MMP-26 gene, as well as MMP-7 gene, is a target of Wnt-signaling pathway, being regulated by β-catenin. There is a striking relation between Wnt pathway and gastric carcinogenesis. Alterations of genes in this pathway, such as mutations of β-catenin and E-cadherin, have been illustrated in gastric carcinoma cases, indicating deregulation of this pathway during gastric carcinogenesis. Design: In this study, the involvement of MMP-26 gene in invasion and metastasis of gastric tumors was analyzed, and the roles of MMP-7 and β-catenin were also studied. Twenty gastric tumor samples were studied. The expression levels of MMP-26, MMP-7, and β-catenin were investigated by semiquantitative RT-PCR. Results: MMP-26 expression was observed neither in normal nor in tumor biopsy specimens. MMP-7 expression was seen in almost half of the gastric tumor samples. β-catenin expression was detected in some tumor tissues as well as in some normal tissues. No biopsy specimen showed MMP-26 expression. MMP-7 expression was observed in 9 of 20 gastric tumor biopsies. β-catenin expression was detected in both normal and tumor tissues. 9 out of 20 samples showed β-catenin expression in tumor biopsies. Among those only 3 samples expressed only β-catenin while the rest had expression in also normal biopsy samples. Among 9 β-catenin positive samples, 3 also had MMP-7 expression. Conclusion: MMP-26 was not expressed in gastric carcinomas, however MMP-7 was positive. Our reults are preliminary and this project is continuing until significant results will be found. This project is supported by the Scientific Research Fund of Fatih University under the project number P50030503 and by TUBITAK under the project number 105S352 (SBAG-K-110).

PP1-112
SINONASAL LEIOMYOMA. REPORT OF A NEW CASE.

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Most of the sinonasal tumours are of epithelial origin. Leiomyomas are benign smooth-muscle tumours that are common in the alimentary tract, uterus, skin and subcutaneous tissue. They are very uncommon in the upper respiratory tract and extremely rare in the nasal cavity and paranasal sinuses. They account for less than 2.5% of the mesenchymal neoplasms of the sinonasal tract and the nasopharynx. To the best of our knowledge, only 23 cases of primary leiomyogenic tumours have been reported in the sinonasal tract with almost an equal frequency of benign and malignant types. We report a case of 48-year-old woman presenting a leiomyoma of the sinonasal cavity. The clinical and pathological characteristics of this tumour will be discussed.

PP1-113
SIGNET-RING CELL CARCINOMA OF THE SMALL INTESTINE AROUND CROHN DISEASE : ANALYSE OF BRAF MUTATION

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It has been recently identified a clinically distinct subgroup of colon adenocarcinomas having high incidence of BRAF mutation. We have observed a signet-ring cell carcinoma of the small intestine in an old patient with long- standing chronic inflammatory disease. The aim of this case report is to emphasize the importance of the BRAF gene mutation in the carcinogenesis

occurring in chronic inflammatory disease. BRAF is a member of the MAP kinase pathway, which mediates cellular response to growth signals. T1796A transversion mutation of BRAF gene identified in melanomas and colon carcinomas causes missense coding of glutamic acid rather than valin amino acid at position 599. BRAF mutations appear to be a later event in ulcerative colitis-related carcinogenesis and do not occur in corresponding nondysplastic mucosa of patients with UC-related cancer. In our case, BRAF V599E mutation has been investigated by the amplification of DNA segment including 1796 nucleotide region with polymerase chain reaction and direct DNA sequencing. We did not detect any mutation of BRAF gene neither in tumoral lesion nor in the adjacent inflammatory mucosa. Studies are underway to analyze BRAF mutations including G-to-T transversion at nucleotide 1382, T-to-G transversion at nucleotide 1385, and G-to-A transition at nucleotide 1388.

PP1-114
TUMOR-ASSOCIATED TISSUE EOSINOPHILIA IN COLORECTAL ADENOCARCINOMAS

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Background Tumor-associated tissue eosinophilia has been described in many sites, including colorectal region. The exact role of tumor-associated tissue eosinophilia in tumors has not yet been defined, and its presence has been related to a good and to a poor prognosis or even having no influence on patients outcome. The aim of this study was to examine the presence of tumor-associated tissue eosinophilia in colorectal carcinomas and their relationship with clinicopathologic parameters such as tumor size, histological grade, histological stage and age of the patients. Material and Methods 132 patients (78 males and 54 females) with colorectal carcinomas who have been treated surgically were examined retrospectively. The tumor specimens have been examined for tumor-associated tissue eosinophilia and reviewed by two pathologists. The carcinomas were 62 well differentiated, 48 moderate differentiated and 22 poorly differentiated neoplasms. According to the modified Dukes classification the carcinomas were A:13 , B:54, C:35, D:30 respectively. The diameter of the tumors was 1,1 to 6 cm (mean size 3,2 cm). The age of the patients ranged from 32 to 80 years (mean age 58 years). Eosinophil infiltration was classified as “marked”, “moderate” and “mild” according to density of the stromal infiltrate. These indicate eosinophil infiltration over 200 in a high power field (hpf) (x400), that ranged from 100 to 200, or with less than 100 eosinophils, respectively. Results Marked to moderate eosinophilic infiltration was found in 51 (82,2%) of poorly differentiated, 30 (62,5%) moderately differentiated and 13 (59%) of well differantiated adenocarcinomas. Marked to moderate eosinophilic infiltration was observed in 4 cases (30,7%) of adenocarcinomas stage A, in 23 cases (42,5%) stage B, in 25 (71,4%) stage C and 28 (93,3%) stage D. There was no correlation between marked to moderate eosinophilic infiltration and size of the tumors. An association of marked to moderate eosinophilic infiltration with advanced age was noticed. Conclusions Our data have shown that high rates of tumor-associated tissue eosinophilia could represent a favourable prognostic factor in colorectal adenocarcinomas. These findings could help to elaborate a therapeutic strategy in specific subgroups of patients.

PP1-115
TH1/TH2 SERUM CYTOKINES IMBALANCE AND THEIR RELEVANCE TO SEVERITY OF DISEASE IN HCV- RELATED LIVER CIRRHOSIS

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Objective: Hepatitis C virus is a RNA virus responsible for the majority of post transfusion non-A, non-B hepatitis worldwide. T lymphocytes and immunoregulatory cytokines may be important in the host response to hepatitis C virus (HCV) infection. T helper type one (Th1) cytokines (interleukin [IL]-2, interferon gamma [IFN-γ] and tumor necrosing factor alpha [TNF-α]) are required for host antiviral immune responses, including cytotoxic T-cell generation and natural killer cell activation, while T-helper type 2 [Th2] cytokines (IL-4, IL-6 and IL-10) can inhibit the development of these effector mechanisms. Cytokines’ pattern was suggested to play an important role in the development of liver fibrosis and cirrhotic transformation in chronic viral infection. Aim of this study is to clarify the relationship between the balance of Th1 and Th2 cytokines production and liver damage in HCV infection. Materials and methods: The study was conducted on 62 patients with chronic HCV infection and 20 apparently healthy subjects as a control group. Patients were classified according to histopathological examination and staging of liver fibrosis into three groups [S2 (22 patients), S3 (18 patients) and S4 (22 patients)]. Circulating Th1 and Th2 cytokines were measured by enzyme linked immunoassay (ELISA) in all subjects. Collagen I and III reflecting stage of liver fibrosis, were evaluated by using indirect immuno-histochemical technique in all patients. Results: Serum Th1 cytokines (TNF-a, IL-2, IFN-g) are significantly elevated in all patient groups versus control group. Regarding IL2 level, it shows non significant changes in all studied groups. Levels of serum Th2 cytokines are dramatically elevated in all patient groups compared to normal group. Serum cytokine levels are correlated directly with progression of hepatic fibrosis stage and parameters of liver injury (ALT & bilirubin levels) but inversely with hepatic synthetic parameters (Prothrombin concentration & albumin level). Conclusion: A continued balance between Th1 and Th2 cytokine levels must be critical for normal immunoregulation. Consequently, further extensive studies on the imbalance of the cytokines network are required to improve the therapeutical approach in patients with chronic HCV infection.

PP1-116
STUDY OF DNA PATTERN CHANGES UNDER THE USE OF 5% ETHANOL AMINE OLEATE AND HISTOACRYL DURING ERADICATION OF ESOPHAGEAL AND GASTRIC VARICES

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Esophageal varices are one the most health emergencies in Egypt. Although bleeding from gastric varies is not as common as esophageal varices, it is always a serious and difficult problem. Aim of the work : to evaluate the use of the hypertonic glucose water as a sclerosing material in esophageal, esophageogastric and gastric varices with studying the DNA pattern of the mucosa , and its effect on the outcome of sclerotherapy and the proliferating index present in correlation with S phase fraction of cell cycle. Material and Methods: This study was carried out on 75 patients , they were grouped into:Group I: esophageal varices :Subgroup 20 patients treated with injection of glucose 50%.Subgroup IB: 20 patients treated with injection of Ethanol amine oliate. Group II: Fundal varices:Subgroup IIA: 10 patients treated with injection of glucose 50%.Subgroup IIB: 10 patients treated with injection of Histoacryle. Group III: 15 patients are control. Endoscopic biopsies taken. Sections were stained by

haematoxyline and eosin for routine examination, feulgen stain for DNA evaluation using Cell Image analyser and indirect imuonohistocheistry for proliferating cell nuclear (PCN). Results: Esophageal varices cases, the S phase fraction showed significant increase in both subgroup IA and IB compared to control group ($p<0.01$) on the expense of cells at 2C. PCNA nuclei increased in both subgroups IA+IB and subgroups IIA+IIB compared to control cases $p<0.01$. In esophageal varieces patients injected by 50% glucose (subgroup IA), showed the majority of histogram are diploid histogram with marked increase in S phase (60%), while patients treated by injection of ethanolamine oliate, elaborated aneuploid histogram in (30%). In fundal varieces cases treated by injection of 50% glucose elaborated aneuploid histogram in 50% of the cases, while patients treated by injection of histoacryle, elaborated aneuploid histogram in 70% of cases. Conclusion: Glucose 50% and ethanolamine oliate were coparable to each other as regard the cessation of bleeding but Glucose 50%had less hazardous effect on the mucosa of the esophagus as regards histopathological study, did not cause any marked lesions of the mucosa whether acute or chronic and had lesser damage on the DNA content of mucosal cells than ethanolamine oliate as regards the cell image analysis.

PP1-117

DNA PLOIDY AND P53 OVEREXPRESSION IN PAPILLA OF VATER IN CASES WITH OBSTRUCTIVE JAUNDICE: IMMUNOHISTOCHEMICAL STUDY

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Carcinoma of the papilla of Vater has a relatively good prognosis when compared with the case of other biliary tract neoplasm such as pancreatic carcinoma or bile duct carcinoma. Our aim is to evaluate the clinicopathologic features and the alteration of the p53 tumor suppressor gene using p53 and the DNA ploidy of papilla of Vater epithelium. Patients and methods: The study included 70 patients had obstructive jaundice due to papillary lesions. The patient's were 40 adenocarcinoma, 10 cases pure adenoma of the papilla of Vater and 15 chronic papillitis as well as 5 patients had normal papilla as a control. We used imunohistochemistry technique for detection of p53 suppressor gene and Cell image analyzer to detect the DNA content of the epithelium through staining the tissue by feulgen stain. Results: The patients were 34 to 76 years, 42 male and 28 female. The papillary adenocarcinoma was well differentiated in 10 cases, moderately differentiated in 22 cases, and poorly differentiated in 8 cases. Positive staining for p53 protein was found only in the nuclei of tumor cells .Positive reaction for p53 were 0% on papillitis and in normal mucosal tissue , 20%(2/10) in adenomas , 50% (20/40) in adenocarcinoma. The percentage of p53 overexpression in adenocarcinoma was 48% (7/15) for early carcinomas and 51.8% (13/25) for advanced carcinomas. All cases of papillitis and adenoma exhibit DNA diploid histogram, while 16/40 (40%) of adenocarcinoma cases exhibit diploid histogram and the rest of cases 60% (24/40) exhibit aneuploid histogram. There was increase in the number of cells at the proliferative S phase in the chronic papillitis, adenoma and adenocarcinoma compared to control cases $p<0.01$and $p<0.05$ respectively.In conclusion; The p53 may be useful in the diagnosis of carcinoma of the ampulla of Vater.The DNA ploidy can help in diagnosis and outcome of papillary neoplasms. Our results confirm that this group of tumors are heterogeneous and underline the need for earlier markers of an aggressive behavior.

PP1-118

THE ROLE OF EPIDERMAL AND TRANSFORMATING GROWTH FACTOR β IN GENESIS OF CHRONIC H.PYLORI - ASSOCIATED GASTRITIS IN CHILDREN

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Aim – to study the role of epidermal growth factor (EGF) and transformating grow factor-β (TGF-β) in the development of chronic HP-associated gastritis in children. Material and methods. The gastrobioptats of 45 children of school age with chronic HP-associated gastritis were examined. Comparing group gastrobioptats of 15 pupils with functional dyspepsy. To assess the proliferative stomach epithelium activity, intensiveness of expression of marker Ki-67 were counted, calculating the proliferative index (PI). Primary antibodies to EGF and TGF-β ("Novocastra", UK) were used. Morphologic researches were made with the help of computer system of colour picture analyses "Diamorph-cito" (Russia). Results. While HP- associated gastritis Ki-67 a small amount of stomach epitheliocytes were found in nucleus, although PI increased in 1.5 times in comparison with homonamed index of functional dyspepsia. Besides, Ki-67 were registred in pericytes of vessels of mucous membrane stroma. EGF were found in membranes and cytoplasm of external epithelium, stomach glandulocytes, pericytes and endoteliocytes, besides in 1.3times more often than while functional dyspepsy. TGF-β was found in same cells (macrophages, fibroblasts) of inflammatory infiltrate. TGF-β assortment in cytoplasm and nucleus of epitheliocytes of gastrit pits and glands was unhomogeneous, but reliable did not reach the level of this parameter in bioptats of people from comparing group. Conclusion. Conducting research showed homodizected representation in the structures of stomach mucous membrane chronic HP-associated children gastritis Ki-67 and EGF. The localization of the last one (vessels endothelium, pericytes) points at the role of this cytokine in stimulating angiogenesis processes during the development of chronic inflammation. The decreasing of TGF-β, taking into account functional load of this growing factor, also promoted the increasing of proliferative epithelium activity and cells of stroma of stomach walls, oppression of monocyte migration to the focus of inflammation, phagocyte macrophage activity, supporting the chronic development of inflammatory process.

PP1-119

ROLE OF NK RECEPTORS IN CELIAC DISEASE COMPLICATIONS

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Background: Celiac disease (CD) has an increased risk of non-Hodgkin's lymphoma (NHL). Although CD was initially associated with enteropathy- associated T-NHL, patients with CD are also at increased risk for other NHLs, B and T cells. Long-standing CD in adult patients or gluten-free diet (GFD) non-responsive CD, clonal γ/δ T-cells, severe mucosal inflammation, are predisposing conditions for NHL. The diagnosis of CD-associated-NHL may be difficult and the prognosis is usually poor. Therefore, there is a need of markers for the early diagnosis and prognosis of NHL in CD. KIRs (Natural Killer cell immunoglobulin-like receptors) are specific receptors involved in the functional T/NK cell regulation contributing to the mucosal damage and possibly to the lymphoma development. Methods 33 patients with suspected or ascertained diagnosis of CD, and 50 blood donors, were enrolled. Oberhuber-modified Marsh criteria, ranging from Marsh type 0 to type 4, were used. KIRs were analyzed by DNA genotyping and KIR/HLA-I ligand matching

with the addition of HLA-I typing. Results 28 pts were at first evaluation. 5 had 8 or more months GFD, and 4/5 developed complication (1 intestinal carcinoma, 1 intestinal DLBCL, 1 bone marrow DLBCL, 1 refractory CD). At first diagnosis, we found 12 Marsh 0, and 16 Marsh 1-3 (3 type 1, 2 type 2, 11 type 3). When 16 CD Marsh 1-3 and 5 CD-GFD were compared with 50 donors, KIR2DS1 was more frequent in CD (14/21 vs 15/50, p=0,004), while in donors and CD Marsh 0 (6/12) was similar. When, at the first visit, CD Marsh 0 were compared to CD Marsh 3, KIR2DL5 was more frequent (10/12) in Marsh 0, than in Marsh 3 (4/11), p=0,021. In all CD with GFD, KIR2DS1 and KIR2DL5 were both present. However, KIR2DS1/HLA-C2 match was present in the 4/4 CD with complications, while it was existent in 11/29 of suspected or ascertained diagnosis of CD, including GFD-responsive patients, p=0,019. Conclusion KIR2DS1 is associated with overall symptomatic CD in comparison to donors. The highest match frequency of KIR2DS1/HLA-C2 in the CD patients with complications suggests the hypothesis that a functional KIR2DS1 receptor is associated with tumor development. Instead, KIR2DL5, is present in almost all Marsh 0, where it may have a protective effect against inflammation, and in all CD with complications. Since inflammatory response could also be involved in the immune control of the neoplastic process, KIR2DL5 might favor directly or indirectly the immuno-escape of neoplastic cells.

PP1-120
LOOP AMPLIFICATION OF THE INFLAMMATORY PROCESS INDUCED BY P2X7 ACTIVATION IN INTESTINAL EPITHELIAL CELLS THROUGH NEUTROPHIL TRANSEPITHELIAL MIGRATION

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Inflammatory bowel diseases (ulcerative colitis and Crohn's disease) in humans are chronic disorders inducing intestinal inflammation. These inflammatory diseases are mainly characterized by a large afflux of neutrophils into the mucosa causing the release of several cytokines. Moreover, during neutrophil transepithelial migration, large amounts of extracellular ATP are released both by neutrophils and epithelia. The P2X7 receptor is a ligand-gated cation channel activated by extracellular ATP and highly expressed on monocytes, macrophages and lymphocytes. P2X7 activation leads to release and maturation of IL1 beta. Moreover, extracellular ATP causes apoptosis and/or necrosis of the hematopoietic lineage through the activation of P2X7 receptors. There is strong evidence from P2X7 gene-deleted mice suggesting that the P2X7 receptor amplifies the inflammatory response, but little is known of the mechanism. Expression of P2X7 on intestinal epithelial cells is not well determinated as well as its putative function during the cross talk with the neutrophils. The aim of this study was to identify the presence of P2X7 in intestinal epithelial cells, to look for the level of P2X7 expression during the neutrophil transepithelial migration, and to see if P2X7 stimulation may induce release of cytokines by epithelial cells. For this purpose we used the T84 model which allow in vitro a human neutrophil transepithelial migration in a physiological direction. P2X7 expression was studied by confocal microscopy, Western blotting and qPCR. Release of cytokines by the T84 monolayers was looked by Elisa method. Results: P2X7 is strongly expressed in T84 cells and the signal is observed both at the apical side and at the basolateral side. Transepithelial migration of neutrophil induces an upregulation of P2X7 at the mRNA and protein levels. Incubation of T84 cells by extracellular ATP in T84 cells induces activation of caspase 1 and IL1 beta as well as different cytokines synthesis (in particular, TNF alpha and TGF beta). Finally, altogether these data showed that activation of P2X7 receptor in intestinal epithelial cells by extracellular ATP can provoked release of proinflammatory cytokines at the basolateral side of the T84 monolayers. We can speculate that amount of extracellular ATP produced during the neutrophil transepithelial migration may activate the P2X7 receptor and thus may amplify the inflammatory response.

PP1-121
MICROVESSEL DENSITY IS AN INDEPENDENT PROGNOSTIC FACTOR OF GASTRIC CANCER

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BACKGROUND: Tumor induced generation of new blood vessels is an important process in growth, prognosis and metastasis of tumors. We have done some research works to find out the relationship between the microvascular density and clinicopathologic features related with prognosis in gastric cancer patients. METHODS: To asses tumor angiogenesis, microvascular density (MVD) were analyzed immunohistochemically in 83 primary gastric cancers. Any single brown-stained cell with Factor VIII, that indicates an endothelial cell, was counted as a single vessel. Branching structures were counted as a single vessel unless there was a discontinuity in the structure. Vessels were counted in 4 regions, with the highest vascular density, at 200-magnification, and the average number of microvessels was recorded. RESULTS: The microvascular density for 83 tumor speciment ranged from 17,5-51,5 with a mean MVD of 33,17±7,95. Mean microvascular density was chosen as the cut-off point, 38 patients were categorized as a low MVD and 45 patients as a high MVD. There was no significant relationship between MVD and age, sex, and histological type of gastric cancer. A significant correlation was found between the MVD in early and advanced gastric cancer, and between gastric cancer with and without regional lymph node metastasis. High MVD was significantly associated with poor survival. Multivariate survival analysis showed that stage of dissease and MVD were independent prognostic factors. CONCLUSION: MVD is one of the most important prognostic factors for gastric cancer patients. Generally, poor prognosis of gastric cancer patients may be enhanced by therapeutic inhibition of angiogenesis.

PP1-122
PROGNOSTIC IMPORTANCE OF VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN GASTRIC CANCER

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BACKGROUND: Angiogenesis plays a central role in cancer growth, survival and development of distant metastasis. One of the most important regulator of the angiogenic process is vascular endothelial growth factor (VEGF), which induce endothelial cell proliferation, migration and invasion. VEGF also has been demonstrated to increase vascular permeability. METHOD:

VEGF was investigated by using immunohistochemical staining in 83 formalin-fixed paraffin embedded tissue sample patients with gastric carcinoma who underwent surgical resection between 2000. and 2003. at the Clinical Center of Montenegro. The relationship among VEGF expression and clinicopathological parameters was analyzed. RESULTS: Positive staining was obtained in 59 out of 83 cases (71,1%). VEGF immunoreactivity was observed mainly in the cytoplasm of tumor cells, and also frequently in stromal cells. No significant correlation between VEGF expression and age, sex, localization of tumors, and Lauren histological type of gastric carcinoma. Positive expression of VEGF correlated significantly with lymph node metastasis ($\chi2=4,27$; $p<0,05$). VEGF expression was more intense in poorly differentiated adenocarcinoma in comparison with other tumors ($\chi2=3,95$; $p<0,05$). According to prognosis, patients with VEGF-positive tumors had significantly worse survival and lowest 5-year survival rate than those with VEGF-negative tumors ($\chi2=11,22$; $p<0,01$). In multivariate analysis, status of VEGF were not an independent prognostic factor. CONCLUSION: VEGF may be a useful marker for predicting metastasis in gastric cancer, and inhibition of VEGF activity may have an important therapeutic benefit in the control of gastric cancer.

PP1-123

VALIDATION OF AUTOMATED IMAGE ANALYSIS (HISTOQUANT) IN COLON CANCER USING DIGITAL SLIDES OF EGFR, COX-2, BETA-CATENIN, AND CYCLIN D1 IMMUNOSTAININGS

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Background: Colon cancer is one of the leading causes of tumour mortality in developed countries. Recent efforts in diagnostic and clinical research of colon cancer have resulted in potential marker molecules, which may be associated with disease progression, prognosis and/or clinical response to therapy. However, evaluating of these markers in situ using immunohistochemistry, frequently suffers from lack of consistency, preventing intra- and inter-laboratory standardization of the diagnostic procedure and proper patient selection for treatment. Method: In this work we tested 68 cases of primary and metastatic colon cancers included in tissue microarrays (TMAs) for prognostic and predictive biomarkers localized to different compartments of tumour cells, including EGFR, beta-Catenin, Cox-2 and Cyclin D1. Immunostaining results were parallel evaluated by 2 independent assessors using both an empirical scoring based on negative, weak, moderate and strongly positive categories and an image-segmentation based automated analysis software, HistoQuant® (3DHISTECH, Budapest, Hungary). Altogether 116 representative areas of 58 colon cancers including all markers and score groups were analysed and measured along 56 parameters including color/intensity segmentation, absolute and relative values of positive and negative areas and cell numbers withing tumors. Discriminant analysis (Statistica®, Statsoft, USA) was used to find out if there was a correlation between the human classification and measured data. Results: From the examined areas 110 were classified correctly using HistoQuant program(overall 95%; EGFR 97%, beta-Catenin 79%, Cox-2 100%, Cyclin D1 100%). Negative cases were revealed in 100% agreement between the analyses. At disagreement, some cases were ranked only to adjacent classes, e.g. some weak positive areas were upgraded to be moderate using the automated measurement, but there was no transition between the weak and strong positive cases. The most discriminative parameters were the positive area within selected area and the intensity values, resulting in very similar differences between groups compared to empirical assessment. Conclusion: Based on this study, we suggest, that that quantitative measurements on digital slides may correlate very well with properly set up empirical scoring in colon cancer. The HistoQuant software can be a reliable tool of accurate marker assessment for diagnostic, prognostic and/or therapeutic utilization.

PP1-124

4 CASES OF UNUSUAL METASTASIS TO DUODENUM

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Background: Small bowel involvement by metastatic tumors is very rare and the most common primaries known are malignant melanoma, lung cell carcinoma, renal cell carcinoma, testicular germ cell tumor, hepatocellular carcinoma, cervical carcinoma, thyroid carcinoma and Merkel cell carcinoma. We found 4 cases of metastatic tumor in the duodenum in the archives of Izmir Ataturk Training and Research Hospital between 2000 and 2006. Case 1: A 54-year old man who had undergone right nephrectomy for a renal mass 9 months before, presented with severe weight loss and vomiting. Upper endoscopy showed a submucosal mass 5 cm in diameter. Duodenal biopsy was consistent with metastatic renal cell carcinoma similar in morphology with the tumor diagnosed in his nephrectomy material. Case 2: A 69-year old man presented with a pigmented lesion on his left cheek and also with hematemesis and melena at the same time. Biopsy of the pigmented lesion showed a malignant melanoma of the superficial spreading type. Upper endoscopy revealed multipl pigmented lesions in gastric and duodenal mucosal surfaces. Duodenal biopsy was a metastatic melanoma with typical melanoma cell groups in the lamina propria. Case 3: A 42-year old man with a diagnosis of testicular tumor had melana in the postoperative period. Ultrasonography showed multipl metastatic tumors in liver, the largest being 6 cm in diameter and the patient underwent surgery. Choriocarcinoma metastasis to duodenum was confirmed from the surgical specimen which was similar to the testicular tumor diagnosed as mixed germ cell tumor with choriocarcinomatous component. Case 4: A 76-year old man who was a heavy smoker for more than 50 years, presented with severe weight loss and jaundice. Endoscopic retrograd cholangiopancreatography showed a tumor in the second part of the duodenum narrowing the lumen. Duodenal biopsy was a squamous cell carcinoma growing in a polypoid fashion. No other diagnostic procedure could be performed as the patient detoriated rapidly and died. Autopsy was not done. It was thought that the primary site of the squamous cell carcinoma might be of lung origin but could not be proved pathologically. Conclusion: Those patients having severe gastrointestinal symptoms with any prior malignancy should be completely evaluated by the help of endoscopic examination followed by biopsy in order to exclude the possibility of duodenal metastasis.

PP1-125

REVIEW OF 24 APPENDICEAL CARCINOID TUMORS:A CONSECUTIVE SERIES FROM 4848 APPENDECTOMIES IN 13 YEARS

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Background: Primary appendiceal carcinoid tumor is a rare disease but carcinoid tumors are the most common type of neoplasms seen in appendix that is rarely suspected before the pathologic examination. Clinical presentation of the appendiceal carcinoid is usually that of an acute appendicitis and in the majority of cases the diagnosis is incidental. Method: The medical records of patients who underwent appendectomy at

Izmir Ataturk Training and Research Hospital between January 1994 to December 2006 were reviewed retrospectively. Age, gender of the patients, location of the tumor and diameter, depth of invasion, histopathologic type of tumor and also incidence of other pathologic lesions in the appendix were recorded. Immunohistochemically neuron specific enolase (NSE), chromogranin A and synaptophysin were performed in 20 cases, since paraffin blocks were not available in 4. Results: During the study period 4848 appendicectomies were performed and out of these, 24 cases of carcinoid tumor were diagnosed (0.49%). There were 9 female, 15 male patients, mean age 29.73 years (range:15-65). Mean tumor diameter was 9.1 mm (range:1 mm-2 cm) and in 14 patients the tumour was localised at the appendix apex, 10 at the mid portion. Acute appendicitis was the clinical presentation in 9 patients (37.5%), but it was an incidental finding in 15 (62.5%). Goblet cell carcinoid was seen in 2 patients and all 22 patient had classical type carcinoid. Serosal infiltration was seen in 14 cases (58.3%). Immunohistochemical study performed in 20 patients revealed NSE positivity in 20 cases (100%), synapthophsin in 18 (90%) and chromogranin A in 15 (75%) cases. Most of the patients underwent only appendectomy and no adjuvant therapy was performed. Only two patients who were brothers, had a right hemicolectomy due to a synchronous carcinoma located in the ceacum at the same time. 15 patients were alive and disease free during a follow up period of 8 years. 7 patients were lost to follow-up. 1 patient died because of colonic adenocarcinoma. Conclusion: Carcinoid tumors of the appendix are extremely rare lesions with no specific clinical symptom but some may present with acute appendicitis. The treatment of choice may be appendectomy especially if the tumor diameter is <1 cm.

PP1-126
COX-2 EXPRESSION IN INTESTINAL AND DIFFUSE TYPE GASTRIC ADENOCARCINOMA

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Cyclooxygenase (COX) is an enzyme that catalyzes the conversion of arachidonic acid into prostaglandin. It has 2 isoforms: constitutive (COX-1) and inducible (COX-2). It is reported that COX-2 expression has a role in inflammation and carcinogenesis. The expression of COX-2 in gastric adenocarcinomas and precancerous lesions proposes its role in gastric carcinogenesis. In this study, 2 different types (intestinal, diffuse) of gastric adenocarcinomas have been compared in respect of COX-2 expression with regard to possible different carcinogenetic pathways. Immunohistochemically COX-2 has been performed on tissue sections of 20 intestinal type and 20 diffuse type gastric adenocarcinomas. COX-2 expression has been scored as; staining intensity (weak:1, moderate:2, strong: 3) and staining extension (no staining:0, <10 %:1, 10-50 %:2, >50 %:3). The staining is (+) in case the total score is over 4 and (-) in case the total score is 4 or less than 4. Statistically chi-square test and Mann Whitney U test have been used. Results for $p<0.05$ are statistically signicant. Staining is observed in 42,5% cases of the total gastric adenocarcinomas while in 53,5% no staining was observed. In 55% of intestinal type and in 30% of diffuse type adenocarcinomas cytoplasmic staining was detected. Considering the total score, a higher level of positivity was observed in intestinal type tumors compared to the diffuse type. Yet, the difference was not statistically significant (p: 0142). COX-2 might play an important role in the evaluation of carcinogenesis in stomach and might be related to carcinogenesis pathway of intestinal type. COX-2 inhibitors might be important in the prevention of carcinoma development in patients with precancerous lesions and in the treatment of patients with gastric adenocarcinoma. However, further studies are needed to detect this fact.

PP1-127
HEPATOCYTE ANTIGEN EXPRESSION IN SUBTYPES OF INTESTINAL METAPLASIA OF THE STOMACH

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Background: Intestinal metaplasia (IM) is generally regarded as a premalignant lesion that is classified by mucin modifications confirmed by histochemical and immunohistochemical methods. Recently, hepatocyte antigen (Hep) introduced as a sensitive and reliable marker of IM. However, the distribution of Hep expression in subtypes of IM are not described. Method: We examined the expression of Hep in 58 cases of chronic gastritis (CG) associated with IM by immunohistochemical staining. According to the mucin expression patterns cases were classified as; 19 cases of IM type I, 16 cases of type II and 23 cases of type III. The distribution of Hep expression were classified into four groups according to the rate of Hep expressing metaplastic cells: negative, low (between 1% and <30%), moderate (between 30% and <60%), high (over 60%). We also compared expression of Hep with that of MUC-1, MUC 2 and MUC 5AC. Results: Hep expression showed a granular cytoplasmic staining and specifically identified in columnar cells, but not in goblet cells. There was no significant difference between Hep expression and subtypes of IM ($p>0.05$). However, the difference between the distribution of Hep expression among three subtypes of IM was significant ($p<0.001$). In type I IM most of the cases expressed Hep in >60% (high) of columnar cells. In contrast in type II IM, the expression was moderate in a grate majority of cases with only 4 cases with high expression. In both group low expression was not observed. In contrast in type III IM low expression was observed in 17 of the 19 positive cases. In the same group moderate expression was noted in two cases and any case displayed a high Hep expression. A strong correlation between the distribution of Hep expression and subtypes of IM was also noted ($r = -0.844$). Any relationship was observed among the expression of Hep, MUC-1, MUC 2 and MUC 5AC. Conclusion: Results of the present study revealed that the distribution of Hep expression is higher in the complete type (type I) of IM and gradually decreases in the incomplete types (type II and type III) and suggest that besides its role as a sensitive marker in IM, the evaluation of the distribution of Hep expression might be useful in the classification of IM.

PP1-128
POLYP SIZE DETERMINES THE PATHOLOGIC FEATURES THAT DISTINGUISH SESSILE SERRATED ADENOMAS AND HYPERPLASTIC POLYPS.

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Background: Large sessile serrated adenomas (SSAs) and hyperplastic polyps (HPs) are morphologically distinctive, whereas small polyps often lack these distinctive features and are often difficult to classify. This study correlates polyp size with pathologic features of serrated polyps in order to establish diagnostic criteria to separate small SSAs and HPs. Methods: The study included 1162 SSAs and HPs were retrieved from our file and grouped by mm size (2mm, 3mm, 4mm, 5mm, 6mm, 7mm, 8mm; N=75, 267, 270,246,186, 87, 31, respectively). In each mm group, the number of right colon polyps was matched to those of left colon. The number of normal, mildly-, and moderately (mod)-dilated, and flask-shaped crypts was recorded. For each crypt shape, the number with basilar, mid and superficial upwards extension of immature cells, mitoses, serration, and type of cytoplasmic differentiation was recorded. Polyps were blindly re-reviewed at a later date and diagnosed as SSA, HP, Indeterminate (Ind). Results: The percentages of normal crypt bases/ polyp were

86% and 28% in 2 mm and 8 mm polyps, respectively, whereas the percentage of crypts with mod-dilated or flask shaped bases increased from 4% and 2% respectively in 2 mm polyps to 44% and 21% in 8 mm polyps. The percentage of polyp crypts with mid-level immature cells and mitoses was <10% in all 2 and 3 mm and most 4 mm polyps to >50% in >5 mm polyps. There was uniform cytoplasmic maturation in all 2 and 3 mm, whereas almost all >5mm polyps had small clusters of crypts with similar cytoplasmic maturational features that were different from the features of adjacent crypts. The percentage of 2mm, 3mm, 4mm, and 5mm polyps diagnosed as HP, Ind, SSA were (94%,6%,0), (55%,42%,3%), (31%,46%,23%), and (10%,25%, 65%), respectively. Conclusions: There was progressive increase in the extent of changes with increasing polyp size beginning with basilar crypt dilation, followed by upwards expansion of immature epithelial cells and mitoses, and ending with flask-shaped serrated crypt bases and full-thickness immature epithelial cells. Small polyps were singularly HP-like. 46% of 4 mm polyps had indeterminate features, whereas whereas 65% of 5mm polyps had predominantly SSA features. Polyp size may be an marker of the extent of underlying molecular alterations. SSAs and HPs may be related entities and derive from a common pool of lesions. SSAs emerge from this pool due to greater levels of cell cycle control slippage and cell signaling pathway perturbations.

PP1-129

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) EXPRESSION IN LATE STAGE METASTATIC COLORECTAL CANCER. COMPARATIVE IMMUNOHISTOCHEMICAL STUDY USING TWO COMMERCIALLY AVAILABLE ANTIBODIES

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BACKGROUND AND OBJECTIVE : Colorectal carcinoma is one of the most common malignancies worldwide and manifests a high mortality, especially at late stages of the disease. Currently available therapeutic strategies have a modest effect on overall survival of patients with advanced and/or metastatic disease. Recently, targeted therapy drugs, such as EGFR inhibitors, are in use for the management of advanced-stage colorectal cancer. It is well known that the dysregulation of the epidermal growth factor receptor (EGFR) signal transduction pathway is involved in colon cancer development and progression, and overexpression of the receptor confers a poor prognosis. Thus targeting the EGFR has become a rational approach for the treatment of colorectal carcinoma. In this context we investigated the expression of EGFR in advanced/metastatic colorectal cancer using two different antibodies against EGFR and compared the incidence, the cellular distribution and the intensity of its expression as given by the two antibodies. We also correlated the EGFR expression with differentiation grade, Dukes' stage at primary site, age and gender. MATERIALS AND METHODS: In this study, 121 archival specimens of metastatic colorectal cancer were analysed immunohistochemically, using two different antibodies against EGFR , the mouse monoclonal anti-human EGFR clone 2-18C9 of the EGFR pharmDx kit (DAKO) and clone 31G7 (Zymed). Immunostaining was evaluated as membranous, and the intensity of the immunostaining was graded 0 (negative), 1+ (weak), 2+ (moderate) and 3+ (strong). RESULTS AND CONCLUSIONS: Overall, 34.7% of the adenocarcinoma cases had membranous EGFR reactivity; 9.1% of the tumors had 3+ reactivity in 3-30% of the neoplastic cells, 17.4% of them displayed 2+ reactivity and 8.3% exhibited 1+ in the same tumor cell percentage. EGFR expression in colorectal carcinomas correlated with differentiation grade showing higher levels in high grade tumors ($p < 0.0001$). However, there were not statistically significant association with Dukes' stage, site, patient age or gender. There was high concordance (92%, $p < 0.0001$) in evaluating EGFR expression between the two antibodies used in this study, with no difference in the incidence, the cellular distribution and the intensity of EGFR expression.

PP1-130

PRELIMINARY STUDY OF INFILTRATIVE COLON CANCER BY USING MICROIMAGING FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY

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BACKGROUND AND OBJECTIVE: Fourier Transform infrared (FT-IR) spectroscopy represents a reliable method for the resolution of the molecular structure and determination of chemical composition of biologic materials. There is evidence that this technique could be used in the early diagnosis of several neoplasms in combination with the conventional histopathologic diagnosis because of the possibility to provide information on the molecular changes in limited areas of lesions. MATERIALS AND METHODS: In this context we examined using FT-IR spectroscopy a limited number of infiltrative colon adenocarcinomas , of variant histologic grade of malignancy, along with adjacent normal colon tissue. Thin paraffin sections of 5μm were obtained and spectral data were collected by a Perkin-Elmer Spectrum One FT-IR equipped with a Perkin-Elmer Auto-image microscope. RESULTS AND CONCLUSIONS: The analysis and the comparison between the spectra obtained from neoplastic and normal tissue zones indicated that there were significant differences of the spectral absorption signatures in the infrared region: i) in the Amide I band, the spectra of normal tissues if compared with the ones of malignant zone, were sharper and were found at higher frequencies with a maximum at 1661 cm-1), ii) in the Amide II band, the normal region was broader and red shifted 5 cm-1 with two maxima around 1658 and 1635 cm-1, differences that could arise from a change of the secondary structure of proteins during carcinogenesis, iii)differences in the CH2 and CH3 modes at 1400, 1466, 2849 and 2920 cm-1 (hypomethylation process in neoplastic cells), iv) small blue shift of the C-OH mode from 1164 cm-1 to 1172 cm-1 indicating an increased phosphorylation induced by carcinogenesis v) in PO^2 modes of nucleic acid and carbohydrates in the region 1100-1000 cm-1 in which the bands of the neoplastic tissue were convoluted with only two maxima at 1080 cm-1 (vsPO^2) and 1117 cm-1 (mucin), while in the normal tissue, the peaks at 1048, 1080 and the one of mucin at 1121 cm-1 were easily discernible (reduction of mucin production in cancer). Specific spectral signatures were also obtained from the connective tissue and from necrotic and inflammation areas.

PP1-131

KIT EXON 11 CODON 557/558 MUTATIONS DEFINES A SUBSET OF MALIGNANT CLINICAL BEHAVIOR IN GASTROINTESTINAL STROMAL TUMORS

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Aim: Most gastrointestinal stromal tumors (GISTs) express activated mutant isoforms of KIT or PDFFRA genes that are potential therapeutic targets. The significance of these mutations for the prediction of GISTs biological and clinical behavior is still under consideration. In this study we have analysed the frequency and pattern of KIT and PDFFRA mutations in a group of patients with GISTs and the association of these mutations with other clinicopathological factors. Materials and methods : Thirty patients with GISTs were examined. DNA were extracted from paraffin sections. Exons 9, 11, 13, and 17 of the KIT-gene and exons 12 and 18 of the PDGFRA-gene were evaluated for the presence of mutations by PCR amplification and direct sequencing. Results : Overall KIT or PDGFRA mutations (MT) were detected in 21 of the 30 patients (70%). Sixteen patients had MT within KIT exon 11, three patients within KIT exon 9 and two patients within PDGFRA exon 18. The detected MT within KIT exon 11were heterogeneous and consisted of 10 deletions, 4 point mutations and 2 insertions. Codons 557 and 558 were involved in 8 cases (50%) of the KIT exon 11 mutations. KIT-MT exon 9 were almost detected in GISTs located in small intestine, showing spindle cells phenotype and they were classified as uncertain malignant potential or malignant. KIT-MT exon 11 were largely confined located in small and large intestine, showing spindle cells phenotype ($p<0.05$) and they were classified as malignant potential. KIT-MT exon 11 codon 557/558 were associated with malignant clinical behavior ($p=0.032$). PDGFRA mutations were all detected in GISTs located in stomach, showing mixed type phenotype and they were classified as uncertain malignant potential. Conclusion: Integration of histological features and determination of KIT and PDGFRA mutations should be additional parameters for the better prediction of GISTs clinical behavior. Tumors with mutations affecting codons 557/558 of the KIT gene seems to represent a distinct subset of malignant GISTs.

PP1-132
EXPRESSION OF RETINOBLASTOMA PROTEIN AND CYCLIN D1 IN GASTRIC CARCINOMA

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BACKGROUND: Abnormal regulation of the cell cycle is a feature of many neoplasms. Regulation of the G1/S checkpoint, which is controlled by the retinoblastoma protein (pRb), is critical event and cyclin D1 plays a positive regulation of the cell cycle. The role of cell cycle regulators in oncogenesis has been investigated in many human tumors. Alteration of the pRb and cyclin D1 disrupt the Rb pathway and occur in many carcinomas. However the expression of the pRb and cyclin D1 in intestinal type gastric carcinoma, is unclear. In this study, our aim was to investigate the expression of pRb and cyclinD1 in resected gastric carcinoma, their adjacent nonneoplastic mucosa and normal gastric mucosa. Also relationship with histopathological features were analyzed. METHOD: The study consisted of 43 patients (32 men, 11 women; mean age: 64) who underwent surgery for primary gastric adenocarcinoma at our institute between 2004–2006. Immunohistochemistry was performed by streptavidin biotin peroxidase method. Using immunohistochemical methods, the expression of pRb and cyclinD1 was investigated in 43 resected intestinal type gastric carcinoma specimens, adjacent noneoplastic mucosa and normal gastric mucosa. Adjacent nonneoplastic mucosa consisted of atrophy, dyplasia, intestinal metaplasia and gastritis. RESULTS: Adjacent nonneoplastic mucosa showed atrophy, dysplasia and intestinal metaplasia in 33(76.7%), 31(72%) and 34(77.2%) of the cases, respectively. Gastritis was detected in all cases. Expression of pRb was detected in 30 (69.7%) of gastric carcinoma, 18 (41.8%) of the adjacent nonneoplastic mucosa. Expression of cyclinD1 protein was detected in 31 (72%) of gastric carcinoma, 24 (55.8%) of adjacent nonneoplastic mucosa. Expression of pRb and cyclinD1 was not detected in normal gastric mucosa. The positive rate of pRb and cyclin D1 expression in gastric carcinoma was significantly higher than that adjacent noneoplastic mucosa ($p<0.05$). pRb and cyclinD1 expression were not significantly associated with histopathological features ($p>0.05$). CONCLUSION: There were significant trends for increased expression of pRb and cyclinD1 from normal mucosa to precursor lesions, detected adjacent noneoplastic mucosa, and carcinoma. These results suggested that positive expression of pRb and cyclinD1 might be an early event in gastric carcinoma and it tended to begin at precursor lesions and maintain throughout the progression of infiltration.

PP1-133
COLORECTAL CARCINOMAS: CLINICOPATHOLOGIC INVESTIGATION, CORRELATION WITH EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS

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Background: Colorectal adenocarcinoma is the forth most frequently diagnosed visceral and the second leading cause of cancer mortality in both genders. The correlation in mortality data between breast cancer and colonic cancer, and the increased incidence of colonic cancer in individual women with breast cancer suggest common factors in their etiology. The protective effect of increasing parity from colonic cancer, similar to breast cancer and relatively better prognosis in women may also imply a common role for sex steroid hormones. Method: In this study, clinicopathologic parameters and estrogen and progesterone receptor expressions of 60 cases previously diagnosed as colorectal adenocarcinoma in the Department of Pathology in Zonguldak Karaelmas University Faculty of Medicine from November 2001 to March 2006 were investigated. Results: 31 cases were men and 29 were women with an age distribution ranging from 33 to 90 (average 65.8; SD: ± 13.55). 18 (30%) cases were from rectosigmoid area, followed in decreasing order by tumors limited in rectum, and sigmoid; ascending colon; descending colon; transverse colon; tumors with multiple sites at presentation and cecum. Histologic grading for well, moderately and poorly differentiated tumors were consecutively found 17 (28.3%), 37 (61.7%) and 6 (10.0%). Most cases were classified either in B2 or C2 according to the Astler Coller staging. Immunohistochemistry was carried out by using antibodies against estrogen and progesterone receptors; tumors with more than 5% nuclear staining were considered positive. Six cases displayed cytoplasmic reaction for estrogen receptor; no nuclear staining was demonstrated for estrogen or progesterone receptors. Conclusion: Epidemiologic parameters and sites of involvement in this study were almost akin to previous reports, except lower incidence of ceacal tumors. Nuclear staining for neither estrogen nor progesterone receptors had been demonstrated in the current study. It is vague whether to exclude cytoplasmic staining. Moreover, although potential importance of estrogen and progesterone receptors has been emphasized by certain reports, its importance is still controversial in terms of both prognosis and management. Therefore, there remains much to do to estimate the significance of these receptors in colorectal carcinoma.

PP1-134
HELICOBACTER PYLORI PREVALENCE IN GASTRIC ADENOCARCINOMA

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Background: Helicobacter pylori colonizes in the gastric mucosa, and may cause various degrees of epithelial damage contributing to the pathogenesis of not only chronic inflammatory changes but also malignancies including gastric lymphoma and carcinoma. Considering its high incidence, approximately more than 50% of asymptomatic the population in most countries (exceeding 80% in developing countries), medical eradication of this organism may trim down inflammatory changes, and thus prevent carcinogenic cascade. In this study, we aimed to determine the prevalence and the value of Helicobacter pylori in gastric adenocarcinomas. Method: Gastrectomy specimens of 45 patients with gastric adenocarcinoma between July 2001 and December 2006 in Karaelmas University Faculty of Medicine were retrospectively re-evaluated. Histological classification of adenocarcinoma and Helicobacter pylori assessment were accomplished by applying Lauren classification and Sydney system, respectively. Results: The study group consisted of 32 males and 13 females with an average age of 61.84, ranging from 35 to 84. Intestinal type adenocarcinoma was detected in 40 cases while the rest five were diffuse type. Helicobacter pylori was present in 20 (44.4%) cases regardless of histologic type; when considered in 18 of 40 (45%) intestinal type, and in 2 of 5 (40%) diffuse adenocarcinoma. Conclusion: The overall Helicobacter pylori incidence in gastric carcinoma patients was generically consistent with literature. Although conflicting results have been reported, in contrast to most previous studies suggesting a closer association with intestinal type carcinoma than diffuse type, no such relation was determined in this study, probably because of the limited number of cases.

PP1-135
INCREASED NUMBER OF NEUROENDOCRINE CELLS IN METASTATIC COLORECTAL ADENOCARCINOMA FOLLOWING CHEMO/RADIOTHERAPY

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Background. Previous reports demonstrated a positive modulation of neuroendocrine (NE) phenotype in rectal adenocarcinomas receiving neoadjuvant therapy prior to radical surgery. However, to our knowledge no data are available on the role of post surgical adjuvant therapy in determining NE differentiation in recurrent/metastatising colo-rectal carcinoma (CRC). Methods. We analysed 15 randomly selected cases of primary CRC (9 cases from colon and 6 from rectum) and the corresponding metastases (8 liver, 4 peritoneal and 3 lung) developed during tumor progression. These were resected 1 to 3 years following adjuvant chemotherapy (all cases) and radiotherapy (5 cases, all from the rectum). The presence of NE cells was evaluated by means of Chromogranin A (CgA) immunohistochemistry, which was scored on the basis on the percentage of neoplastic cells as follows: 0 (no cell staining), 1+ (1-20% positive tumor cells) and 2+ (more than 20% positive tumor cells). Results. In primary tumor samples, focal NE differentiation (CgA score 1+) was evident in 3/15 cases (2 colonic and 1 rectal). Two of these cases had predominant mucinous features. Metastatic tumors resected after adjuvant chemotherapy were stained in parallel, and found to contain CgA positive cells in 8/15 cases (1 of the three CgA positive primary CRC, and 7 additional cases that were negative in the primary tumor (4 from the colon and 3 from the rectum). Radiotherapy treated cases were not significantly correlated with an increased number of NE cells. The presence of NE phenotype was not correlated to clinical outcome, although the two patients with the highest number of endocrine cells in metastatic tissue (score 2) were both died of the disease. Conclusion. Our data support the hypothesis that adjuvant therapy might modulate the NE phenotype in CRC, possibly selecting a cell population less responsive to current therapeutic regimens for CRC with possible biological and prognostic implications.

PP1-301
IMMUNOHISTOCHEMICAL STUDY OF E-CADHERIN AND BETA-CATENIN EXPRESSION IN COLORECTAL CARCINOMAS

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AIM: Parameters as histologic type, differentiation, lymph node metastasis and stages defining the prognosis of colorectal carcinomas have been observed. This study was executed observe E-Cadherin and beta-catenin expressions of tumor cells and using these parameters and the results to define life expectancy, response to the treatment, metastatic illness and recurrence of tumor. MATERIAL AND METHOD: 60 cases diagnosed as colorectal adenocarcinoma between 2004-2005 were selected to study retrospectively. 5 of these cases were left out because of technical reasons. H&E stained preparates were examined and the differentiation and the prognostic parameters of the tumors were evaluated. Along with the tumor, a block which has normal tissue was selected. Immunohistochemistry was performed using E-cadherin (36B5 clone, Neomarkers) and beta-catenin (Cat-a.1. clone, Immunovision) primary antibodies and avidin-biotin-peroxidase. RESULTS: 53 of 60 adenocarcinoma tissues were evaluated as classical type adenocarcinoma and 7 of them as mucinous carcinoma . 48 classical type adenocarcinoma tissues showed membranous staining for E-Cadherin, 13 tissues showed cytoplasmic staining and no staining was observed in 5 tissues 4 of Mucinous carsinoma tissues showed membranous type staining. Three of them showed no staining. All 53 adenocarcinoma tissues expressed nuclear or membranous type beta-catenin in different intensities. In all of 7 mucinous carcinoma tissues nuclear or membranous staining was observed. CONCLUSION: E-cadherin and beta-catenin showed membranous, beta-catenin nuclear, and E-cadherin cytoplasmic expressions. The staining intensity of E cadherin and Beta catenin were compared separately with prognostic parameters (tumor type: mucinous or classical adenocarcinoma, degree of differentation, vascular invasion, existence of lymph node metastasis, metastatic illness and recurrence of tumor) When E-cadherin and beta-catenin expressions were compared, no significant difference was observed. On the other hand, there was a significant difference between the tumor stage and the existence of lymph node metastasis.

Neuropathology

PP1-136
CEREBROVASCULAR DISEASES: A REPORT OF 175 AUTOPSY CASES
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The aim of this study was to determine the incidence of cerebrovascular diseases (CVD) on pathoanatomical material of the Municipal Institution "Taldom Central Regional Hospital" over the period from 1989 to 2006. The obtained findings revealed 175 cases of CVD (91 females and 84 males, mean age 63 years) from a total of 1,071 postmortem examinations of adults. In the majority of cases, the primary disease had been diagnosed as intracerebral haemorrhage (121 subjects) and intracerebral infarction (54 cases), with all the CVD having been registered on the background of arterial hypertension and/or progressing diffuse atherosclerosis, however, not all the patients had even been on ambulatory medical records. Besides these risk factors, obesity(59 cases)and diabetes mellitus (12 cases)were revealed as concomitant diseases. The direct causes of death were the following complications of the combined primary disease: haemorrhage into the cerebral ventricles (102 cases), compression and dislocation of the truncus cerebri due to oedema or swelling of the substantia medullaris (39), thromboembolism of the pulmonary artery (18), pneumonia (14), rupture of the ascending aortic wall followed by haemorrhage into the pericardial cavity (1 case), and renal insufficiency (1 observation). Hence, CVD on the background of arterial hypertension and disseminated progressing atherosclerosis amounted to 16% amongst the 1,071 postmortem examinations of adult subjects of the general somatic hospital over the above-mentioned eighteen-year period. In order to decrease and prevent CVD morbidity, it is necessary to systematically and thoroughly meticulously follow-up the health status of patients with cardiovascular diseases, to improve the organization of preventive measures, and to remove the risk factors amongst the adult population of the Taldom region by means of cardiological screening to be followed by ambulatory registration of the cohort revealed.

PP1-137
ALVEOLAR SOFT PART SARCOMA METASTASIS TO CEREBELLUM: REPORT OF AN UNUSUAL CASE
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Background: Alveolar soft part sarcoma is a malignant soft tissue tumor which it;s metastasis to other organs like lung and brain as primary presenting symptom is not unusual.In the review of literatures there are reports of metastasis to different parts of brain,but to our knowledge cerebellar metastasis is not reported.We present an extremely rare case of metastatic alveolar soft part sarcoma to cerebrum and cerebellum at the same time as primary manifestation of the tumor. Case report:A 18 year old lady admitted due to nausea,vertigo,severe headache and blurred vision.MRI shows multiple lesions in cerebral hemispheres and cerebellum.Craniotomy was done.Pathologic diagnosis was metastatic alveolar soft part sarcoma which was confirmed immunohistochemically. Metastasis of this type of sarcoma to cerebellum is extremely unusual.

PP1-138
METHANOL TOXICITY: GFAP AND CD34 EXPRESSIONS IN HUMAN OPTIC NERVE AND BRAIN
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Methanol is used in variety of commercial products and production of illegal alcoholic beverages. The toxicity of methanol is due to its metabolites, which are formaldehyde and formic acid. The optic nerve consists mainly of ganglion cell axons, glial cells blood vessels and mesodermic tissue. Astrocytes are particularly important, they contribute to the blood-optic nevre, blood-brain barrier and participate in the scarring and repair of the nervous system GFAP immunostaining is the most commonly used method for examining astrocyte proliferation and hypertrophy after various central nervous system (CNS) injuries. The antigen CD34 is expressed widely on vascular endothelium, including that of the central nervous system (CNS) and high endothelial venules. The aim of the present study was to investigate the effects of methanol exposure on GFAP positive astrocytes in brain and optic nerve, and to show changes in CD34 expression in endothelial cells. 12 cases (all male; individual ages, mean 47.6±15.6 median 46 range 20–76 years) and 12 controls were identified and included in the study. According to toxicological results, the blood methanol concentration ranges widely from 14 to 202 mg per 100 ml. There were 6 cases (50%) with the methanol concentration over mean (98.35 mg per 100 ml). In the 10 of 12 cases (83.3%) also ethyl alcohol was detected in their blood samples. The ethyl alcohol concentrations ranged from 75 to 230 mg per 100 ml. Of these cases, six died before admittance to hospital. In the case group there was positive significant correlation between GFAP and CD34 intensity scores, furthermore there was positive statistically significant correlation between brain and optic nerve GFAP extent of expression (Spearman's correlation coefficient = 0.711, p=0.010, and Spearman's correlation coefficient = 0.720, p=0.008). Intensity and extent for every one of the two markers and groups were evaluated according after dividing score groups in 0-2 and 3-4 scores. There was no statistically significant differences between cases and controls brain GFAP and CD34 intensity and extent scores (p= 0.05).

PP1-139
CEREBRAL ASPERGILLOSIS PRESENTING AS A MASS LESION IN AN IMMUNOCOMPETENT HOST
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Fungal infections of the central nervous system (CNS) are almost always surprising findings. Their presentation is subtle, often without any diagnostic characteristics, and they are frequently mistaken for pyogenic abscess, or brain tumor.Aspergillosis of the central nervous system is an uncommon infection, mainly occurring in immunocompromised patients. It may be presented in several forms: meningitis, mycotic aneurysms, infarcts and the tumoral form. In this case, we reported an intracranial granuloma caused by Aspergillus fumigatus involving the anterior cranial fossa and the frontal lobe. In this case, clinical symptoms developed since 1 year before. The final diagnosis made after craniotomy. The patient treated with an extensive excision and medical antifungal therapy, but finally he failed to respond to these treatments.

PP1-140
EGFR STATUS IN PEDIATRIC EPENDYMOMAS USING IMMUNOHISTOCHEMISTRY (IHC) AND CHROMOGENIC IN SITU HYBRIDIZATION (CISH)
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Background: The epidermal growth factor receptor (EGFR), a member of the family of receptor tyrosine kinases, plays an important role in a wide variety of pathophysiological disorders including cancer. Studies have shown that EGFR amplification correlate with shorter survival in glioma patients receiving adjuvant therapies. In ependymomas, EGFR status remains unclear. Different EGFR targeted therapies using agents directed toward the intracellular tyrosine kinase domains are currently in phase III clinical trials. The optimal assay to evaluate EGFR status in possible candidates for possible anti-EGFR therapy remains to be determined. In this study, we evaluated nine pediatric ependymomas for both EGFR gene amplification and protein expression by CISH and IHC. Design: Nine pediatric ependymomas were evaluated. CISH and IHC were performed using paraffin-embedded tissue. High EGFR expression was detected when total membranous staining was seen in more than 10% of the cells. CISH amplification was seen when 10 or more copies or larger clusters were seen in more than 50% of the nuclei; low amplification was seen in the cases with 6-10 copies in more than 50% of the nuclei. Result: EGFR amplification was not detected in any case. Protein EGFR eoverexpression was present in a single ependymoma. Conclusion: EGFR amplification/protein overexpression is a rare phenomenon in pediatric ependymomas. These findings suggest that EGFR abnormalities are not associated with pediatric ependymomas tumorigenesis; therefore, it is unlikely that patients with these tumors will benefit from EGFR targeted therapy.

PP1-141
EVALUATION OF THE RELATIONSHIP BETWEEN UNDESCENDED TESTES AND NEUROMUSCULAR DISORDERS

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Background/aim: Because of striated muscle origin; the cremaster muscle may be possibly affected by neuromuscular disease and may cause the increased incidence of undescended testes and other urogenital malformations. Therefore we aim to determine the frequency of undescended testes in children with muscle disease. Material and methods: Seventy boys as having neuromuscular disorders confirmed with muscle biopsy were included in this study. Fresh striated muscle samples of all cases were frozen in liquid nitrogen and frozen sections were evaluated with several histochemical, enzyme and immune histochemical stains. Detailed clinical information, physical and laboratory examination findings were obtained from patients files. Clinical relevance of undescended testes was evaluated. All findings were searched statistically. Results: The mean age of the boys was 6.73 (± 3.71). Muscular dystrophies were the most common disease (57.2 %). Other diseases determined in the cases were 17 primary myopathies, 6 inflammatory myopathies, 4 neuropathies, and 3 have metabolic/ mitochondrial myopathies. Sixteen patients (22.9 %) had similarly affected family members. Consanguinity rate was 28.6 % (n= 20). Sixty two patients (88.6 %) presented at least one of the muscle serum enzymes altered. Forty four patients (62.6 %) had pathological EMG findings. Fourteen cases (20 %) had fiber type disproportion. Urogenital malformations were determined in 7 patients (10 %). In Spearman's Correlation Analysis, there were statistical significances between the types of neuromuscular disease and the presence of undescended testes (p= 0.040) and urogenital malformations (p= 0.017). In Chi Square test, no statistical significance was found (p= 0.284). The odds ratios for undescended testes and urogenital pathologies were computed and they were found 4.03 and 4.51 respectively. These results supported that urogenital abnormalities were more common in children with neuromuscular disorders than population. Conclusion: We thought that congenital muscle disease may create a disturbance of testicular descend possibly with influence of cremaster muscle or other inguinal tissues. Therefore children with muscle diseases must be more carefully physical examined for presence of urogenital pathologies and paradoxically children with urogenital pathologies must be neurologically examined for presence of neuromuscular disorders.

PP1-142
CYCLOOXYGENASE-2 (COX-2) EXPRESSION AND ANGIOGENESIS IN GLIOBLASTOMA

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Background: Cyclooxygenase-2 (Cox-2) (the key enzyme that catalyzes the first steps in the biosynthesis of the prostaglandins from arachidonic acid) appears to play a role in the regulation of progression, invasiveness and angiogenesis of various neoplasms. Method: We analyzed the imunohistochemical expression of Cox-2 and angiogenic parameters (microvessel density and vascular patterns) in 54 glioblastomas. We also examined their relation with prognosis. Results: Cox-2 immunohistochemical expression was observed in 48 tumors (89%). There was no staining in six tumors (11%). On univariate analysis, MVD was correlated with a poor outcome (MVD>70, hazard ratio:0.441, 95% confidence interval: 0.200-0.975, p=0.041). But MVD showed no prognostic impact on multivariate analysis. Neither Cox-2 expression nor vascular pattern showed prognostic value. The difference in Cox-2 expression between the classical and bizarre vascular pattern in glioblastomas was statistically significant (p=0.047). But, no correlation was found between Cox-2 expression and MVD. Conclusion: These findings suggest that Cox-2 is heterogeneously expressed in glioblastomas without a significant association with microvessel density. However, Cox-2 expression may be related with vascular pattern in glioblastomas.

PP1-143
BRAIN PARENCHYMA CLEAR CELL EPENDYMOMA

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Background Ependymoma is a slow growing glial tumor originating from the ventricular or central canal lining and composed of neoplastic ependymal cells. In children, it usually arises in the posterior fossa. Exceptionally, it may occur in brain parenchyma, without any connection to the ventricular lining. Clear cell ependymoma is an uncommon and diagnostically challenging variant. Method We report a rare case of brain parenchyma clear cell ependymoma arising outside the ventricular system and occurring in a 12-year-old-boy and we describe the epidemiologic, clinicoradiologic and pathologic features of clear cell ependymoma. The differential diagnosis is discussed and similar published cases are reviewed. Results The patient was presented with 4-months history of symptoms related to intracranial hypertension. MRI brain demonstrated a heterogenous lobulated solid and cystic parietal mass, which measured 7 cm at high diameter. Tumor was partially excised. Histology, showed variable density of tumor cells. Many of these cells had a clear perinuclear halo. Perivascular pseudorosettes were obvious in most of the sections. Immunohistochemistry revealed positivity for GFAP, S-100. EMA showed granular cytoplamic positivity. Conclusion Two particularities are highlighted in this case: first the localization on brain parenchyma without connection to ventricular system, second the predominance of clear cells in this tumor.

PP1-144
ANTI-INFLAMMATORY AND ANTI-APOPTOTIC ACTION OF ATYPICAL 1,4-DIHYDROPYRIDINE COMPOUNDS IN NEUROTOXICITY MODELS

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Background. Our concept suggests that atypical (non-calcium antagonistic) 1,4-dihydropyridine (DHP) compounds which previously demonstrated anti-neurodeficiency activities in vivo models, may open new possibilities of neuroprotection provided by targeting mitochondrial and oxidative stress processes. These DHPs are: cerebrocrast (comprizing classical bi-cyclic DHP structure), glutapyrone and tauropyrone (glutamate- and taurine-containing monocyclic DHP structures). The present studies investigated ability of these drugs to protect neuropathologic changes in cultured nerve cells and cerebral cortex (ex vivo) caused by different neurotoxic agents which induce mitochondrial dysfunctions. Methods. 1.Assessment of cell death, free radical production and mitochondrial membrane potential in neurotoxicity model in cerebellar granule cells by use of neurotoxins: a) MPP+, b) oxygen-glucose deprivation (OGD), and c) glutamate; 2. Immunohistochemical assessment of apoptosis and astrocyte proliferation in cerebral cortex: caspase-3 and GFAP, a glial fibrillary acidic protein, expression in mice brain tissue after repeated i.p. administration (for two weeks) of DHP drugs per se, and DHP + anti-HIV drug azidothymidine (AZT), a well-known mitochondria-compromizing drug. Results. In MPP+ model, only cerebrocrast acted as neuroprotective agent by complete prevention of cell death, free radical production and drop in mitochondrial membrane potential. Amino acid-containing DHPs lacked these activities, however they showed neuroprotection in OGD (ischemia) and glutamate models. In the mice cerebral cortex, AZT induced major histopathological changes (pericellular and perivascular oedema, perivascular inflammatory infiltration), increased caspase-3 expression and caused an upregulation of astrocytes. Cerebrocrast considerably decreased these changes and reduced caspase-3 expression in AZT-induced neurotoxicity model (9 ± 1 vs.16 ± 2 cells/mm2, $P<0.004$), whereas glutapyrone and tauropyrone lacked this effect. Besides, tauropyrone demonstrated pro-apoptotic activity. Conclusion. The data obtained clearly demonstrate a beneficial neuroprotective - anti-apoptotic and anti-neuroinflammatory - action of cerebrocrast, a representative drug of atypical DHP series, opening new vistas in novel mitochondria-targeted drug design for the treatment of neurodegenerative disease related to mitochondrial and apoptotic damages (e.g. Parkinson's disease, Alzheimer's disease).

PP1-145
CD44, MEMBRANE TYPE 1- MATRIX METALLOPROTEINASE (MT1-MMP) AND KI–67 EXPRESSION IN ASTROCYTIC TUMORS

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BACKGROUND: Astrocytic tumors are the most common primary tumors of the brain. Astrocytic tumors include astrocytoma (Grade II), anaplastic astrocytoma (Grade III), and glioblastoma multiforme (Grade IV). Astrocytomas and glioblastomas are known to have high capacity of local invasiveness with uncommon extracranial metastatic ability. CD44's expression shows poor prognosis in breast and colon carcinomas and proposed to be an invasion marker in glioblastomas. Matrix Metalloproteinase (MT1-MMP) is an important factor promoting the cerebral invasion and neovascularisation of the gliomas. METHOD: H&E slides obtained from Cumhuriyet University Faculty of Medicine Pathology Department archieve belonging to 70 patients who were diagnosed as having astyrocytic tumor between years 1993 to 2005 were reevaluated according to the criterias of WHO classification accepted in 1993. Slides obtained from paraffin blocks of the cases were stained with CD44, MT1-MMP and Ki-67 immunohistochemically. RESULTS: Nine of the cases were pilocytic astrocytoma, 14 were diffuse astrocytoma three were anaplastic astrocytoma and 44 were glioblastoma multiforme. Statistical analysis of the study were performed between Low Grade Astrocytic Tumors (LGAT) (Grade I + Grade II) and High Grade Astrocytic Tumors (HGAT) (Grade III + Grade IV). CD44 and MT1-MMP expressions showed weak cytoplasmic and membranous staining in LGATs whereas they showed strong cytoplasmic and membranous staining in HGATs. There was a significant difference in cytoplasmic staining ratios and membranous staining intensities in MT1-MMP and CD44 expressions between LGATs and HGATs ($p<0.05$). Ki-67 proliferation index was found to be 4.54 ± 7.81 in LGATs and 18.31 ± 9.51 in HGATs ($p<0.05$). CONCLUSION: Ki-67 proliferation index was found to be directly correlated with increased tumor grade. As a result it is concluded that, CD44 and MT1-MMP may be useful as an invasion marker in astrocytic tumors and they are related with malign progression of gliomas.

PP1-146
FATAL FAMILIAL INSOMNIA (FFI): CLINICAL AND NEUROPATHOLOGICAL STUDY OF ANOTHER SPANISH FAMILY

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Fatal familial insomnia (FFI) is a transmissible prion disease linked to a mutation in codon 178 of the prion protein gene (PRNP) on chromosome 20. It is characterized by disturbed sleep-wake cycle, resulting in daily vigilance perturbations and a variety of other neurological symptoms. We present the clinical and neuropathological findings, and genotyping of three relatives of a Spanish family affected with fatal familial insomnia. The patients were maternal cousins, (43, 49 and 53 years old) and the mother of two of them was previously diagnosed of Gerstmann–Straüssler–Scheinker disease (GSS) in another institution based exclusively on neuropathologic study. The patients developed a rapidly evolving disease with alterations of sleep-wake cycle and behavioural disorders as the initial symptoms and died between 4 and 12 months after the onset of the illness. A full neuropathological examination done at the Navarre Neurological Tissue Bank showed thalamic and olivary degeneration with isolated and mildfocal cortical spongiosis. In the genetic examination all the patients harboured the prion protein (PrP) D178N (ASP178ASN) mutation and were homozygous Met/Met in codon 129. Based on the cases presented here, we discuss typical clinical symptoms and neuropathological findings. In particular, we outline how genetic testing is mandatory in the diagnosis and may lead to the identification of a risk of a genetic prion disease.

PP1-147

THE STATUS OF MGMT METHYLATION AND TP53 MUTATIONS IN PATIENTS WITH GLIOBLASTOMA

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Background: MGMT (O – 6 – methylguanine – DNA - methyltransferase) is a DNA-repair enzyme that specifically removes promutagenic alkyl groups from the O-6 position of guanine and protects cells from cytotoxic and mutagenic effects. Epigenetic silencing of O (6) – methylguanine - DNA methyltransferase (MGMT) by promoter methylation can leads to lack of DNA-repair, increased sensitivity to alkylating chemotherapeutic agents and a higher susceptibility to TP53 transition mutations, especially G:C/ A:T transitions. The aim of our study was to assess the correlation of promoter methylation of the MGMT gene with TP53 mutations and the clinical characteristics of glioblastomas. . Methods : Tumor samples were obtained from 32 patients with primary glioblastoma (15 males and 17 females, aged from 40 to 75 years). The methylation-specific PCR (MSP) was performed in a two-step approach .The PCR products were separated on 3% agarose gel containing ethidium bromide and documented using the Gel Doc1000 Bio Rad Image System. For each PCR reaction, methylated and unmethylated DNA were included as positive and negative control. Results : Methylation of the MGMT promoter was detected in 72% (23 of 32) of tumors. 31% (10 of 32) of all tumors presented TP53 mutations. 90 % of TP53 mutations were detected in tumors that also presented MGMT methylation. We further correlated the results with survival. Conclusions: Methylation of the MGMT promoter gene is a freguent molecular event in glioblastoma.There was no statistical correlation between TP53 mutations MGMT methylation (Fisher exact test p=0.223). Coexistence of MGMT methylation and TP53 mutations in patients with glioblastoma was not associated with the best prognosis.

PP1-148

ANGIOLIPOMA OF SUPRASELLAR AND SELLAR REGION – CASE REPORT

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INTRODUCTION Angiolipomas are benign mesenchymal neoplasms. The forearm is the most common site, followed by the trunk and upper arm. Within the craniospinal axis, angiolipomas are extremely rare, and almost all have been found in the spinal extradural space. Here we present two patients with suprasellar and sellar angiolipoma. CLINICAL SUMMARY Patient 1 – A 53-year old man presented to neurologist six months before initial neurological evaluation with sudden intensive headache, vertigo and deficit in lower part of visual field. Magnetic resonance imaging disclosed an expansive process in pituitary gland that was isointense compared to brain tissue on T1-weighted images, whereas T2-weighted images and the gadolinium enhanced T1-weighted images showed a strongly hyperintense, homogeneous lesion. Patient 2 – A 49-year old woman was admitted to hospital because of intensive headache, fatigue, vertigo and visual deficit. Magnetic resonance showed a expansive process within sellar region extending to the adjacent cavernous sinus. T1 and T2-weighted and the gadolinum enhanced images were identical to Patient's 1. METHODS AND RESULTS Paraffin embedded tumors of both patients were examined by staining section with hematoxylin and eosin and with Mallory, and immunohistochemistry method with vimentin and actin. Light microscopy revealed a very vascular tumor composed of dilated capillaries and mature adipose tissue. Vessels were composed somewhere of only flattened single layer of endothelial cells and others showed a few layers of smooth muscle cells. Interstitium consisted of mature adipose tissue with mast cells and collagenous stroma. CONCLUSION Angiolipomas represent mesenchymal hamartomas composed of abnormal blood vessels and mature adipose tissue. These tumors are extremely rare within craniospinal axis. According to the literature, only four patients have been reported with angiolipoma in suprasellar and sellar region.

PP1-149

USEFULNESS OF IN SITU DEMONSTRATION OF 1P LOSS AND P53 OVEREXPRESSION IN PATHOLOGIC DIAGNOSIS OF OLIGODENDROGLIAL TUMORS

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BACKGROUND: Recent development of adjuvant chemotherapy effective to oligodendroglial tumor increases the requirement of an accurate pathologic diagnosis of this tumor. However, the differentiation between astrocytic and oligodendroglial tumors is not always straightforward. To improve the diagnostic accuracy of diffuse gliomas, we intended to compare pathologic findings to chromosome 1p status and overexpression of p53. DESIGN: The cases studied included 12 oligodendrogliomas (6 grade II, 6 grade III), 6 oligoastrocytomas (1 grade II, 5 grade III), 1 pilocytic astrocytoma, 9 astrocytomas (4 grade II, 5 grade III), and 10 glioblastomas. On the paraffin sections, 1p loss was analyzed using the fluorescence in situ hybridization (FISH) method. When the ratio of nuclei containing a single 1p signal and two centromere 1 signals is more than 50%, the case was evaluated to be positive for 1p loss. Immunohistochemically, the expression of p53, GFAP and Olig2 was studied. In addition, double immunostaining for GFAP and Olig2 was carried out. RESULTS: FISH analyses demonstrated the 1p loss in 83% of oligodendrogliomas and 50% of oligoastrocytomas, but not in any astrocytic tumors except for 1 grade II astrocytoma and 1 glioblastoma. No detectable microscopic differences were noticed between 1p loss-positive and negative cases in oligodendroglial tumors. Overexpression of p53 was detected in 5 oligodendroglial tumors, all of which corresponded to 1p loss-negative cases, as well as in 40% of astrocytic tumors including glioblastoma. Olig2 expression was seen in all gliomas studied and GFAP was also commonly expressed in most oligodendroglial (78%) and all astrocytic tumors. Double immunostaining for Olig2 and GFAP demonstrated that the expression of these antigens was mutually exclusive in most cases and that the coexistence in a single cell was noted in only 30% of glioblastomas. CONCLUSION: The present study confirmed that 1p loss was almost specific to oligodendroglial tumors and demonstrated that the overexpression of p53 in oligodendroglial tumors was restricted to those without 1p loss. Although it seems hard to predict the 1p status microscopically, p53 immunohistochemistry may provide useful information about the chromosomal abnormality. Olig2 and GFAP were frequently expressed in both gliomas, indicating a limited role for the differential diagnosis. Considering the mutually exclusive localization of these antigens in low and moderate grade gliomas, the coexistence may indicate the severe disruption of cell differentiation in glioblastoma.

PP1-150
FASCIN EXPRESSION IN GLIAL TUMORS AND PROGNOSTIC VALUE
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Backround: Glial tumors, especially high grade forms are known as aggressive malignancies, with generally short survival time. So, determination of prognostic parameters is always important. But prognostic parameters of glial tumors are quite limited and histologic grade of the tumor is accepted as the most important prognostic factor. While tumor histologic grade increases, mean survival time remarkably shortens. In addition to tumor histologic grade, to detect new prognostic parameters, especially molecular and genetic alterations are interesting study subjects. Fascin, an actin-bundling protein, is one of those molecules and its contribution to prognosis investigated in many tumors. Method: In this study, fascin expression of glial tumors correlated with histologic grade and investigated prognostic value. 76 glial tumors (44 glioblastomas with known survival time, 18 anaplastic astocytomas, 6 diffuse astrocytomas, 8 pilocytic astrocytomas) were examined immunohistochemically for fascin expression. Results: As results for this study, significant correlations have been found between "distribution and intensity of fascin expression" and tumor histologic grade. In glioblastoma group; survival rates were statistically higher for cases that ≤ 50 year-old and express weak fascin intensity ($p< 0.05$). In addition, age and "distribution-intensity score of fascin expression" found as independent prognostic factors in glioblastomas ($p< 0.05$). Conclusion: In conclusion, fascin expression levels are correlated with tumor grade and fascin overexpression may play an important role in the biologic behavior of glial astrocytic tumors and in their prognosis

PP1-151
FASCIN EXPRESSION IN ASTROCYTIC NEOPLASMS
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BACKGROUND: Fascin is a 55-kd actin-bundling protein encoded by the human homolog for sn (hsn) gene. It is found in cell membranes and promoting cellular motility. Fascin has been linked to the malignant biological behavior of some tumors. We investigated immunohistochemically fascin expression in astrocytomas and in gliosis cases. METHOD: Immunohistochemical study was performed in 28 astrocytic tumors and in 6 gliosis. Fascin positivity of tumor cells was graded as follows: no staining or less than 5% as 0, 5% to 25% as (1+), 26% to 50% as (2+), 51% to 75% as (3+), more than 75% as (4+). RESULTS: There were 18 males and 10 females (mean age, 47.6 years) in astrocytoma group. Eighteen of 28 cases showed positive fascin staining (64%). Two of seven WHO grade I cases showed positive fascin staining (28.5%). Four of six WHO grade II cases showed positive fascin staining (66%). Four of eight anaplastic astrocytomas cases showed positive fascin staining (50%). All of GBM cases (7 cases) were positive (100%). All of gliosis cases (3 males, 3 females) were positive. Immunohistochemically fascin staining was intracytoplasmic in tumor cells whereas paranuclear staining was seen in astrocytic cells in gliosis cases. Fifteen tumors showed more than 75% positive staining tumor cells, 3 tumors had 50% to 75% staining. In comparison, 6 of 13 WHO grade I and grade II astrocytomas had fascin positivity (46%), 12 of 15 WHO grade III and IV astrocytomas had fascin staining (80%). CONCLUSION: Higher grade astrocytic tumors generally expressed a greater degree of fascin staining. But for it shows reactivity in gliosis too, it is not helpfull in distinguishing tumor and gliosis. Fascin expression may play a role in malignant biological behavior of astrocytic neoplasms.

PP1-152
HDAC, MGMT, P53 AND ACTIVATED AKT/PKB PROFILES IN ASTROCYTIC GLIOMAS
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BACKGROUND/AIM: Response to alkylating and methylating agents currently in use for astrocytic glioma treatment is considered to depend on p53 status or MGMT (O6-methylguanine DNA methyltransferase) expression. MGMT silencing, usually a result of promoter methylation, can also be achieved by HDACs (histone deacetylases), which, in addition, promote survival by keeping AKT/PKB in the phosphorylated and p53 in the deacetylated status. The aim of this study was to identify immunophenotypic/functional profiles of astrocytic gliomas in regard to proteins targeted by newly developed anticancer drugs. METHODS: Eleven protein markers (acetylated histones H2A, H2B, H3, H4, lysine specific histone demethylase-1 [LSD1], HDAC1, 3 and 5, MGMT, p53, and phosphorylated / activated AKT/PKB [pAkt-Ser473]) were investigated in 42 astrocytomas (28 grade IV, 7 grade III and 7 grade II). Immunostaining evaluation cut-offs were set at 10% strong staining in tumor cells for all markers except acetylated histones and LSD1. RESULTS: Acetylated histones and LSD1 were ubiquitously expressed in all categories of tissues tested, benign and malignant, whereby strong positivity for H2B was predominantly found in grades II and III ($p=.013$). Aberrant expression of HDACs, MGMT, p53 and pAkt was collectively observed in 39/42 (92.9%) astrocytic gliomas. HDAC1 and/or HDAC5 expression were observed in 29/42 (69.1%) cases, wherefrom in 5/14 (35.7%) grade II/III and in 24/28 (85.7%) grade IV tumors ($p=.001$). HDAC1 positive tumors were exclusively grade IV (12/28 [42.9%], $p=.004$), while coexpression of both HDACs occurred in 9 cases. MGMT and p53 expression (19/42 [45.2%] for both, different cases) were unrelated to tumor grade but showed inverse correlation to each other ($p=.025$), justifying the reported need of wild-type p53 for MGMT expression. The same incidence (45.2%) was observed for strong cytoplasmic pAkt-Ser473 positivity, which was associated with grade IV tumors ($p<.001$). HDAC expressing tumors with phosphorylated AKT/PKB (15/42 [35.7%]) were either MGMT or p53 positive ($p<.0001$ and $p<.001$, respectively). CONCLUSIONS: This study shows that the majority of astrocytic gliomas, mainly those of grade IV, express HDACs 1 and/or 5, and that half of these tumors have activated AKT/PKB while simultaneously expressing MGMT or p53. The above profiles may explain the commonly observed failure in classic and recently established astrocytic glioma treatment and, if validated in larger studies, they seem to justify the need for therapeutic interventions with HDAC activity in this group of tumors.

PP1-153
ANGIODYSGENETIC NECROTIZING MYELOPATHY (FOIX – ALAJOUANINE SYNDROME): REPORT OF TWO AUTOPSY CASES
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In 1926 Foix and Alajouanine described for the first time two postmortem cases with spinal arteriovenous malformations. These occur in adult males, clinically they often involve low thoracic and lumbosacral spinal segments, are manifested with paraparesis, gradual sensory disturbances in the lower limbs, sphincters disfunction and impotence (Foix-Alajouanine syndrome or angiodysgenetic necrotizing myelopathy). Two

postmortem cases are reported in which the clinical manifestations and the arteriovenous malformations were present. Both patients died of pulmonary embolism. There are around 50 case reports in the world literature about this entity. Two cases with the clinical features above described and in which a spinal arteriovenous malformation was documented by autopsy, are presented here. The first was that of a 60 year old man with severe pain and inability to stand up. Later on had weakness of the lower limbs, alterations of sensation and difficulty to urinate. Died 23 days after admission from pulmonary emboli. The spinal cord had a large number of dilated and tortuous subarachnoid veins on its dorsal surface. The second case was that of a 46 year old male with motor deficit of the left lower limb which evolved to paralysis of both pelvic extremities, sensitivity disturbances and urinary retention. Died on day 13 of his hospitalization from pulmonary emboli. An extensive intraspinal haemorrhage was found. In both cases abnormal intraspinal and subarachnoid vessels were identified. They showed tortuosity, dilatation and collagenization of their walls. Changes of hypoxic myelopathy were present. It has been proposed that there is a direct communication between arterial and venous systems throughout abnormal vessels ("dilated" veins) without passing through capillaries as is seen in normal conditions. The blood flows from arteries to veins, so the blood pressure of venous system increases and an ischemic damage is produced in the spinal cord because of degeneration of the walls of vessels that are not designed to support elevated pressure, besides there is a progressive occlusion. Venous hypertension per se predisposes to stasis and thrombosis, so there is spinal necrosis and irreversible neurological damage. In conclusion it should be emphasized angiodysgenetic necrotizing myelopathy is an entity with a prognosis that depends of a correct and early diagnosis, so it is very important to use imaging techniques as myelography and spinal angiography in those patients that present a spinal cord syndrome, and to establish timely surgical management.

PP1-154
GIANT ARTERIOVENOUS MALFORMATION IN THE BRAIN. REPORT OF SEVEN AUTOPSY CASES

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Arteriovenous malformation (AVM) is an abnormal communication between arteries and veins without an intermediate capillary network. They are usually seen in the fourth and fifth decades of life and more commonly in males. They cause approximately 5% of the parenchymatous hemorrhages in the brain. They can cause ischemic damage by the "steal phenomenon" which results from the arteriovenous shunt. Classically they have been divided in capillary telangiectasias, cavernous angiomas and arteriovenous malformations, but now the are all considered in one group and behave like pseudotumoral lesions. The usual sequence in the formation of the AVMs is first dilatation of the veins with compression of the neighboring tissue, then replacement of the muscular wall by collagen which confers to the cut surface a sand paper texture. Seven cases of giant arteriovenous malformations of the brain are presented. Four were in males and three in females. In all of them chronic headache was present. Three manifested with haemorrhage, one with multiple infarcts due to "steal phenomenon" and one with hydrocephalus due to compression of the aqueduct. Five were clinically diagnosed and treated and two were autopsy findings. About 1% of parenchymal hemorrhages are due to AVMs. Clinically they manifest by parenchymal bleeding in 50% of cases, with seizures in 20% to 25%, headache in 15% and focal deficits in <5%. In children of less than 2 years of age heart failure is not uncommon. The "steal phenomenon" is rare at any age. Nowadays the diagnosis is made by CT, MRI and arteriography both carotid and vertebral. The size and topography of the AVM are essential to institute the correct treatment. In 1986 Spetzler and Martin proposed a system of grading based on the size, location and pattern of venous drainage. The scale has 5 grades and is used to evaluate the relative risk and the selection of the appropriate therapy. Four modalities of treatment exist, namely monitorized follow up, microsurgery, endovascular surgery and radiosurgery. Deterioration due to treatment has been observed in 19% in patients with grades I and II, in 25% in those with grades III and in 42% of patients with grades IV and V. Therefore, surgical treatment is not recommended in high grade cases. The cases presented here show that the size of the lesion is the most important factor in the outcome, which can be hemorrhage, "steal phenomenon" and massive dilatation of the vein of Galen.

PP1-155
THE DETECTION OF DIFFUSE AXONAL INJURY IN HEAD TRAUMAS, THE ROLE OF BETA AMYLOID PRECURSOR PROTEIN IN DIAGNOSIS

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One of the most important factors affecting mortality and morbidity in head trauma cases is diffuse axonal injury (DAI). The aim of this study is to prospectively study DAI both macroscopically and microscopically in brain samples obtained from head trauma cases whose autopsies were carried out in Mortuary department of the Council of Forensic Medicine. We also aimed to analyze the relationships between the DAI, survival time and other traumatic findings in autopsy. Of the 70 cases involved in this study, 64 (91,4 %) were male and 6 (8,6 %) were female and those having 3-24 hours survival time after the trauma constituted the most crowded group (28,6 %). Traffic accidents were found to be the major trauma type with a frequency of 54,4 %. While DAI was detected in 22 cases (31,4 %) by microscopic examination of Hematoxyline- Eosin (HE) sections, it was detected in 33 cases (47,1 %) by evaluation of the immunohistochemical Beta-Amyloid Precursor Protein (β-APP) treated sections. When the regions of the brain were taken into consideration in DAI cases, axonal bulbs as a finding of the axonal injury were detected to be in corpus callosum in all cases (100%), rostral brainstem in 77,2 %, internal capsule in 63,6 % and parasagittal white matter in 9 % of the cases. In evaluation of the relation of the morphological findings of axonal injury to the survival time after the trauma, the early morphological findings of the injury was demonstrated to be present at about 12 hours by an examination with HE but at 4-5 hours by an examination using β-APP. In none of the cases with a survival time of 3 hours or less, axonal injury was detected by using the both techniques. There seemed an increase of the density and degree of widespread in relation to survival time after the trauma and the most intensive β-APP positivity was detected in the cases with a survival time of over 8 days. The relationship between the development of axonal injury in the brain and type of head trauma, post-mortem interval, scalp lesions detected at autopsy and other parameters such as skull fracture, bleeding and contusion was insignificant. In conclusion, in all head trauma cases, regardless of the type of the trauma, DAI must be taken into consideration and the brain must be thoroughly examined both macroscopically and microscopically.

PP1-156
CORRELATION OF PROLIFERATIVE CAPACITY (MIB-1) AND ANGIOGENESIS (VEFG AND BFGF) IN HISTOLOGICAL SUBTYPES OF MENINGOTHELIAL TUMORS

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Introduction: Tumors of the central nervous system show highly complex growth capacity that is end result of interaction between cellular proliferation and growth factors controlling angiogenesis. By and large, growth factors of controlling vasculogenesis have crucial importance in neurooncology. Methods: In a cohort of 37 cases containing typical (n=20) [grade I]; atypical (n=11) [grade II and malignant (n=6) [grade III] meningiomas classified according to demographic (age, gender, site) and histological subtypes. Proliferative capacities of each individual tumor were assessed by MIB-1 whereas angiogenetic markers bFGF and VEFG for their growth manipulation and angiogenesis, respectively. Results: WHO 2000 classification was used not only for grading but for subtyping in meningothelial tumors. Higher the grade of tumors, more apparent hypercellularity, necrosis, mitosis, sheeting and brain invasion were. By and large, vascular, nuclear and cytoplasmic immunoreactivity of bFGF correlated as higher the grade, more prominent the expressivity. It should be emphasized intratumoral vascular bFGF correlated with tumor grade ($p<0.05$). Furthermore, MIB-1 LI correlated with vascular bFGF immunoreactivity positively and it appeared to be statistically significant ($r=0,543$, $p<0,001$). Neither nuclear bFGF nor cytoplasmic bFGF immunoreactivity correlated with different grades ($p>0,05$). VEFG immunoreactivity was found to be highest expressivity in malignant meningioma group, particularly in papillary subtype. Conclusion: MIB-1 LI is a precise marker for cell kinetics that directly proportional between grade I vs. grade II, but not useful for differentiating grade III from the former two. There was no significant correlation between cytoplasmic VEGF with that of cytoplasmic and nuclear bFGF immunoreactivity suggesting the meager interaction between these two molecules. Moreover, tumors profoundly proliferating showed a proportional and selectively higher vascular bFGF proposing close correlation between proliferating tumor cell and its vascular supply (angiogenesis). Atypical and malignant meningiomas those bearing microvascular rich matrix were under control of not only high cell kinetics but also vascular growth factor, i.e., bFGF and VEGF. Abbreviations: LI: Labeling index / WHO: World Health Organization / VEGF: vascular endothelial growth factor / bFGF: basic fibroblast growth factor /

PP1-157
LOH ON 1P/19Q AND 10Q AND Ki-67 LABELLING INDEX IN GLIAL TUMORS

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Backgrounds: Many studies have shown that molecular and cytogenetic findings offer a more encouraging view of the behavior of glial tumors. The loss of special genes can be related to tumor progression. LOH of the oligodendroglioma-associated tumour suppressor genes suspected on 1p and 19q have been shown in mostly oligodendrogliomas and closely correlated with the oligodendroglioma phenotype and the survival of patients with these aberrations considered more favorable. Same chromosomal aberrations can also occur in some astrocytomas as well.. Also loss of chromosome 10 is common in glioblastoma multiforme (GBM) and pivotal events as the lesion passes from grade III to IV . About Ki-67 labelling index (LI), various percentages are being reported in various studies depending on the grade of glial tumors. In this study, LOH of chr 1p/19q and 10q , their relation with survival and Ki-67 LI in GBMs have been investigated Also a few oligodendroglioma cases included to compare . Method: To study allelic deletions at chromosomes 1p,19q and 10q, we obtained DNA from peripheral blood and also tumor samples of 19 patients. 8 microsatellite markers were used for PCR-LOH analysis. LOH analysis were performed by ABI 31O Genotype. Data were collected automatically and analysed by Gene- Scan programme. Results: There were 13 GBMs, 3 anaplastic astrocytomas and 3 oligodendrogliomas. In 2 oligodendrogliomas, neither 1p/19q LOH nor 10qLOH were found but LOH 1p was seen only in one oligodendroglioma.Ki-67 LI was found 1% in two of them, interestingly 10% in one who has no LOH of these chromosomes. Among anaplastic astrocytomas LOH at 10q was detected only in one patients. Ki-67 LI was greater than 10% for all anaplastic astrocytomas. In 13 GBMs only one patient showed LOH of 1p and 10q . In this case Ki-67 LI was lower than 10% conversly from others whose LI were greater than 10%. Conclusion: Although LOH 10q is the most frequent genetic alteration in primary GBMs, LOH of chromosome 19q is common in secondary GBMs and loss of both the whole p arm of chromosome 1 and q arm of 19 is closely correlated with the oligodendroglial phenotype; not all oligodendrogliomas and GBMs have these chromosomal deficiency and the unpredictable cases do. These data demonstrate that despite rapid progress with these molecular features, histologic features still the most important characteristics of glial tumors. Ki-67 LI is objective grading marker for glial tumors.

PP1-158
VEGF AND VEGF RECEPTORS IN HUMAN MEDULLOBLASTOMA

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BACKGROUND: Medulloblastoma (MB) is the most common childhood malignant brain tumour. Despite recent progress in prognosis, a third of these children succumb to their disease whereas the remaining ones suffer significant long-term side effects. Neoangiogenesis seems to be a critical factor for tumour growth and progression and a pre-requisite for metastases. VEGF is one of the most powerful mitogen for endothelial cells in central nervous system tumours. The different isoforms bind to two tyrosine kinase receptors(Flt-1 or VEGFR-1 and KDR or VEGFR-2) and, upon binding, the receptor is phosphorylated thus triggering the intracellular transduction signal pathway. Although VEGF receptors were initially found on endothelial cells, recent studies have shown that tumour cells of different origins express VEGF receptors too. This suggest that VEGF may act as an autocrine signal. Starting from these considerations, we investigated the role of VEGF/VEGFR signalling on MB growth. METHOD: 13 paraffin embedded cases of classic MB were analyzed to determine expression of VEGF, VEGFR-1 and VEGFR-2. Transcript and protein expression of the same targets were also analyzed by RT-PCR and western-blot techniques on 3 MB cell lines, along with their functional status. RESULTS: Cells of our MB cases showed an intense and diffuse cytoplasmic expression of VEGF. VEGFR-1 expression was lower and more heterogeneous than VEGF but with several cells reacting intensively. VEGFR-2 expression was

lower than VEGF and VEGFR-1. All targets showed a stronger staining in the interfollicular than in the follicular areas. Positive reaction for VEGF and its receptors was also seen around the endothelia of tumour-supplying vessels. Semiquantitative analysis of VEGF and VEGFR expression was also performed and generally showed moderate to high expression levels. VEGF and its receptors were expressed in all the MB cell lines and were functional. CONCLUSION: Immunohistochemical analysis of MB specimens and the in vitro studies of MB cell lines support an autocrine role of VEGF in vivo given the concomitant expression of VEGF and its cognate receptors in MB cells.

PP1-159
ANALYSIS OF PURKINJE CELLS AND CEREBELLUM VOLUME IN FEMALE OBESE RATS: A STEREOLOGICAL AND HISTOPATHOLOGICAL STUDY

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Background: It was previously demonstrated that obese people have reduced grey matter (GM) in several brain areas, including regions implicated in the regulation of taste (i.e., inferior frontal operculum and postcentral gyrus), reward (i.e., putamen), and behavioural processing (i.e., middle frontal gyrus), compared with their lean counterparts. It is well established that the brain may serve as a direct target for adiposity signals, one of the most important being leptin, adiponectin, resistin i.e… We investigated the relationships between high fat diet intake and cerebellum morphology. Methods: In this study, twenty adult female Sprague Dawley rats (150-200 g each) from the Experimental Research and Application Center (Ataturk University, Erzurum, Turkey) were randomly allocated into two equal-sized groups. In the control group, rats were fed a commercial rat diet (7-10 % fat, 68-70% carbohydrate, 18-20% protein, 1-2% vitamins and minerals; 210 kcal/100 g/day) for 3 months, and rats in the high-fat diet group were fed a high-fat diet (30% calories as fat) for the same period of time. Cerebellum of control and high fat diet fed rats were analyzed stereologically and histopathologically. Results: Both numerical density and total number of Purkinje cells was significantly reduced ($p < 0.01$). Also volume of the cerebella was increased when compared with those of the controls due to edema. Conclusion: As a result of the study, it was thought that high fat intake may cause some of the abnormalities in brain morphology and morphometry associated with excess body fatness.

PP1-160
CELL SIGNALLING FACTORS IN GLIOMAS: HIGH P4EBP1 AND TGFB-PSMAD2 ACTIVITY ASSOCIATE WITH MALIGNANT ASTROCYTOMAS AND GLIOBLASTOMAS

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BACKGROUND: Gliomas are the most frequent primary CNS tumors. Four grade of malignancy are actually admitted by the WHO classification and Glioblastoma multiforme (GBM), the most frequent grade IV glioma, is considered one of the most aggressive neoplasms. It can derive from low grade (AGII) and anaplastic astrocytomas (AGIII),or arise de novo. Secondary glioblastoma usually harbour overexpression of PDGFR, a mutated p53 (over 60%) and PTEN alteration (around 10% of cases). GBM de novo show EGFR amplification (30%), p53 mutations in less than 30% and loss of heterozygosity 10q or PTEN mutations in over 70% of cases. Moreover, it has been recently described that high TGFβ-pSMAD2 activity confers poor prognosis in glioma affected patients. In previous studies we identified that the factor p4EBP1, a protein that plays a control role in selective protein synthesis, acts as a funnel factor in breast and ovarian carcinomas, reflecting the oncogenic role of this signalling pathway. The aim of this study is to analyse the role of these pathways in astrocytic tumors evaluating involved factors expression. DESIGN: tissue microarrays of 67 astrocytic tumours were performed, including 40 GBM (59.7%), 9 AGIII (13.4%), 7 AGII (10.5%) and 11 (16.4%) pilocytic astrocytomas (PA). Immunohistochemistry for p53, p4EBP1, MAPK, pSMAD2 and AKT was done. Levels of expression were semiquantitatively evaluated as percentage and intensity of stained tumoral cells (histo-score [H score]). RESULTS: high expression of p4EBP1 was found in 100% of GBM and more than 60% of AGIII, while all the AGII and PA showed the lowest scores. Almost 100% of GBM, >90% of AGIII and AGII showed high expression of pSMAD2, nevertheless 85% of PA showed lower levels. Conversely no differences ERK1-2 expression were seen among the four groups of gliomas. CONCLUSIONS: These results show that overexpression of p4EBP1 is strongly related to anaplastic astrocytomas and glioblastomas (more than 60% and 100%) and may also discriminate tumoral progression between grade II and III astrocytomas. Furthermore, we could observe that high TGFβ-pSMAD2 expression correlates with diffuse astrocytomas and GBM, while this molecular pathway shows lower activity in better prognosis tumours as PA, therefore TGFβ-pSMAD2 may be a prognostic molecular marker. Ongoing studies are analyzing the association between p4EBP1 and its upstream markers and TGFβ-SMAD2 activity as well as with survival.

PP1-161
OLFACTORY NEUROBLASTOMA: A STUDY OF 7 CASES

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Background : Olfactory neuroblastoma is a rare malignant neuroectodermal tumor, accounting for nearly 1,2 % of all naso-sinusal malignancy. It can occur in all age groups, without sex or racial predilection. The aim of our study is to specify the clinico-pathological, the differential diagnosis, the treatment and the behaviour of this tumor. Material and Methods : We studied seven cases of olfactory neuroblastoma diagnosed in the department of pathology during 20 years. Clinical and radiological informations were collected from patient files. Morphologic characteristics provided from histopathological reports. The grade of the tumors was established according to the grading scheme proposed by Hyams, based on tumor differentiation and varying from I to IV in surgical samples. We evaluated the tumor extension according to the Kadish staging system. Results : Our population is composed by fives women and two men with a mean age of 36 years. The presenting symptoms were mainly nasal obstruction and epistaxis in all cases associated to ophthalmic or neurological symptoms due to loco-regional involvement in two cases. Two cases were classed grade I, Three grade II, one grade III and one case grade IV according to the Hyams grading. On Immunohistochemistry, NSE (Neuron Specific Enolase) was expressed in all cases. The other markers were expressed differently in cases. Four cases were classified stage B, two stage C and one stage A according to the Kadish staging system. All patients were treated by surgery

followed by radiotherapy in four cases. Recurrence was seen in three cases (respectively after 2 months, 3 months and one year). Two patients (grade II, Stage B and grade II, stage C) were alive respectively after a period of one and three years, one patient (grade I, stage A) was alive after a period of 9 years. No information was available on the outcome of four cases. Conclusion: Olfactory neuroblastoma is a locally aggressive tumor. Craniofacial resection is presently the preferred surgical therapy. Multiples local recurrences are frequent. Postoperative adjuvant radiation therapy is useful for cases with close margins or for recurrent disease. Chemotherapy is indicated for advanced stages. Our study confirm us the useful of the grade and the stage as a prognostic factors. Since, we noted a correlation between theses parameters and the behaviour of patients.

PP1-162
AMOEBIC CEREBRAL ABSCESS MIMICKING BACTERIAL MENINGITIS: CASE REPORT

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Background: Entamoeba Histolytica is a well-known cause of infectious colitis, typically producing diarrhea and occasionally frank dysantery. This organism may also spread to involve extraintestinal sites such as the liver, lung, central nervous system, usually in the form of amoebic abscess Cerebral Amoebiasis is a rare and dreadful complication of E. Histolytica infection. Case Presentation: We report a case of an amoebic brain abscess (due to Entamoeba Histolytica) in a 2-year-old-girl, with symptoms mimicking meningitis with no evidence of disease elsewhere. Aspiration of the abscess revealed the organism, and the abscess regressed in response to specific medical treatment. This article reviews the rarity of cerebral amoebic abscesses and the difficulty of the diagnosis. Conclusion: As it is demonstrated by this case, the alert surgical pathologist has the potential to be the first to suspect and establish the diagnosis. This case report is to note that the patients with cerebral amoebic abscess may show any clinical evidence of extracerebral amoebic lesions which is important for early diagnosis and management to achieve cure.

PP1-163
SELECTIVE ATROPHY OF TYPE II MUSCLE FIBERS IN MYASTHENIA GRAVIS

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Background: Selective atrophy of type II muscle fibers has been reported in the literature as a common change in muscle biopsy in limited series of myasthenia gravis (MG) patients and it was considered as a possible prognostic marker. We performed histomorphometric study to confirm the previous findings in a larger cohort of patients and we searched for correlations between the fiber atrophy and the clinical features of the disease to evaluate possible prognostic implications. Methods: Muscle biopsies (sternothyroid muscle) from 179 patients with pure MG were obtained during thymectomy. Histochemical staining (myofibrillary ATPase) and immunohistochemistry (myosin heavy chain) was performed for muscle fiber typing in tissue cryosections. Histomorphometrical study with statistical evaluation of histograms was preformed using computer assisted image analysis. Clinical data of the patients were analyzed and correlated with the morphometrical results. Results: The statistically significant type II fiber atrophy was observed in only 23 (12,8%) cases of MG. No significant association was disclosed between the duration and severity of the disease or outcome of the patients. The only significant relationship was demonstrated between the type II fiber atrophy and long-term use of corticosteroids (methylprednisolone in particular). Conclusion: Type II fiber atrophy in MG muscle represents rather a steroid-induced change (i.e. chronic steroid myopathy, either clinically apparent or subclinical) than a morphological feature of the MG proper. Prognostic value of type II fiber atrophy in muscle biopsy in MG could not be demonstrated in our series. Supported by the Grant of Ministry of Health of the Czech Republic No. IGA MZCR NR/8924-3 and by the Research Project VZ FNM 00064203.

PP1-164
MENINGIOMA; A CLINICOPATHOLOGICAL PROFILE OF 300 ARCHIVE CASES

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Background: Meningiomas are the most common benign tumors of the CNS. Meningiomas usually have good long-term prognosis after surgical excision but some manifests aggressive clinical behavior leading to increased patient morbidity and mortality. Method: In this study we retrospectively review meningiomas operated on in SB Ankara Diskapi Y.B. Research and Training Hospital, 2nd Neurosurgery Clinic between years of 1995-2005. There were 300 cases which were pathologically examined in Pathology Clinic of the same hospital. Patient age, sex, tumor localization and tumor subtype are documented and reviewed. Results: The data about age could be obtained for 286 patients. Mean age was 50.72 ±13,394. Mean ages were 51.05±12,91 and 50.56±13,66 for males and females respectively.Over 300 patients, 200 was female and 100 were male with a female/male ratio of 2:1. Among 287 cases with known tumor localization, 49(17,1%) were convexity meningioma, 66(23%) falx-parasagital meningioma, 51(17,8%) tentorium-posterior fossa meningioma, 88(30,7%) skull base meningioma, 28(9,8%) spinal meningioma, 5(1,7%) intraventricular meningioma. Mean ages for these locations were 51.27, 52.08, 49.9, 48.44, 55.46, 47.4 respectively. There was no statistically meaningful association between age and localization (p=0,084). Among 300 pathological examinations, meningiomas were subtyped in 203 cases. The most common meningioma subtype was meningothelial meningioma with 86(43.3%) cases. The others were as follows: transitional 37(18.3%), fibroblastic 29(14.3%), psammomatous 22(10.8%), malignant 10(4.9%), angiomatous 8(3.9%), atipical 7(3.4%), metaplastic 2(1)%, microcystic 1(0.5%) and secretory 1(0.5%). Frequencies for locations of different subtypes demonstrated great variation. The skull base was most frequent site of location for meningothelial, fibroblastic, atipical and malignant. The rate of involvement of skull base in meningothelial, fibroblastic, atipical and malignant meningiomas were 36.1%, 27.6%, 50%, 33.3% respectively. Spinal cord was the most frequent site for psammomatous meningioma with the percentage of 55%. The difference for localizations of different histological subtypes were statistically non significant. Conclusion: Our results are in concordance with the literature data. In conclusion meningiomas are benign tumors seen predominantly in females who were in their 5th and 6th decades of life, commonly located in the skull base. They are mostly grade 1 tumors with the most common subtypes being meningothelial, transitional, and fibroblastic meningiomas

PP1-165
CELL DEATH AND SURVIVAL MECHANISMS IN THE HIPPOCAMPAL TISSUE OF PATIENTS WITH MESIAL TEMPORAL SCLEROSIS

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Background- Mesial temporal lobe epilepsy syndrome due to hippocampal sclerosis is the most common type of focal epilepsy. Surgical resection of the mesial temporal structures renders 60-80% of these patients seizure free. This finding supports the idea that the severe neuronal loss observed in the hippocampus may somehow lead to synaptic reorganization and thus contribute to the progressive nature of the disease. Therefore prevention of cell loss may slow down or prevent epileptogenesis and seizure occurence. Method- In this study we aimed to investigate the pro-apoptotic and anti-apoptotic mechanisms, in cell death and survival respectively, in epileptic human hippocampi. Twenty five patients were selected randomly among those who underwent anterior temporal lobectomy with amygdalohippocampectomy due to mesial temporal lobe epilepsy in our center between 2001-2003. We applied immunohistochemical techniques in the formalin-fixed paraffin embedded hippocampal tissues and for cell death, we investigated the presence of pro-apoptotik molecules Bax (intrinsic apoptotic pathway) and tBid (extrinsic apoptotic pathway) in the tissue. Besides, we looked for the nuclear translocation of AIF, which is presumed to be a caspase independent pathway factor. Regarding the mechansims of cell survival, we searched for the expression profile of the following proteins: c-IAP1, c-IAP2. Results- Cells, that were stained, were counted in different regions of the hippocampus, namely CA1, CA2, CA3, dentate gyrus, hilus and subiculum. The results were compared with non-epileptic autopsy controls. We observed that Bax and tBid immunoreactivity were significantly increased in patient hippocampi. There was no nuclear translocation of AIF. c-IAP1 and c-IAP2 were absent in control hippocampi whereas the expression was dramatic in the patients in all hippocampal subfields. Finally, Hsp70 immunreactivity was significantly increased in all surviving neurons in patient hippocampi and degenerating neurons failed to express Hsp70 at all. Conclusion- Our findings indicate that both pro-apoptotic and anti-apoptotic mechanisms are activated in the hippocampus of patients with mesial temporal lobe epilepsy. Future investigations may provide novel therapeutic strategies that will inhibit pro-apoptotic cell death or encourage anti-apoptotic cell survival mechanisms. Supported by HUBAB (0201101005).

PP1-166
GLIOSARCOMAS: REPORT OF FIVE CASES WITH IMMUNOHISTOCHEMICAL FINDINGS

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Backround: Gliosarcoma is a rare primary central nervous system neoplasm characterized by biphasic histological pattern with both glial and sarcomatous components. It was classified by the World Health Organization as grade IV tumor. Our aim is to describe the clinical, morphological and immunohistochemical features of five cases of gliosarcomas and to discuss its pathogenetic mechanisms. Materials and Methods: We studied five cases of gliosarcomas to describe its clinical and pathological features. Immunohistochemical and histochemical analyses were performed to confirm diagnosis, assess histogenetic origin, and evaluate the proliferation index in all cases. Results: The male:female ratio was 4:1. The mean age was 50,2 years, ranging from 35 to 64. Headache was the commonest clinical symptom. All patients underwent craniotomy with microsurgery and total resection of the tumor. Immunohistochemical analysis was positive for p53 both glial and sarcomatous cells in four cases, however one case was negative for p53 both glial and sarcomatous cells. Conclusions: Our findings support monoclonal origin of gliosarcomas involving the TP53 tumor-supressor gene. However, alternative pathways can not be ruled out.

PP1-167
ATYPICAL TERATOID RHABDOID TUMOR

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INTRODUCTION: Atypical teratoid rhabdoid tumor is a malignant embryonal central nervous system tumor manifesting in children (generally younger than 2 years old). There is a male predominance. This tumor is composed of rhabdoid cells, sometimes with PNET, epitelial tissues or neoplastic mesenchymal tissues in it. It usually occures in the posterior fossa (cerebellum, cerebellopontine angle, brainstem) Its clinical presentation differs depending upon its site, size and localization. Here we present a case of atypical teratoid rhabdoid tumor in the deep parietal lobe of cerebrum. CASE: A 1 year old male infant was admitted to our hospital with right sided convulsion which soon became generalized. MR imaging showed a mass measured 5x4x4 cm in diameter which is localized at the deep parietal lobe, adjacent to the lateral ventricule wall. The initial working diagnosis was dysembryoplastic neuroepithelial tumor. The tumor was totally removed. Macroscopically the tumor was gray-tan coloured and had soft consistency. Two months after the initial operation the tumor recured locally with a bigger mass which is 6x6x5 cm in diameter. The recurrent tumor is totally removed but the patient died during the operation. Histologically the tissues were composed of neoplastic cells that are diffusely arranged in a highly vascularized stroma. These cells were forming short fascicules in focal areas. They were pleomorphic, polygonal, rhabdoid shaped neoplastic cells with large eosinophilic cytoplasm, hiperchromatic nuclei and prominent nucleoli. There were eosinophilic inclusion like bodies in the cytoplasms of these cells and sometimes in extracytoplasmic localizations. There also were large areas of necrosis and common mitotic figures. Tumor cells were diffuse positive for vimentin, S–100, GFAP; focally positive for EMA, SMA, synaptophysin, chromogranin, CD 99 and negative for desmin. Ki–67 proliferation index was %50. CONCLUSION: This case is diagnosed as atypical teratoid rhabdoid tumor and is discussed as it is a rare diagnosis.

PP1-168
HER-2/NEU, ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION IN WHO GRADE I MENINGIOMAS: A PILOT STUDY

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BACKGROUND: Meningiomas are generally benign central nervous system neoplasms, which frequently express progesterone receptor (PR) and rarely express the estrogen receptor(ER). Her-2/ neu is represented a well- known prognostic factor in various tumors such as breast carcinomas. However, the significance of Her-2/ neu expression in meningiomas is unclear. We evaluated the correlation with Her-2/ neu, PR, ER expressions and clinicopathologic parameters in WHO Grade I meningiomas. MATERIALS AND METHODS: In total, 34 cases of meningiomas (WHO grade I) were included in this study. Formaline-fixed, paraffin- embedded tissue sections were immunostained for Her-2/ neu, PR and ER. The level of Her-2/ neu expression in each sample was semiquantatively evaluated as follows: 0=no staining; 1+ = faint, incomplete membranous pattern; 2+ = moderate, complete membranous pattern and 3+ = strong membranous pattern. An immunohistochemistry score of 2+ or greater was considered as Her-2/ neu overexpression. Over 5% nuclear staining of tumor cells was considered positive for hormone receptors. RESULTS: There were 25 females and 10 males in study group. The age of the patients ranged from 38 to 78 (mean 56.9±11.5) years. According to the histological type, 16 (45.7%), 10 (28.6%), 5 (14.3%) and 4 (11.4%) cases had a meningotheliomatous, transitional, psammomatous and fibroblastic type, respectively. Whereas 18 (51.4%) of meningiomas were positive for PR, no staining was determined for ER. Her-2/ neu immunostaining was detected in 27 of the 34 meningiomas. Her-2/ neu overexpression were observed in 20 (58.8%) cases. There were positive correlation between Her-2/ neu and PR expressions (p=0.015). Her-2/neu overexpression was frequently seen in meningotheliomatous type (p=0.03). There were no correlations with Her-2/ neu, ER, PR and other clinicopathologic parameters. CONCLUSION: We observed that Her-2/ neu protein is overexpressed in about 58.8% of the WHO grade I meningiomas that essentially have a meningothelial patterns and PR positivity. The effect of Her-2/neu overexpression on pathogenesis and progressions of meningiomas could be better understanding the further studies which have large group and different histological grade. Thus, different pharmacologic treatment methods could be revealed in meningiomas.

PP1-169
A RARE TUMOR IN AN UNUSUAL SITE: INTRAVENTRICULAR ASTROBLASTOMA. CASE REPORT

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BACKGROUND: Astroblastoma (AB) is a rare central nervous system glioma which mostly occurres in young adults. AB is usually a circumscribed, well-demarcated tumor of the cerebral hemispheres, located close to the surface, with a distinctive perivascular orientation of neoplastic cells. On MRI images, AB typically appears as a solid or cystic, lobulated mass with inhomogeneous contrast enhancement and no oedema or mass effect. The biological behaviour of this tumor is unpredictable. CLINICAL HISTORY: Our patient is a 6 year old girl presented with a 5 week history of progressive headache and vomiting. Brain MRI showed a large demarcated solid-cystic mass in the left temporal-occipital ventricular horn. The patient underwent a left temporal-occipital craniotomy and intraoperatively the tumor appeared as a soft, reddish intraventricular mass. The tumor was totally removed and the postoperative course was uneventful. DISCUSSION: In our case the age of the patient and the intraventricular location of the mass strongly suggested the diagnosis of ependymoma. Histological examination revealed a tumor with solid compact pattern of growth with some papillary areas in which the tumor cells were clustered about the vessels, giving the appearance of pseudorosettes. The cellular processes were however broad and short and the nuclei of tumor cells were located at some distance away from the vessels, without a tapering of glial processes toward the vascular lumen. The vessel walls showed a conspicuous hyaline thickening. We found a brisk mitotic activity (MIB 1: 14%), small discrete foci of necrosis, no vascular endothelial proliferation. Immunohistochemical examination showed a positive staining with GFAP, S-100 and EMA. Our final diagnosi was astroblastoma. CONCLUSION: The very young age of the patient, the intraventricular location of the tumor and the presence of pseudorosettes might have been able to suggest a diagnosis of ependimoma, but the distinctive soap-bubble appearance on MRI and the anchoring of tumor cells by short cytoplasmic processes to vessels with hyaline thickening walls led us to the diagnosis of astroblastoma.

PP1-170
"LEUKOENCEPHALOPATHY OR LYMPHOMA – THAT IS THE QUESTION"

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BACKGROUND: Primary CNS lymphoma (PCNSL) is an uncommon form of non-Hodgkin lymphoma comprising 1-2% of all primary brain tumors.It affects the brain parenchyma, leptomeninges, spinal cord and eyes and typically remains confined to the CNS. It is usually associated with acquired immunodeficiency syndrome(AIDS) or with other causes of long term immunosuppression. However,elderly patients are also affected.These lymphomas are usually of B cell type and the expression of BCL6 appears to be associated with a better prognosis. Although treatment-related leukoencephalopathy is well recognized in PCNSL and other CNS neoplasms,the mechanisms involved in its development are poorly understood.Additionally,there are few reports of focal and diffuse leukoencephalopathy preceding the diagnosis of PCNSL or seen at the time of PCNSL presentation. Radiologic evidence of leukoencephalopathy may lead to a broad differential diagnosis, including inflammatory, infectious, genetic, or neoplastic diseases. Additionally, the histologic finding of demyelinization, without any other diagnostic feature could compromise the pathologic differential diagnosis in some cases. METHODS: We reviewed clinical histories and preoperative MRI of 15 PCNSL that presented at the University Hospital Son Dureta, between the years 2003-2006. Hematoxylin&Eosin slides were examined. Luxol Fast Blue stains for myelin were done in all cases. In addition, we performed a battery of stains to better characterize these neoplasms, as follows: CD20, CD3, BCL2, BCL6, CD8, CD4, CD10, MIB1, ALK, MUM1, and in situ hybridization for EBV. RESULTS: All lymphomas were diagnosed as Diffuse large B-cell lymphoma (DLBCL) and all showed an accompanying T-cell infiltrate in varying degrees, with a larger percentage of CD8+ lymphocytes.The presence of T cell infiltrate did not correlate with the diagnosis of demyelinization. Positive EBV in situ was observed in 5 immunosuppressed patients. Six lymphomas expressed BCL2 and BCL6.Radiologic findings of leukoencephalopathy was seen in 7 cases and correlated with histologic evidence of demyelinization in 4 cases;this was characterized by rarefaction of the white matter,myelin loss,gliosis and partial axonal loss. CONCLUSIONS: 1.The finding of leukoencephalopathy on MRI with histological demonstration of demyelinization seems to be more common than originally thought in PCNSL. 2. Radiologic

leukoencephalopathy and histologic evidence of demyelinization are more common in those lymphomas expressing BCL2 and BCL6 FUTURE DIRECTIONS: We are in the process of investigating the presence of JCV DNA in these tissue samples.

PP1-171
LIPOMATOUS EPENDYMOMA : A CASE REPORT

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BACKGROUND: Ependymomas consitute 3-9 %of all glial tumors. It may develope in all age groups but manifest predominantly in children and young adults. The variants of ependymoma according to the WHO classification are cellular, papillary, clear cell and tanycytic types. And other rare variants are; ependymoma of the filum terminale, ependymoma with extensive tumour cell vocuolation, melanotic ependymoma, signet ring cell ependymoma and ovarian ependymoma. CASE: This 47 year-old man presented with complaint of headache and difficulty in walking for 3 months. CT scan of the head revealed a large hyperdense mass lesion in the posterior fossa. Through a suboccipital craniotomy total excision of tumor was done. In the pathological examination with H&E stained sections, showed a celluler neoplasm with sheets of monotonous cells possesing dark round nuclei. Perivascular pseudo-rosettes, necrotic areas and occasional mitoses were found. In addition, there were large areas where the tumor cells had a signed ring apparance with large vacuoles in the cytoplasm. CONCLUSION: Lipomatous Ependymoma is a rare variant of ependymomas reported in literature.The outcomes for this lipomatous form may be similar to those of ependymomas of similar grade.

PP1-172
CENTRAL NERVOUS SYSTEM METASTASES: AN ANALYSIS OF 169 BIOPSY CASES

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Background: Central nervous system (CNS) metastases are common complications of systemic cancers. They may develop from any primary neoplasm but some tumors have a predilection for the CNS. These are lung and breast carcinoma, melanoma, renal cell carcinoma and adenocarcinoma of colorectal origin. However, at the time of neurosurgery approximately one half of metastases may be of unknown primary. Aim of the study was to evaluate an algorithm of immunohistochemical markers which could be useful in diagnostic routine for identification the origin of the most frequent CNS metastases with unknown primary. Methods: Among the 997 biopsies of CNS tumors operated on during the 2006 at the Institute of Neurosurgery KCS there were 169 (16.9%) cases of CNS metastases. Males were more frequently affected (M: F ratio, 2.5:1). All cases were in adults. The locations were cerebral hemispheres (71.92%), cerebellum (16.95%), meninges (5.26%), spinal cord region (3.51%), brain stem (1.75%) and bones (0.58%). In 75 cases (44.37%) the primary tumor was known and in the majority of cases it was lung carcinoma (24.85%), followed by melanoma (7.10%), breast carcinoma (4.73%), colorectal carcinoma (4.14%), renal cell carcinoma (0.59%) and tumors of uncommon origin such as testis and urinary bladder (2.95%). In 92 cases (54.43%) the primary tumor was unknown. Microscopically, the first step in our investigation was to point out whether the CNS tumor was primary or metastatic and if metastatic to determine its phenotype. The panel of monoclonal antibodies for poorly differentiated CNS metastases derived from the most common primaries was performed. Results: Using the algorithm of monoclonal antibodies we were able to identify the primary tumor in more than 80% of cases. Immunohistochemically, the CNS metastases derived from lung adenocarcinomas were positive to CK7, TTF-1 and CD56; from squamocellular lung carcinomas, positive to CK5/6, negative to CK7 and TTF-1; from colorectal adenocarcinomas, positive to CK20 and CDX2, negative to CK7; from melanomas, positive to melan-A, HMB-45 and S-100; from breast carcinomas, positive to CK7, ER and negative to TTF-1; from renal cell carcinomas, positive to vimentin, CD10 and keratin AE1/AE3. Conclusion: The performed algorithm of immunohistochemical markers may represent an important role in surgical neuropathology taking into consideration that the evaluation of CNS metastases with unknown primary represents a very important process that helps in the strategies to maximize the patient's survival.

PP1-173
AN IMMUNOHISTOCHEMICAL STUDY OF EGFR, p53, Ki67 IN GLIOBLASTOMAS AND THEIR RELEVANCE TO SURVIVAL

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BACKGROUND It is well-documented that the epidermal growth factor receptor (EGFR) gene is involved in the control of cell proliferation and is amplified and overexpressed in more than one third of glioblastoma cases. On the other hand, TP53 mutations as well as overexpression of the corresponding gene are frequent in secondary glioblastomas. In addition, according to some studies, the Ki67 labelling index can be used in order to subdivide glioblastomas on the basis of their proliferative activity. However, there is a great deal of debate about the prognostic significance of these factors in the malignant course of glioblastomas. In our study we analysed immunohistochemically the expression of EGFR, p53 protein and Ki67 in 31 glioblastomas and their relationship to patients' survival. METHOD 31 adult patients with glioblastoma were enrolled in the present study. Formalin-fixed paraffin-embedded tissue sections were immunohistochemically studied. The topography and cellular distribution of the three markers was analysed as well as semi-quantitative assessement of EGFR intensity of staining was made (negative, mild, moderate, high). P53 and Ki-67 were measured using digital morphometry and for the statistical analysis of the results the SPSS 13.0 programme for Windows was used. RESULTS The percentage of p53 and Ki67 nuclear positivity was ranged between 2-90% and 3-35%, respectively. The EGFR immunostaining was evaluated as negative (4 cases), mild (1 case), moderate (14 cases) and high (12 cases) with membranous and cytoplasmic localization. A predominantly heterogeneous pattern of staining was detected, within the specimens. We observed that the patients with Ki67≥15% demonstrated shorter survival periods than the patients with Ki67<15%, at a statistically significant level (p=0,036, log-rank test). There was no correlation among the three markers while EGFR and p53 expression levels were unrelated to survival. CONCLUSION In our study we observed a rather heterogeneous pattern of EGFR staining reaction, in contrast to previous experiments. On the other hand, our findings agree with several published data regarding the absence of prognostic significance of EGFR and p53 expression in glioblastomas. Furthermore, we reconfirm our previous studies that Ki67≥15% is a cut-off point for predicting worse survival. The extensive EGFR expression needs further evaluation as it may be used for targeted treatment.

PP1-174
PRIMARY CENTRAL PRIMITIVE NEUROECTODERMAL TUMOUR OF THE CENTRAL NERVOUS SYSTEM. REPORT OF A CASE AND REVIEW OF THE LITERATURE

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BACKGROUND: Primitive neuroectodermal tumours (PNETs) are a rare group of highly malignant neoplasms which arise from pluripotent neural crest cells. These tumours occur predominantly in childhood and young adults. According to new proposals, based on immunohistochemical, ultrastructural and cytogenetical characteristics they are subdivided in two categories: central PNETs and peripheral PNETs. In this context, we present a rare case of a primary central PNET which was multifocal in the spinal cord and in the posterior fossa of a woman with a review of the literature. METHOD: A 42-year old woman presented with reduced mobility and sensory disturbances of one week duration of her right leg. Neurological examination revealed a paresis of the right leg and increased bilateral tendinous reflexes. MRI of the brain and spinal cord demonstrated a tumour in the roof of the posterior fossa consistent rather with a meningioma and multiple intramedullary and extramedullary obstructive masses along the entire spinal cord. Decompressive surgery took place and formalin-fixed paraffin-embedded tissue was examined with hematoxylin/eosin and immunohistochemically for: GFAP, S-100, Vimentin, Desmin, CD56, NSE, NF, Chromogranin A, Synaptophysin, CD99, MCK, EMA, CD45, C-Kit, Myo-1, Ki67. RESULTS: Hypercellular, small round-cell malignant tumour with ill-defined rosettes (Homer–Wright rosettes), numerous mitoses, Ki67 index >50% and areas of geographic necrosis. The malignant cells were diffusely positive for CD56 and Vimentin, focally for Synaptophysin, NSE, C-Kit and negative for CD99 and for all the other markers. The immunohistochemical profile was compatible with that of central PNET. The patient received adjuvant radiotherapy but she survived a few months after the initial diagnosis. CONCLUSION: Central PNETs are highly aggressive neoplasms without a sex predilection. Bibliographical data regarding the exact incidence of intraspinal localization in central PNETs are confusing. It seems that 10–30% of cases of central PNETs (medulloblastomas) are already disseminated through the CSF at the time of diagnosis, as in our case, and they rarely metastasise outside the CNS (<5%). The distinction between central PNET and peripheral PNET is of great importance as they require different treatment protocols. The main immunophenotypic difference between central and peripheral PNET is the CD99 expression in the latter.

PP1-175
INTRACRANIAL INTRAPARENCHYMAL PSEUDOTUMORS WITH EXTENSIVE CALCIFICATION AND FIBROSIS; CLINICOPATHOLOGICAL SPECTRUM OF THE SO-CALLED CALCIFYING PSEUDONEOPLASM OF NEUROAXIS

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Background: Extensively calcified masses without an associated neoplasm or an underlying systemic process are extremely rare in the central nervous system, and are often defined as “calcifying pseudoneoplasms of the neuraxis”. While this term appears to represent a unique and interesting clinicopathological entity, the lesions within this category appear to be diverse. Patients: We present four patients with extensively calcified intraparenchymal masses not associated with a neoplastic, inflammatory or a systemic process. Results: These lesions occurred in three males and one female, ages 16, 35, 49 and 59. Three patients presented with seizures, while the fourth case was discovered incidentally. Two lesions were located in the temporal lobe, one within the lateral ventricle, and one in the parietal lobe. Radiologically, all masses were dense solid calcifications that were hypointense on T1 and T2-weighted magnetic resonance imaging. The variations in the type and extent of histological features portended a superficial similarity of tumors to one another. The spectrum of histological changes included focal ossification with peripheral striations, proliferation of fibroblast-like cells, extensive psammomatous calcifications (in three cases), and limited reactive changes in the surrounding tissue. We present a brief comparison of our findings with the lesions reported in the literature as “calcifying fibrosseous pseudoneoplasms of neuraxis”. Conclusion: Intracranial non-neoplastic masses with extensive calcifications and fibrosis appear to outline a spectrum of pathological processes rather than a specific entity. It is still possible that the histological diversity reflects minor phenotypic variations in the same etiological process.

PP1-176
CYCLIN D1 IMMUNOREACTIVITY IN MENINGIOMAS

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BACKGROUND: Cyclin D1 is an important nuclear protein required for progression of cells through the G1 phase of the cell cycle. Routine pathologic examination could not predict the clinical course of meningiomas because even histological benign tumors may recur after total surgical resection. Quantifying of their proliferative potential may help in prediction of biological behavior of tumors with comparable histology. Degree and type of the Cyclin D1 expression has been poorly investigated in the meningiomas AIM: The aim of our study was to explore the usefulness of Cyclin D1 expression as a proliferative marker in meningiomas and its relationships to Ki-67/PCNA, WHO grade and mitotic activity. METHODS: We evaluated immunoreactivity for proliferative markers (Cyclin D1, Ki-67 and PCNA) in a consecutive series of 64 meningiomas samples graded according to the WHO classification. Immunohistochemical staining with Ki-67, PCNA and Cyclin D1 was performed using the microwave processing procedure and LSAB+ methodology. The number of positive cells for proliferative markers has been counted in ratio to 1000 cells. The results have been statistically correlated for each other, with tumor grade and in ratio to number of mitosis in 10 and 20 HPF. RESULTS: There was significant correlation in expression of proliferative markers (Ki-67, PCNA, Cyclin D1): for each other ($p< o, o1$), tumor grade ($p< o, o1$), number of mitosis on 20 /HPF ($p<o, o1$) and on 10 /HPF ($p< o, o5$). All meningiomas samples showed Cyclin D1 (nuclear, cytoplasmic and 'ring' shape) immunostaining. CONCLUSION: Cyclin D1 immunostaining was found to be very sensitive and it may be used as a proliferative marker for meningiomas.

PP1-177

PRIMARY DURAL LYMPHOMAS. A CASE REPORT

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Objectives: Primary dural lymphoma (PDL) is exccedingly rare. It arises from dura mater and differs biologically from other primary central nervous system lymphomas (PCNSL). It is usually a low-grade B-cell marginal zone lymphoma, whereas other types of PCNSL are usually high-grade, diffuse, large B-cell lymphomas. The pathogenesis of PDL is not well understood because the dura is devoid of any lymphoid tissue. One hypothesis is that a benign inflammatory condition of the dura could attract polyclonal lymphocytes from which a monoclonal lymphoma could arise. The most common clinical presentations are headache, seizures, focal sensory or motor deficits and visual disturbances. Case Report: We report a case of a 54-year-old woman who admitted to the emergency because of seizures. She had a past medical history of hypothyroidism. A CT and MRI scan before and after contrast medium revealed an enhanced mass arising from the dura localized in the right parietoccipital region and the tentorium as well. The radiological features were considered as a meningioma en plaque. The patient underwent a right parietoccipital craniotomy and a subtotal excision of the tumor was performed. The surgical specimen was histologically Low-grade marginal zone B-cell lymphoma. Immunohistochemically the tumor cells were positive for CD45, CD19, CD20, CD79a, but negative for CD5, CD10, CD23, CD30, EMA, cytokeratins, vimentin, GFAP and S-100p. After additional clinical and laboratory screening there was no other evidence of lymphatic disease. Postoperatively the patient experienced infection of Listeria monocytogenese and after 8 days in intensive care unit she died. Conclusions: PDL is more indolent and has a better prognosis than parenchymal PCNSL or systemic lymphoma with CNS metastasis. However, further studies with longer clinical follow up are necessary to assess the final outcome in these patients.

PP1-178

VIMENTIN AND SURVIVIN EXPRESSION RATES AS PROGNOSTIC FACTORS IN MEDULLOBLASTOMA

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Background: A medulloblastoma is a primitive neuroepithelial tumor of the cerebellum that occurs in children and metastasizes through the cerebrospinal fluid. It is highly malignant and invasive, and the 5-year survival rate is only 60%. Surgical resection techniques, radiation, and chemotherapy have improved the overall survival but the patients suffer life-long cognitive dysfunctions or endocrine abnormalities as the side effects of treatment. Therefore it is essential to identify prognostic markers to determine the appropriate treatment strategy in order to minimize the side effects. Methods: This study evaluated the immunohistochemical differentiation and survival rate with synaptophysin, glial fibrillary acidic protein, epithelial membrane antigen, vimentin and primitive neuroepithelial marker nestin of 55 paraffin-embedded medulloblastomas, using a tissue microarray. The expression of survivin, the apoptotic inhibitor, and the survival rate with regard to the proliferation index of Ki-67 were also investigated. Results: The group testing positive to vimentin, a mesenchymal differentiation marker, had a worse prognosis and there was a strong correlation between vimentin expression and nestin expression. Patients with a survivin expression rate >35% had a significantly poorer clinical course and there was a correlation between the survivin expression rate and Ki-67 expression rate. Conclusion: In conclusion, vimentin and survivin are negative prognostic markers in medulloblastomas.

PP1-179

SPECIFIC EXPRESSION OF COX-2 IN TYPE 1 MUSCLE FIBERS

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[Background] A skeletal muscle biopsy is important for the diagnosis of diseases of the motor unit. Basic pathologic reactions of muscle can be recognized in paraffin sections of formalin fixed biopsy specimens; however, frozen sections for histochemistry to determine patterns of muscle fiber types are essential for a proper pathological evaluation of skeletal muscle. Many Pathology Laboratories cannot perform histochemistry routinely by limitations in having a specially trained technician and low volume of the muscle biopsy. Recently, Troponin C and Troponin T have been developed for muscular fiber typing in formalin fixed, paraffin embedded tissue; however, the antibodies are useful only for skeletal muscle typing, and therefore, low cost-benefit effect still does not allow most general hospitals to run muscle biopsy section in Pathology laboratories. We incidentally discovered expression of cyclooxygenase -2 (COX-2) in skeletal muscle tissue around breast cancer, and the "checkerboard" pattern of COX-2 expression was suggestive of fiber typing of the skeletal muscle. [Objective] We performed this study to verify whether COX-2 is expressed in a specific muscle fiber type and to validate applicability of COX-2 immunohistochemistry in the formalin-fixed, paraffin-embedded muscle biopsy specimens. [Method] We used 58 cases of muscle biopsy specimens in Seoul National University Bundang Hospital, which consisted of 27 cases of neurogenic atrophy, 9 cases of muscular dystrophy, 8 cases of inflammatory myopathy, 7 cases of other muscular diseases, and 7 cases of nonspecific changes. Immunohistochemistry was performed with COX-2, Troponin C and Troponin T monoclonal antibodies. Expression of COX-2 was comparatively analyzed with expression of Troponin C and Troponin T in individual muscle fibers. Expression of COX-2 was also compared with the results of histochemistry in the cases which frozen samples were available. [Results] Compared with immunohistochemical expression of Troponin-C and Troponin-T, and compared with histochemical studies of NADH-TR and ATPase pH9.4, COX-2 was specifically expressed in type 1 muscle fibers in various muscle diseases and normal skeletal muscle. [Conclusion] Specific expression of COX-2 in type 1 myofibers is a brand new finding hitherto undescribed in the literatures. *This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-E00221).

Pediatric Pathology

PP1-180
UNUSUAL PRIMARY OCULAR NEOPLASM IN A CHILD: LEIOMYOSARCOMA OF THE CILIARY BODY

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BACKGROUND: Primary uveal tract neoplasms are extremely rare in childhood; the most common lesions found are melanocytic. Leiomyomas have been reported, occurring mainly at the ciliary body. These tumors are believed to arise from primitive mesectodermal tissue giving rise to the ciliary body muscle, although they may also arise from vascular smooth muscle. METHOD: We study the case of a 7 year-old girl who underwent enucleation of the right eye with clinical suspicion of choroid melanoma due to a ciliary body mass, and describe its histological and immunohistochemical findings. RESULTS: Histologically, the neoplasm featured spindle cell morphology, atypia and mitoses, in addition to invasion of choroid and retina. The tumor expressed smooth muscle alpha actin, pan-actin HHF-35 and desmin, whereas immunohistochemistry for melanocytic markers, such as S-100, Melan-A and HMB-45 was negative. The proliferation index, measured by Ki-67 reactivity, was 9% in tumor cells. Based on these features, the diagnosis of leiomyosarcoma of the ciliary body was established. CONCLUSION: Smooth muscle neoplasms arising from the uveal tract are extremely rare, with few cases having a diagnosis confirmed by immunohistochemical or ultrastructural methods. Many cases formerly diagnosed as leiomyomas were in fact well-differentiated amelanotic melanomas with a fusiform pattern. Immunohistochemistry is an indispensable tool in the diagnosis of uveal tract neoplasms, and expression of myogenic differentiation markers allow distinction from the most common melanocytic tumors arising in this location. Although several leiomyomas have been reported in the literature, there are only two previously reported cases of primary leiomyosarcoma of the uveal tract. Therefore, the case presented here represents an extremely rare ocular neoplasm.

PP1-181
HYALINE MEMBRANE DISEASE: A REPORT OF 62 AUTOPSY CASES

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The present work aimed to reveal the incidence rate at hyaline membrane disease (HMD) based on the pathoanatomical material of the Municipal Institution "Taldom Central Regional Hospital" over 1983-2006. The obtained findings revealed that of a total of 1,967 post-mortem examinations and 245 perinatal cases there were 62 cases of HMD (41 neonatal boys and 21 neonatal girls) which turned out to be the primary cause of death. The immediate cause of death was determined to be the progressing pulmonary insufficiency related to the presence of resorptive atelectasis, and in 13 newborn infants it was accompanied by microfocal pneumonia. Death in HMD more often occured during the first 48 hours (57 cases). HMD was registered in 50 neonates on the background of immaturity, with stage I immaturity being determined in 12 cases, stage II - in 19, and stage III immaturity - in 19 cases. Hence, in the present study, HMD turned out to amount to 3% of the 1,967 post-mortem examinations and to 25% of the 245 perinatal sections performed at the general hospital over 24 years. The incidence rate of HMD was almost 2 times higher in neonatal boys than in neonatal girls, with the majority of cases accounting for premature neonates (81%). Neonatal mortality caused by hyaline membrane pathology more often occurs within the first 48 hours (92%), and the incidence rate thereof increases with the deepening of the immaturity degree.

PP1-182
PULMONARY, LIVER AND SPLEEN HEMOSIDERIN DEPOSITION OBSERVED IN INFANT AND CHILDREN AUTOPSIES

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Background: Hemosiderin deposition is not often recognized on routine examination with hematoxylin and eosin staining; however, iron stains may be helpful in the evaluation of hemosiderin deposition in infant deaths. Methods: This report describes the data obtained from autopsy of 86 infants and children whose deaths were investigated in Forensic Medicine Council Bursa Morgue Department from January 2000 to January 2003. A histochemical technique was used to identify haemosiderin in lung, liver and spleen specimens and correlated the observations with other descriptive variables such as the reported cause of death, postmortem interval, trauma history, gender, age. Results: There was weak positive, significant correlation between lung and liver hemosiderin scores (spaermann correlation coefficient rho= 0,348, p=0,001); with the increased lung hemosiderin scores, an increase in liver hemosiderin scores was observed. Similarly, a positive marked correlation between spleen and liver hemosiderin scores (spaermann correlation coefficient rho= 0,335, p=0,002) was present; with the raised liver hemosiderin scores an increase in spleen hemosiderin scores was seen. When statistical analysis was performed, there was no significant statistical differences between age groups, gender, lung hemosiderin deposition scores ($p > 0.05$). Out of 56 liver hemosiderin positive cases, 35(62,5%) and of 30 liver hemosiderin negative cases, 6(20%) were in the under 6 month age group with significant statistical correlation (person ki-squre test $p<0.001$). Probability of liver hemosiderin positive cases to be in the under 6 month age group was detected to be 6,7 times more than those who were hemosiderin negative (CI %95=2,3-18,9). Likewise, of 25 spleen hemosiderin positive cases, 18(72%) and of 61 spleen hemosiderin negative cases, 23(37,7%) were in the under 6 month age group with remarkable statistical correlation(person ki-squre test $p = 0.004$). Probability of spleen hemosiderin positive cases to be in the under 6 month age group was detected to be 4,3 times more than those who were hemosiderin negative(CI%95=1,6-11,8). Conclusion: This study was undertaken to provide data for further studies of hemosiderin deposition in infant's lung, liver and spleen. After the major differential diagnoses were ruled out, this study demonstrated, depending on statistically assessed morphometric grounds, that the presence of haemosiderin deposits in the liver and spleen were significantly higher in a under 6 month age group.

PP1-183
AMELOBLASTIC FIBROSARCOMA OF THE MAXILLARY SINUS IN AN INFANT: A CASE REPORT WITH LONG-TERM FOLLOW UP

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Ameloblastic fibrosarcoma (AFS) or ameloblastic sarcoma is an extremely rare odontogenic neoplasm. The authors report ameloblastic fibrosarcoma (AFS) in the maxillary sinus of a 4-month-old boy. The tumor composed of odontogenic epithelium, resembling that of ameloblastoma and a mesenchymal part exhibiting features of fibrosarcoma. We also found some areas with deposition of dentinoid material closely adjacent to ameloblastic epithelium. Although AFS has been occurred in a wide age range, this is the first report of this tumor in infancy with long term follow up.

PP1-184
IMMUNOHISTOCHEMICAL EXPRESSION OF P53 PROTEIN IN HISTOLOGICALLY FAVORABLE WILMS TUMOR AND ITS RELATIONSHIP TO TUMOR STAGE AT PRESENTATION

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Background: Wilms tumor as the most common renal tumor of children has been associated with chromosomal abnormalities. Although a correlation between anaplasia and mutations of P53 tumor suppresser gene has been found in Wilms tumor, significance of these mutations in different clinical stages of favorable-histology Wilms tumor remains largely unresolved. Objective: The goal of this study was to determine the frequency of P53 expression in histologicaly favorable Wilms tumors and its correlation to tumor-stage at presentation. Material & Methods: In this retrospective study 48 cases of confirmed Wilms tumor with favorable-histology were retrieved from the files of departments of pathology in 3 hospitals in Mashhad between 1990 and 2004. Histological characteristics and clinicopathological staging were in accordance with NWTS guidelines. P53 expression was determined by immunohistochemical method. For each section, the proportion of neoplastic cells exhibiting nuclear positivity was broady quantified and intensity of staining was charted based on visual impression by two pathologists. Result: A total of 48 cases of histologically favorable Wilms tumor were assessed. 11 cases (23%) showed positivity for P53 which were 3 (27.3%) with stage II, 3 (27.3%) with stage IV, 2 (18.2%) with stage I, 2 (18.2%) with stage III and 1 case (9.1%) with stage V. The P53 immunopositivity was seen in 1-25% of tumor cells in 9 cases (18.8%), in 26 to 50% of tumor cells in 1 case (2.1%) and in >75% of tumor cells in the other one case (2.1%). The intensity of staining was moderate in 6 cases (12.5%), weak in 4 (6.3%) and strong only in one case (4.2%). The most common component with P53 immunoreactivity was blastemal in 11 cases (100%). Conclusion: We found no correlation of P53 immunoreactivity and its intensity to tumor stage at presentation in individuals with histologically favorable Wilms tumors (P-Value=0.66, P-Value=0.52 respectively).

PP1-185
POSTMORTEM STUDY: DENNYS-DRASH SYNDROME

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BACKGROUND: Dennys-Drash syndrome is characterized by male pseudohermaphroditism, Wilms' tumour and glomerulopathy with fast progression to end-stage renal disease. It is caused by a mutation in supressor gene WT1, located in 11p13. MATERIAL: Two year-old male newborn that presented acute respiratory insufficiency and acute renal failure. As previous history he had bilateral chriptorchidism partially treated with surgery; the right testicle was brought near the entrance of scrotum, waiting for a second operation. He also presented a hormonal dysfunction that was being studied. Unfortunately, he dies and a necropsy is performed. RESULTS: External exam shows the absence of testicles inside the scrotum, penile curvature and hypospadias penile-scrotal. The rest of external exam is normal. After opening cavities, bilateral pleural effusion stands out, with congestive lungs. Testicles are seen in abdominal cavity, one of them adherent to abdominal wall and the other attached to a rudimentary uterine structure, which shows continuity with a short and ill-developed vaginal channel. The remaining organs are normal in appearance and situation. Microscopic study reveals dysgenetic testicles, structures derived from müllerian conducts (uterus and vagina), and a nephropathy consisting in diffuse glomerular and mesangial sclerosis with increased sclerosed glomeruli. Bilateral pneumonia, oedema and pulmonary congestion are also found. CONCLUSION: According to the original description, Dennys-Drash syndrome (DDS) is constituted by male pseudohermaphroditism, Wilms' tumour and progressive glomerulopathy. After this original description, patients with incomplete forms were found, so this syndrome was divided into three categories: 1) male genotype with all three abnormalities, 2) male genotype, nephropathy and ambiguous external genitals, and 3) female genotype, nephropathy and Wilms' tumour. Clinically, it is characterized by massive proteinuria with nephrotic syndrome at short age and fast evolution to renal insufficiency; the histological lesion is diffuse mesangial sclerosis (DMS). From a clinical (ambiguous genitals, nephrotic syndrome with early appearing symptoms and fast progression to renal failure) and histological (DMS) point of view, our patient has the features of DDS. Cases like ours have a high risk of developing Wilms' tumour subsequently.

PP1-186
MECKEL'S DIVERTICULUM IN PEDIATRIC PATHOLOGY OWN EXPERIENCE

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Introduction: Meckel Diverticulum is the most common anomaly of the gastro-intestinal tract, with an incidence between 0, 5-2% and is more frequent in males. It is the result of the persistence of the proximal portion of the yolk sac. Macroscopically it consists in a figer-like structure, 2 to 8 cm in length, located on the antero-mesenteric border of the ileum, usually 30 cm proximal to the ileo-cecal valve in children and 90 cm in adults. From 1986 to 2006 in "Grigore Alexandrescu"Hospital 156 children have been diagnosed and treated , 104 of them boys and 52 girls, aged 1 month - 16 years . Fifty two cases were asymptomatic, usually an incidence finding in laparatomy, 104 cases presented colonic obstruction (volvulus or intussusception), bleedings, diverticulitis, perforation. Colonoscopy and gastroscopy excluded other causes of bleeding. Surgical excision was performed. Material and methods: Fragments of the diverticulum were fixed in formaldehyde 10%, included in paraffin and the sections were stained with HE and VG. Results: Histologically, colonic diverticulum had a wall lined by intestinal mucosa, in 18 cases had an ectopical gastric mucosa features , in 5 cases pancreatic tissue, in 4 cases intermingled aspects: 2 cases intestinal and gastric mucosa and 2 cases gastric mucosa and pancreatic tissue. Conclusions: Because of malignancy risk (adenocarcinoma, carcinoid, leiomyosarcoma, etc) and the complications that may occur, resection is indicated and also the carefully analysis for the discovery of nodular or papillary zones with this potential .

PP1-187
VALUE OF ELECTRON MICROSCOPY EPON BLOCK BANKING IN PAEDIATRIC PATHOLOGY
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Background: The use of electron microscopy (EM) in human medicine has been decreasing in recent years. But in pediatric pathology, EM is still required in the diagnosis of some skeletal muscle diseases, renal biopsies, metabolic and storage diseases, some undifferentiated tumors and research. EM laboratories are very seldom planned. The reasons of this are expensive prices of electron microscope and the other equipments, housing problems of the EM, educated technician and pathologist supply. In pediatric pathology laboratories where EM is not available, it is a problem to send specimen of the cases that requires EM. EM tissue processing and blocking is not very difficult and expensive. In this prospective study, we aimed to evaluate the value of tissue blocking for EM in which EM required disorders have been suspected before the biopsy was fixed in formalin. Method: In this study 48 biopsies were EM blocked between 2004-2006. The fresh tissues in 1mm diameter were fixed in Karnovsky fixative at 4C°, dehydrated in increasing concentrations of alcohol, infiltrated with liquid embedding medium, polymerized and produced solid blocks from each case. After histochemically and immunohistochemically light microscopic evaluation of the cases; the EM blocks of the patients which required to evaluate with EM was sent to different centers where EM is possible. The final decision of diagnosis was reevaluated by pathologic, clinical and EM findings. Results: Between these 48 biopsies EM blocked nine blocks from skeletal biopsies were studied for EM. One of them brought the diagnosis. One was not satisfactory. In the other seven cases EM helped in differential diagnosis together with the findings of light microscopy and other techniques. Two cases were sent for evaluation for EM from renal biopsies. One of them did not contain glomeruli and considered as unsatisfactory. The other was diagnosed brought the diagnosis. One biopsy from subcutaneous nodule helped in the diagnosis of storage disease. The cases sent for EM from EM epon block banking collection consisted of 25% of the cases. Among them 16% were unsatisfactory, 16% brought the diagnosis and 68% helped the diagnosis. Conclusion: Our results indicate that separating fresh tissue for EM epon block banking might be useful in pediatric pathology.

PP1-188
TENASCIN EXPRESSION IN PAPULOSQUAMOUS DISORDERS OTHER THAN PSORIASIS IN PEDIATRIC PATIENTS: A PATHOGENIC FEATURE?
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BACKGROUND: Tenascin is a large extracellular matrix protein that is expressed in the basal membrane zone during embryonic development, tissue repair, and oncogenesis. In vitro studies suggest that proliferating epithelium induces the production of tenascin by mesenchymal cells. In this study, our goal was to compare the expression of tenascin in psoriasis with other papulosquamous disorders of skin in pediatric patients. METHOD: The study was conducted on skin biopsy samples of 37 pediatric patients [twenty male (54.1%) seventeen female (45.9%), mean age 8.35 (range from 3 to 14 years)] suffering from psoriasis or other papulosquamous disorders. Of the 37 skin biopsy samples, 17 (45.9%) were diagnosed as psoriasis and 20 (54.1%) were diagnosed as other papulosquamous disorders [6 (16.2%) pityriais rosea, 5 (13.5%) pityriasis lichenoides, 3 (8.1%) lichen planus, 2 (5.4%) lichen nitidus, 2 (5.4%) lichen striatus, and 2 (5.4%) pityriasis rubra pilaris] histopathologically. The expression of tenascin was evaluated in all biopsy samples immunhistochemically. Data analysis was carried out by Spearman correlation analysis. RESULTS: Among the total of 37 skin biopsy samples, tenascin expression was positive in 16 (43.2%) and negative in 21 (56.7%) at perilesional superficial dermis. Of the 17 biopsy samples diagnosed as psoriasis histopathologically, 16 (94.1%) were negative and 1 (5.8%) was positive for tenascin; and of the 20 biopsy samples diagnosed as other papulosquamous disorders, 15 (75.0%) were positive and 5 (25.0%) were negative for tenascin (p=0,001). CONCLUSION: Our results suggest that immunohistochemically tenascin expression might be a pathogenic feature and an indicator for the papulosquamous disorders other than psoriasis in pediatric patients.

PP1-189
MONOZYGOTIC TWINS AND METASTASIS OF NEUROBLASTOMA TWIN-TO-TWIN THROUGH FOETOPLACENTAL CIRCULATION
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Introduction: Neuroblastoma is the common childhood solid tumor of neural crest cells. It may arise at any site in the sympathetic nervous system, most commonly in the adrenal medulla. We report a case of congenital neuroblastoma in a pair of monozygotic twins which is rare and only few cases have been described. Clinical History:The patients were two baby girls born at 37 week' gestation following a riskfull pregnancy. There was no significant family history and parents had one older healthy child. The twins were born asymptomatic. The first twin was admitted to our hospital at the age of 10 days with marked abdominal distension and firmness, weight loss and hiperbilirubinemia. Abdominal ultrasound showed large liver with numerous hyperchoic nodules. A needle core biopsy of the liver revealed a histological and immunohistochemical diagnosis of neuroblastoma. The tumor is composed of small blue cells partially arranged in Homer-Wright pseudorosettes. Immunohistochemicaly tumor cells were positive for NSE and synaptophysin, but negative for LCA. Treatment commenced with standard chemotherapy protocol but soon the therapeutic course was complicated with respiratory symptoms and liver failure. After 20 days of therapy she died at age of 1 month. Autopsy showed icteric skin, general edema, well defined nodular mass in left adrenal gland measuring 2.5x2.5x2 cm and large liver with multiple nodules. A few days after her death, twin sister was admitted to hospital with similar symptoms. An extensive physical, laboratory and radiological examination and liver biopsy confirmed diagnosis of metastasis neuroblastoma in the liver. Treatment commenced with chemotherapy and after two cycles, the therapeutic course was complicated with liver failure. The biopsy specimen showed extensive toxic damage of liver with fibrosis and hemosiderosis. At 6 months of age she died. Autopsy showed postnecrotic cirrhotic changes in liver without metastasis, hypersplenism, diffuse alveolar damage of lungs and ascites. Conclusion: This is a rare case of metastasis neuroblastoma from twin-to-twin through foetoplacental circulation in utero that is confirmed at autopsy. First twin had primary tumor in adrenal gland with liver metastasis while second twin had liver metastasis without a primary tumor site.

PP1-190
ARTHROGRYPOSIS, RENAL TUBULAR DYSFUNCTION AND CHOLESTASIS AS A RARE ARC SYNDROME – REPORT OF ONE CASE ON AUTOPSY

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AIM: To present a rare case of ARC syndrome (only one on autopsy in 2006). We present arthrogryposis, renal tubular dysfunction and cholestasis as ARC syndrome with additional features of dysmorphio, hypoplastic lungs and variation of blood vessels on arch of aorta. METHODS: Standard protocol of autopsy was preformed with additional Rtg examination. Specimens of all organ tissues were taken and proceeded in standard H&E staining. RESULTS: It was a female newborn that lived 45 minutes, weight 2350gr, length 43cm with abnormal position of left leg (end flexion). Left foot lies on the left shoulder and has equinovarus position. Rtg was preformed and showed bilateral fractures of both humeral and femoral bones. There is universal hypotonia with neurogenic muscle atrophy. Renal histology reveals dysplasia changes with cystic dilatation of many tubules and concentric layers of immature mesenchyme. Bowman's space is enlarged and some glomerules show segmental or complete sclerosis. There are focuses of cartilage bars and many proliferated blood vessels. Microscopic analysis of liver sections showed both cholestasis and giant cell transformation of hepatocytes. CONCLUSION: It is considered that arthrogryposis, renal tubular dysfunction and cholestasis give picture of ARC syndrome with many additional features. As a rare syndrome, it should be put in differential diagnoses of all cases with muscular or/and skeletal deformities.

PP1-191
IMMUNOHISTOCHEMICAL EXPRESSION OF CELL CYCLE, APOPTOSIS REGULATORS AND EPIDERMAL GROWTH FACTOR RECEPTOR [EGFR] IN PEDIATRIC INTRACRANIAL EPENDYMOMAS

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Background: Intracranial ependymomas are the third most common primary brain tumor in children. Since conflicting data have been reported regarding the prognostic significance of grading in ependymomas,especially in children ,the expression of proliferation and apoptosis regulators may contribute in the assessment of grading and prognosis The aim of this study was to investigate the expression of: p-53 tumor-suppressor oncoprotein, p-27/kip-1 cyclin/CDK inhibitor,bcl-2 anti-apoptotic protein and EGFR in pediatric ependymomas in correlation with Ki-67/MIB-1 proliferative index and prognosis Method:Our material comprised 17 ependymomas[14 cerebellar,3 supratentorial] from 13 children. One child suffered from two tumor recurrences and 2 children from one recurrence each.The ependymal tumors were classified into 3 classical ependymomas [grade II,WHO],4 anaplastic ependymomas[grade III,WHO] and 10 anaplastic with areas of classical ependymoma. The Streptavidin-Biotin method was performed on paraffin sections for the detection of Ki-67/MIB-1,p-53[DO-7],bcl-2[100/D5],p-27[1B4,EGFR [cl.EGFR25] Results: Ki-67 was detected in all cases,[range 5-50% of the nuclei]showing a high[>20%]expression in 14 anaplastic ependymomas[10 of them with a classical ependymal component]p-53 was detected in 15/17 ependymomas,showing a parallel high[>10%]expression with Ki-67 in 10 anaplastic ependymomas[7 with a classical ependymal component] EGFR was detected in a considerable number of ependymomas [88%15/17], showing a heterogenous high[>20%] expression in 11 anaplastic tumors[8 with a classical ependymal component]p-27 and bcl-2 proteins were detected in all ependymomas,showing a parallel high expression[>50% and >30% respectively]in 12 tumors[2 classical,10 anaplastic],while a parallel high Ki-67/p-27/EGFR expression was observed in 64,7%of the cases[11/17,3 anaplastic, 8 anaplastic with a classical ependymal component] The 2 cases of anaplastic ependymomas with a classical component showing recurrences showed high Ki-67/p-27/EGFR expression at initial presentation. In conclusion,high Ki-67/p-27/EGFR expression was correlated with the anaplastic phenotype of ependymal tumors suggesting that cell cycle deregulation may play an important role in their pathogenesis. EGFR detection in a considerable number of ependymomas probably reflects its involvement in the neoplastic transformation and may serve as a therapeutic target

PP1-192
MULTICYSTIC RENAL DYSPLASIA: A MISSING SPECIMEN FOR PATHOLOGIST

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Background: Multicystic renal dysplasia (MCRD) is the most common form of cystic renal disease in children and it is a clinically important consequence of abnormal nephrogenesis. Surgery was used as the treatment of choice for MCRD because of the potential complications of hypertension, infection and malignant change. After the results of prolonged follow-up of children with conservatively managed MCRD suggested that clinical approach was safe, the incidence of complications was small, and that there was a clear tendency for MCRD to decrease in size, surgery is not the preferred therapy. As a result the pediatric pathologists nowadays seldom find opportunity to examine a specimen of nephrectomy with MCRD. The aim of this study is to report a retrospective study of MCRD in children assessing the clinicopathologic properties. Method: The cases of MCRD diagnosed, treated and followed up in Dr.Behcet Uz Children Hospital Izmir Turkey between 1990 and 2007 were retrospectively reevaluated for clinicopathologic parameters and statistically analyzed. Results: Among 422 pediatric uropathologic specimens, 77 consisted of nontumoral nephrectomy specimens of which 17 (22.1%) were MCRD. The mean age is 2.58±3.59 (2 months-14 ages). The cases were left-sided in 8 and right-sided in 9 of the cases. The cases were male in 9 and female in 8 of the cases. The weight of the kidneys with MCRD was above normal in five cases, lower than normal in six cases and in normal limits in six cases according to the age. Associated renal pathology (ureteropelvic obstruction etc.) existed in eight cases (47%). Sixteen of the cases were operated before 2002 and only one case after 2002 till 2007. There are 41 cases diagnosed as MCRD in follow-up without operation in recent five years. Following surgical removal of the affected kidney, these cases progressed normally. Conclusion: Nowadays all children diagnosed as MCRD are submitted to conservative management with follow-up visits every six months following clinical parameters, blood pressure, urinary tract infection, renal function, involution of MCRD kidney, contra lateral renal growth. That is why MCRD is becoming a missing or "ancient" specimen for pathologist.

PP1-193
PEUTZ-JEGHERS SYNDROME IN CHILDREN, HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY, A CASE REPORT

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Introduction: Most polyps in children are sporadic, usually they are isolated or like colorectal juvenile polyposis, they don't require any supervision after surgical treatment. It has been noticed the increase frequency of some syndromes like: familial adenomatous polyposis, juvenile polyposis coli, hereditary mixed polyposis, Peutz-Jeghers syndrome, etc. We present the case of a 12 years old girl hospitalized to "Grigore Alexandrescu" Hospital for black pigmentation of the oral mucose, vomiting, signs of obstruction. The X-ray and computer tomografic exams have shown the presence of two polypoid masses located at the antrum level. Resection of the masses was performed. Material and methods: Fragments of the polyps were fixed in formaldehyde 10%, included in paraffin, the sections were stained with HE and VG and processed for immunohistochemical marker (ACT). Results: The microscopical exam revealed mucose glands separated and intermingled by thick smooth muscular fibers, this aspect being characteristic for hamartomatous polyps in Peutz-Jeghers syndrome. Conclusions: According to the latest literature data, the patients with this syndrome are supervised for long time because the malignancy risk. We agree for the necessity of screening protocols as well as the supervision of the risk organs.

PP1-194
NEONATAL GIANT CELL HEPATITIS AN UNUSUAL CAUSE OF NEW-BORN CHILD DEATH. REPORT OF TWO CASES

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Intorduction Neonatal giant cell hepatitis is a very rare condition in a clinical praxis. It is characterised by syncytial giant cell hepatocytes. Case report Both children with neonatal giant cell hepatitis were born in the same family in a two year interval and both died in the first 24 hour period. In the first case the female child's authopsy finding showed haemorrhagic diathesis: intraalveolar, kidney and ventricular mucosae bleeding. In the second case the male new-born child's authopsy finding showed petechial cutaneous bleeding of the head, trunck and extremities, fetal hidrops and ascites. Both cases share the same histological findings in the liver. Severe disorder of the liver parenchima with syncicial giant cell transformation of the hepatocytes. Transformed giant cell hepatocytes contained yellow pigment wich react with Berlin blue. Conclusion As hemosiderin deposites were found in the multinucleated transformed hepatocytes both of our cases we concluded that metabolic disorder in transportation and storage of iron is in the background of both cases so that hepatocellurar damage caused fatal haemorrhagic disorder.

PP1-195
ASSESSMENT OF DUCTAL PLATE MALFORMATION IN BILIARY ATRESIA. A HISTOLOGICAL STUDY OF 50 CASES

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Background. Biliary Atresia (BA), one of the commonest cause of neonatal cholestasis, is characterized by a necroinflammatory process affecting the entire biliary tree (sclerosing pan-colangitis). The process leads to the obstruction of the extrahepatic bile ducts and to the disappearance of intrahepatic ducts. The porto-enterostomy (Kasai operation), aimed to re-establish the biliary flow, can be curative on the sole condition that the basic disease process is subsiding after the corrective operation. If not the disease evolves unavoidably into biliary cirrhosis even after initially successful operation. Two distinct histological forms of BA have been described: the embryonic type or antenatal-fetal (early-severe BA) and the neonatal-perinatal form (classic BA). The early-severe (ES) form is characterized by arrest of remodelling of the embryonic ductal plate or ductal plate malformation. Incidence, clinical implications and outcome of the two forms are still controversial. Methods. We have reviewed the histology of 50 BA cases undergoing Kasai operation over a ten years period (1996-2006). According to the age at the time of Kasai operation the patients were divided in two groups: group A: <60 days, 20 patients (range: 22-58 days; median: 44); Group B: >60 days, 30 patients (range: 60-173 days; madian: 82). The main histological evaluation parameters were: persistence of ductal plate (lack of resorption and remodelling of the excess epithelial structures), degree of fibrosis, giant cell transformation and extramedullary haematopoiesis. Results. The distribution of histological forms was found to be: ES form 40 cases (80%), classic form 10 cases (20%). Severe biliary fibrosis was found in all ES forms; whereas fibrosis was mild in classic forms. Extramedullary haematopoiesis and giant cell transformation was equally distributed among the two forms. With regard to the age, the cases were distributed as follows: group A, 17 ES (85%) and 3 classic forms (15%); group B: 23 ES (77%) and 7 classic forms (23%). Conclusions: This study has yield three main results: 1) the ES form has turned to represent the vast majority of BA (up to 80%); 2) the patients age at Kasai operation does not differ between the two histological forms; 3) accompanying histological features such as extramedullary haematopoiesis and giant cell transformation are not useful histological criteria in differentiating the two forms.

PP1-196
EOSINOPHILIC ESOPHAGITIS IN CHILDREN: HISTOLOGY AND THERAPY COMPARISON.

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Introduction: Eosinophilic esophagitis (EE) is a chronic inflammatory immuno-allergic disease presenting with solid food dysphagia, food impaction, vomiting and abdominal pain. On endoscopy EE may appear as a patchy panesophagitis with white

spikes, vertical linear strictures, concentric rings up to esophageal stenosis. The histological hallmark is more than 20 eosinophils per high power field (HPF) and/or eosinophilic micro-abscesses in the superficial epithelium. EE is unresponsive to acid suppression. Therapy is aimed to eliminate the antigenic stimuli. Methods: To evaluate the histological response to current treatments, we reviewed 110 mid and distal esophageal biopsies from 32 pediatric patients (20M/12F, range 2-19 years, median 7) with gastro-esophageal symptoms unresponsive to Proton Pomp Inhibitor (PPI) undergoing upper gastrointestinal endoscopy between 2002-2007. Seven patients had esophageal motor disorder: 3 with esophageal atresia repaired, 3 with neurologically impairment and one with dismobility. Biopsies were stained with hematoxylin-eosin and alcian blue. Results: Twenty-nine patients were diagnosed as having EE on the basis of the eosinophil count (median eosinophil count 42 per HPF, range 21-100) and/or presence of eosinophilic micro-abscesses. Twenty-four patients underwent follow-up biopsies after treatment (prednisone, fluticasone oral swallowed, elemental or restricted diet). Five patients had just diagnostic biopsy. According to the diagnostic criteria, histological remission was obtained in 12 out 24 patients. In nine children the persistence of high number of eosinophils was concomitant with reintroduction of food in the restricted or elemental diet, whereas 3 patients did not gain either histological remission or symptom improvement. Finally in three patients although endoscopic features were suggestive for EE, the diagnosis was not confirmed histologically. As four patients had associated reflux esophagitis, PPI treatment was given. Two patients received surgical therapy. Two of these patients received a diagnosis of Barrett's esophagus. Conclusions: EE is a recent entity, almost rare in children. Our experience suggest that: 1) current treatment (restricted diet and/or corticosteroid drugs) seems to be effective in most patients with EE; 2) persistence of high level of eosinophils may be due to food reintroduction in elemental or restricted diet; 3) the unresponsiveness could be due to an incomplete or not compliance therapy and 4) occasionally reflux esophagitis can coexist; in this case PPI therapy or surgery is suggested.

PP1-197

DESMOPLASTIC SMALL ROUND CELL TUMOR [DSRT] IN CHILDREN CORRELATION OF IMMUNOHISTOCHEMICAL WITH GENETIC FEATURES

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Background: Desmoplastic small round cell tumor[DSRT] is a rare aggressive,usually intra-abdominal neoplasm with divergent differentiation affecting children and young adults,which may present with unusual clinical and histological features.The aim of this study was to investigate the immunophenotypic features of 8 pediatric DSRT in correlation with the expression of Ki-67,proliferative index,p-53,p-27/kip-1,bcl-2 proteins and genetic findings. Method:Our material comprised 7 intra-abdominal and 1 pleural DRST from 7 boys and 1 girl,aged 8-16 years. The Streptavidin-Biotin method was performed on paraffin sections for the detection of epithelial,mesenchymal and neural markers, CA-125,MyoD1, WT-1[cl.6FH2], Fli-1[SC-C19], CD99/MIC-2[12E7], c-kit/CD117, Ki-67/MIB-1, p-53[DO7], bcl-2[100/D5], p-27[cl.1B4] RT-PCR was performed on frozen and paraffin-embedded tissues of 7 cases for the detection of the EWS/WT-1 fusion gene.Results:The immunophenotype of DSRT was :8+/8 Desmin and Vimentin,6+/8 Cytokeratin AE1/AE3,Cytokeratin 8.18 and Cytokeratin 19, 8+/8 EMA, 8+/8 NSE,7+/8 CD/NCAM and/or CD57,0/8 Synaptophysin and S-100,5+/8 Neurofilaments 2F11,1+/8 Chromogranin A,2+/8 CA-125,1+/8 Smooth muscle actin,0/3 Calretinin and Cytokeratin 5/6,1+/8 bcl-2. CD99/MIC-2 was detected in 4/8 DSRT showing mainly a cytoplasmic expression and a membranous one in the pleural case with a parallel nuclear Fli-1 expression. There was no nuclear expression of WT-1 by the use of the 6FH2 clone.CD117/c-kit was detected in 2 DSRT showing a heterogenous membranous expression. Ki-67/MIB-1 was detected in >60% of the nuclei in 7 cases showing an inverse correlation with p-27 expression and a parallel one with p-53 protein. RT-PCR revealed the EWS/WT-1 fusion gene in 5/7 cAses regardless of the tissue used [frozen or paraffin-embedded] Cases with an unusual phenotype [2 cases Cytokeratin -, 1 Smooth muscle actin +, 1 pleural case Fli-1+] showed no particular variant of the EWS/WT-1 fusion gene. Six children died, 1 child is alive with disease and the boy with the pleural DSRT is alive without disease. Although there was no correlation of the immunophenotype with prognosis, the pleural DSRT showed a lower proliferative index [Ki-67:10-20%] In conclusion, the combination of clinical, immunohistochemical and genetic features is necessary for the diagnosis of DSRT. The detection of the specific EWS/WT-1 fusion gene on fresh and archival tissue contributes significantly in the diagnosis of unusual cases.

PP1-198

A THORACIC LIPOBLASTOMA IN 11-MONTH-OLD GIRL WITH REARRANGEMENT OF CHROMOSOMAL REGION 8Q11-13

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Background : Lipoblastoma is a rare benign mesenchymal tumor of embryonic fat with a good prognosis despite its potential for rapid growth and local invasion. It occurs in children under the age of 3 years (M/F, 3:1) and has predilection for the (lower) extremities. Although the first published lipoblastomatosis originated from the thorax, lipoblastoma arising from the chest wall is rare, often associated with extrathoracic component. The circumscribed form is named lipoblastoma and the diffuse form lipoblastomatosis. The main differential diagnosis is myxoid liposarcoma, which may have the same clinical and radiological presentation, but in liposarcoma, exceedingly rare in this age group, lipoblasts present nuclear atypia and pleomorphism. Karyotypic analysis showing the characteristic alterations in 8q11-13.may be of use when diagnosis is difficult Design: An 11-month-old girl presented with bronchiolitis. At chest X-ray a mass with a large intrathoracic component was present, associated with pulmonary and tracheal displacement and costal involvement. The tumour extended outside the thorax in the axillary region. CT scan displayed areas of low attenuation suggesting a fatty component. Fine needle biopsy displayed a benign lesion with admixture of fat and fibro-connective tissue. A surgical resection was performed, incorporating the segment of the third rib included in the mass. Results: The tumour weighed 514 gr and measured 13 x 9 x 5 cm. It was firm, fatty, partially encapsulated, with a central buldging zone corresponding to extrathoracic extension. Histologic examination showed typical lipoblastoma with a focal myxoid component. Conventional cytogenetics analysis showed in all tumoral cells the following

caryotype: 46,XX,ins(11;8)(q22-23;q12q24). The insertion had been confirmed by molecular cytogenetics. The breakpoints on the chromosome 8 were those involved in lipoblastoma. In situ hybridization is ongoing to confirm the rearrangment of the interest genes. Conclusions : Although most lipogenic tumours are relatively easily dignosed histologically, pathological diagnosis based on purely morphological parameters may be difficult. Cytogenetic studies have shown that the various dignostic entities among lipogenic and even lipomatous tumours are characterized by different, more or less specific chromosomal abnormalities. This information is used to establish a more biologically meaningful pathogenetic classification of these tumours.

PP1-199
MORPHOMETRICAL CRITERIA OF PATHOLOGY AND ADAPTATION IN HELICOBACTER PYLORI (HP) - ASSOCIATED GASTRITIS IN CHILDREN, TEENAGERS AND YOUTH

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Background.Peculiarities of Hp-associated gastritis during the first and second decades of life are brought about by the continuing development of immune system, formation of antiinfectional resistance and morfo-functional maturation in prepubertal and pubertal periods. Morphometrical criteria of ontogenetic evolution, pathological changes and adaptive reformation of GM at Hp-assosiated gastritis in children, teenagers and youth have been studied. Methods.Antral biopsies of 9 children under 7 years, 14 children 8-13 years, 30 teenagers and 9 youth were studied according to the Sydney systen. The following morphometrical findings analysed: the quantities of pits and glands per 1 mm of GM length, GM thickness, dimensions of GM components (the depth of pits, length of neck and glands). Metrical indicators are given in mm. Results. Morphometrical indicators of GM with aging tend to decrease (in the number of pits and glands) or increase (thickness of GM), and they do not change (depth of pits, length of neck) or statistically reliable ($p<0,05$) increase (number of glands) in GM. In groups 1 and 4 pit-glandular index amounted to 2,74 and 2,70. Percentage of pit-glandular complex components (pits, necks, glands) in groups 1 and 4 amounted to 43,7%, 23,0%, 33,3% and 40,0%, 20,0%, 40,0%. In groups with minimal and maximal manifestations of inflammation pits number ($p<0,05$) and glands number ($p<0,001$)decrease, thickness of GM ($p<0,001$) and glands length ($p<0,001$) increase. Pit-glandular index in both groups amounted to 2,92 and 2,60 and pit-glandular complex components percentage amounted to 41,7%, 20,8%, 37,5% and 37,9%, 19,0%, 43,1%. Conclusions. Received groups data reflect of ontogenetical evolution of GM: the decreasing of pits and glands number testifyes to growth of stomach. The increase of GM glandular part satisfies a requirements of growing organism.The reduction of pits number per GM is because of increase of inflammatory infiltrate in GM. In this case some of the glands undergo destruction and the remaining structures provide for adaptive reformation: increase of GM glandular part. Morthometrical indicators of GM at minimal manifestation of inflammation could be considered at close to normal. In this cases Hp-gastritis manifested itself for the first time and had short duration and changes in GM were completely reversible. Maximal manifestation of inflammation are typical for long term (recurring) course of Hp-gastritis but changes in GM are only partially reversible and go with the adaptive reformations.

PP1-200
PRIMARY ANAPLASTIC LARGE CELL LYMPHOMA OF SKELETAL MUSCLE SIMULATING ALVEOLAR RHABDOMYOSARCOMA

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Background: Primary skeletal muscle involvement by anaplastic large cell lymphoma (ALCL) is quite uncommon and few cases studies were reported in the international literature. We report a new case of CD30/ALK positive anaplastic large cell lymphoma simulating clinically and pathologically an alveolar rhabdomyosarcoma. Case: An 8–year-old boy presented with an enlarging painful mass of the right buttock without associated lymphadenopathy. A diagnosis of rhabdomyosarcoma was initially suspected. Results: Tumor biopsy revealed large anaplastic cells appearing as ill-defined cellular aggregates that show central loss of cellular cohesion reminiscing of alveolar areas. A panel of immunohistochemistry markers showed these large cells to be CD30 and ALK positive. Conclusion: Anaplastic large cell lymphoma should be included in the differential diagnoses of tumors in the soft tissue and pertinent immunohistochemestry is recommended.

PP1-201
RETROPERITONEAL MALIGNANT TERATOMA IN INFANCY

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Retroperitoneal teratomas are uncommon germ cell tumors in children. They account for less than 10% of all primary retroperitoneal tumors and 3% of the tumours in childhood. They occur mainly below 2 years of age. Majority of tumors are benign, situated on the left side and pararenal in location. Malignant teratoma was higher in adults than in children, with incidence of 26% and 10% respectively. We report an unusual case of a 4 months-old infant presented with abdominal pain and a left-sided abdominal mass. His alpha-fetoprotein was 79,49 mg/ml, β HCG was 0,54 mUl/ml. Abdominal ultrasound and CT scan showed a well circumscribed retroperitoneal mass of 84x80 mm in size with a variety of tissues and calcifications and located on the left side. During surgery, he was found to have a very large left retroperitoneal tumor adherent to the kidney. The entire tumor and the left kidney were excised. Pathological examination revealed a malignant cystic teratoma. Postoperative course was uneventful and the patient was well and asymptomatic at the 7months follow-up.

PP1-202
SOLID PSEUDOPAPILARY TUMOR: A CASE REPORTS

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BACKGROUND: We report SPT of pancreas, which was diagnosed in the period of last 10 years in Department of Pathology of our Institute. SPT is benign neoplasm of low malignant potential, with clear morphological charecteristics, unclear origin and recurrency. Most frequently appearing at young women and adolescnts. METHOD: We analysed clinical

symptoms and performed diagnostic methods: radiography and ultrasound imaging, MRI, as well as patohistology of tissue samples, stained by HE and using immunohistochemistry. RESULTS: During the period of 1997 to 2007 there was diagnosed two SPT-s on girls at the age of thirteen. First girl was operated with diagnosis of insulinoma, two years before admittion to our hospital. We suspect on cystic change on pancreas. Second girl was admitted at our hospital after abdominal trauma because of shoulder ache, pallor, perspiration and tachicardia. Lungs radiographic shows us no significant changes for the both of girls. Ultrasound for the first girl had shown that pancreas was not accessible, and MRI has shown the presence of tumor mass, for second girl hipoechogenic, oval mass of 6 cm diameter was noticed at the epigastric area. By laparatomic interventions, significant amount of blood and haemorrhagic-necrotic tumor tissue were evacuated from the head and pancreas body. Multiple fragments of dark-red, soft, structureless tissue, and pale-grey, homogeneous, partly compound solid mass, were sent at the pathology department. Microscopic findings: tumor tissue was imbibed by blood, partly necrotic, consisted of nests of tumor cells, medium-sized, oval, pale-eosinophylic cytoplasms with uniformly grooved nuclei within the stroma capillary rich. Around the stromal capillaries, tumor cells are cohesive and further away partly destructed, discohesive, pseudopapillary arranged. Tumor cells have shown excessive immunopositivity towards α-1-antitripsin and α-1-antihimotripsin, weaker immunopositivity towards NSE and vimentin, while negative towards citokeratin. Both patients are free of disease till now. CONCLUSION: With thorough microscopic and immunohistochemic analyses and correlation with clinical symptoms, the differential diagnosis was made easier in contrast with other, more frequent pancreas tumors. Complete surgical resection of SPT is treatment which insures long survival period after operation and good prognosis. It is necessity to monitor patients for long period of time in order to avoid rare metastatic deposits and frequent recurrency.

PP1-203
CLEAR CELL ADENOCARCINOMA IN DUPLICATION OF THE URINARY BLADDER - CASE REPORT

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BACKGROUND: Clear cell adenocarcinomas of the urinary bladder are rare tumors with an unknown histogenesis. Since these tumors appear histologically similar to clear cell tumors of the female genital tract, a mullerian histogenesis has been proposed. The isolated incident of vesical complete duplication is a very rare event that is characterized for the formation of two bladders with mucous membrane and muscular wall, each unity receiving ureter and presenting urethral duplication. The authors report an important case with atypical presentation of adenocarcinoma of clear cells when there is appearing in patient bearer of the rare one evil-formation of the urinary appliance. CASE REPORT: Patient of the masculine sex, 10 years, asymptomatic up to that time, presented suddenly a picture of urinary obstruction. He carried out computerized tomography of abdomen, when what revealed bulky cystic formation intraperitoneal and retroperitoneal, besides accented hydronephrosis to the straight one and absence of left kidney, confirmed by secretory urography. He was subjected to a surgery, taking two vesical bags as a find, one of them completely occupied by neoplasic vegetating injury. The duplicated and bearer of the neoplasic process bladder was excised. Macroscopically, the surgical specimen was consisting of cystic cavity with 9.0 x 7.0 x 5.0cm, occupied by neoplastic injury brownish violet and friable, not infiltrative and measuring 6.5 x 4.5 x 2.5cm. Microscopically, it was a papillary standard neoplasia with tubular and cystic areas, presenting columnar and cubic flattened cells with spacious and clearly cytoplasm and vesicular nucleolus. This morphological context associated to expression of CK7 and absence of calretinin and WT-1, confirm the diagnosis of adenocarcinoma of clear cells. The patient evolved well in the postoperative one, without urinary complaints. CONCLUSION: The primary adenocarcinoma of clear cells of bladder (mesonefric carcinoma) is a very rare tumor in this topography and it has like differential diagnosis since kind injuries, like the nephrogenic adenoma, but also malignant tumors as the urotelial carcinoma with clear cells or the metastatic carcinoma of renal cells. There is still not a long time of continuation in a great deal of these tumors, but the experience accumulated in the literature indicates that the adenocarcinoma of clear cells cannot be so aggressive like initially it was believed. The majority has a model of exophytic growth and they can be diagnosed in precocious traineeships with a better relative prediction.

PP1-204
ALLOGRAFT REJECTION AFTER LIVER TRANSPLANTATION IN CHILDREN

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Purpose: We performed this study to assess the clinical course of acute rejection and chronic rejection after liver transplantation in children. Methods: Seventy-six liver transplantations were performed in 75 children between December 1994 and March 2002. Twenty-five boys and 50 girls were included in this study, and the mean age was 20 months old. We analyzed the incidence, clinical course and outcome of acute and chronic rejection after liver transplantation retrospectively. Results: Forty out of 75 children (53%) experienced 45 episodes of acute rejection, and 32 episodes (71%) of them occurred within 1 month after transplantation. The degree of acute rejection was mild in 16 (36%), moderate in 14 (31%) and severe in 2 (4%) cases. Younger (＜2 years old) recipient experienced higher incidence of acute rejection. But there was no association with recipient´s sex, ABO matching, type of donor, and the kind of immunosuppressant. All 40 children with acute rejection improved with conventional treatment. There was no significant statistical relation between acute rejection and recipient´s survival. Chronic rejection occurred in 7 (9%) children, and 3 of them died of chronic rejection itself but another 3 children improved during the follow-up periods. Conclusion: Acute rejection occurred in more than half of the pediatric liver transplantation recipients. Most rejection episodes were mild and occurred within 1 month after transplantation. Acute rejection did not affect the recipient´s survival and graft function.

PP1-205
CHILDHOOD DEATHS IN MEDICO – LEGAL AUTOPSY CASES, IN ADANA – TURKEY
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Adana city is one of the major cities in Turkey. Forensic Sciences mostly practiced by the University although autopsies mostly held at the City Morgue by The Forensic Medicine Council Branch of the Ministry of Justice within consultation and laboratory supports of the University. The Department of Forensic Medicine at the University carries the autopsy histopathology of the 12 cities at the Eastern Mediterranean region of Turkey. In this study we analyzed the childhood age group of cases (0 –6 ages) during the 2005 – 2006 period. A total of 1197 cases are yielded to our laboratory for histopathological examination of the tissues and organ samples which had been taken during the autopsies at this region of the country. 216 (18%) of those cases were in the 0 – 6 age groups. 95 (44%) of them were natural deaths. 81 (37.5%) of them were unnatural deaths. 25 (11.6%) of them were stillborn cases whilst 15 (6.9%), remained unexplained. Of those childhood cases, 129 (59.7%) were female whilst 87 (40.3%) of it were male. Infectious diseases were the main cause of death in all cases with 57 (26.4%). Drowning had taken the second stage of all cases.

PP1-206
ACUTE PLASTIC BRONCHITIS: A CASE REPORT
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Introduction: Plastic bronchitis is a rare disorder characterized by the formation of branching mucoid bronchial casts. It is usually associated with underlying pulmonary diseases like bronchial asthma, allergic bronchopulmonary aspergillosis, cystic fibrosis, bronchiectasis and at times other system diseases like congenital heart defects and sickle cell disease. It is presented as acute respiratory distress with wheezing, breathlessness and cough mimicking foreign body aspiration. Plastic bronchitis has been reported from newborn period up to seventh decade but its exact pathophysiology is unknown. The bronchial casts may be divided into two types. Type 1, inflammatory cast with fibrin with cellular infiltrates associated with inflammatory diseases of the lung and Type 2, cellular cast made up of mucin with few cells usually associated with congenital cyanotic heart disease. Case report: A two-year-old boy was admitted to the hospital with complaints of cough, fever for two days and progressively increasing breathlessness. One month prior to the admission he had upper respiratory tract infection which was treated with antibiotics. There was no history of asthma; the chest X-ray showed atelectasis of the right upper lobe. Sweat chloride and tuberculin tests were negative. Serum immunoglobulins IgA, IgG and IgM were normal. Bronchial cast of the right main bronchus was extracted by forceps during bronchoscopy. It resembled a wax model of bronchial tree; was stained with H.E., PAS, Alcian Blue, Masson Goldner, Alcian PAS and Weighert for fibrin. The pathologic examination of this cast showed mucin, epithelial cells, fibrin and inflammatory cells neutrophils. Conclusion: Any child with acute respiratory distress refractory to conventional medical therapy with unusual radiographic picture needs intraluminal evaluation with bronchoscopy and pathologic examination.

PP1-207
UNUSUAL ELECTRON MICROSCOPIC FINDINGS IN MICROVILLUS INCLUSION DISEASE
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Background: The underlying causes of protracted diarrhea of infancy can be divided into those entities having a normal villus-crypt ratio and those associated with villus flattening. Microvillus inclusion disease (MID) is one of the causes of intractable diarrhoea in infants. Although ultrastructural detection of pathognomonic microvillous inclusions in the enterocyte cytoplasm is essential for the diagnosis of MID, unusual electron microscopic (EM) features such as intermediate structures between microvillous inclusions and lysosomes, inclusions containing few microvilli, dense apical granules in the apical cytoplasm can be observed in some cases. Therefore, two cases of MID with interesting EM findings are discussed further in order to draw attention to the variation in the ultrastructure of MID cases. Method: A 45-day-old boy and a 56-day-old boy were admitted to hospital with complaints of onset of diarrhea since the first and second weeks of their birth. Physical examination revealed abdominal distention and malnutrition in both babies. Laboratory findings showed anemia and hypoalbuminemia. They both underwent endoscopic duodenal biopsies. Formalin fixed biopsy specimens were stained with H&E, PAS and CD10 using streptavidin-Biotin peroxidase technique whereas gluteraldehyde fixed specimens were routinely processed for EM. Results: Duodenal biopsies showed partial to total villus flattening with brush border loss as demonstrated by PAS stain and CD10 antibody. PAS and CD10 also showed intracytoplasmic positivity in the form of apical vacuoles in both cases. EM showed marked brush border loss, and scattered typical apical intracytoplasmic vesicles containing microvillus inclusions in the enterocytes. Some vesicles had microvillus-like structures also located in the apical cytoplasm. There were also numerous dens apical granules. Conclusion: Though the diagnosis of MID depends upon the typical microvillus inclusions observed in EM, some cases may show less specific findings such as dens apical granules and intermediate structures resembling microvillus inclusions and also some atypical inclusions. Further studies seem to be necessary to evaluate the clinical significance of these unusual EM findings.

PP1-208
CONGENITAL RHABDOMYOMA OF HEART
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Background: Rhabdomyoma is reported to be the most common tumor of the heart in the pediatric age group. Tuberous sclerosis of the brain is the most frequently associated lesion, noted in approximately 50% of the reported cases. Case report: A male baby was born in the 29th week of gestation with birth weight 1200 g, birth length 36 cm and Apgar 1/1.The family and gestational histories were not remarcable. Physical examination showed bradycardy, ascites and anasarca. There were no neurologic signs and the skin was normal. The newborn died 9 min. after the delivery. The post-mortem examination revealed rhabdomyoma in the heart`s left ventricle (2,5x2x1,5 cm), multiple glial tubera with foci of calcification in the cerebellum and internal hydrocephalus. The cause of death was heart failure.

Tissue specimens from myocardium were fixed in 10% neutral formaline and processed by usual methods; paraffin sections were cut at 5 μm and stained with: H.E., PTAH, PAS, Alcian blue, Trichrom and lipid stain. Histological examination showed nodules of large, vacuolated spider cells, with PTAH positive cross-striation in myofibrills. The PAS stain revealed scanty amounts of cytoplasmic material with granular appearance. Lipid and alcian blue stainings were negative. Rhabdomyoma of the heart, responsible for death, accompanied with brain lesions known as tuberous sclerosis is rarely diagnosed in the newborn period.

PP1-209
EXPRESSION OF EGFR AND C-ERBB2 IN NEUROBLASTOMA

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Neuroblastoma, one of the most common extracranial solid tumor in children, has a highly heterogeneous clinical presentation and course. Current risk-based therapy is effective in patients who have low or intermediate risk features, however, intensive treatment of advanced neuroblastoma in children over one year of age is far from satisfactory. Expression of epidermal growth factor (EGF) receptors (EGFRs) has been associated with aggressive behavior in some tumors. The study was based on 40 tumors, including 32 neuroblastomas,4 ganglioneuroblastomas and 4 ganglioneuromas. The clinicopathological data comprised: patients' age, gender, tumour site and stage, tumour histology and MYCN status. The immunohistochemical analysis of expression of EGFR and c-erbB2 was performed with monoclonal antibodies on tumor sections from paraffin blocks. The staining was considered as positive if more than 10% of neoplastic cells were immunoreactive. The proliferation index was established on Ki-67 staining. 30 of examined tumors showed EGFR expression and statistical analysis showed tendency for significant relations between this and lower tumor differentiation and proliferation index (p=0,08), older age (p=0,11) and higher stage (p=0,14). In 24 tumors immunoreactivity for c-erbB2 was found and statistical analysis showed its siginificant correlations with proliferation index (p=0,04) and lower tumor differentiation (p=0,05). The tendency for significant c-erbB2 expression relations with tumor stage (p=0,09) was also found. Our results reveal frequent expression of EGFRs in neuroblastoma and show its relations to some clinicopathological features. These facts suggest that specific inhibitors of EGFRs can create the new promising therapeutic agents in neuroblastoma therapy. Work supported by grant of Polish State Ministery of Education No N40117631/3867

PP1-210
ANGIOGENIC POTENTIAL AND CLINICOPATHOLOGICAL FEATURES IN NEUROBLASTOMA

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The study was performed on tissue samples from 62 neuroblastoma (NB) Schwannian stroma-poor tumours including 39 untreated and 23 chemotherapy pretreated tumours. The aim of the study was to determine microscopic angiogenic parameters and looking for the associations between vascular parameters and clinicopathological features of tumours and basic prognostic factors. The clinicopathological data comprised: patients' age, gender, survival, tumour site and stage, tumour histology and MYCN status. The morphological analysis of the angiogenic potential concentrated on examination of vascular patterns - classical type or pathological angiogenesis with mural microvascular proliferation (MVP). The quantitative study included semi-automatic assessment of vascular density (VD) in CD34 stained tumour sections. Pathologic angiogenesis with MVP, including simple and/or glomeruloid type, was encountered in 25 cases and was more frequent in differentiating histology subtype and extraadrenal tumours. VD value ranged from 56 to 385 vessels/mm2 (median 149). Higher VD was connected with younger patient's age. In untreated tumours VD was significantly higher in infants than in children over one year of age. Pathologic type angiogenesis and lower VD were found to be associated with shorter patients survival. Our study confirmed high vascularization of NB and revealed common occurrence of pathological vascular pattern with MVP. Angiogenic potential in the analysed group showed diversity related to some clinicopathological tumour features. These facts show heterogeneity of NB tumours in vascular aspects, which possibly influence on tumours' response to antiangiogenic therapy.

PP1-211
P53 EXPRESSION IN DEVELOPING AND MATURE HUMAN KIDNEY

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Background: p53 is a negative regulator of the G1-S phase transition in the cell cycle. In the case of DNA damage it induces growth arrest or apoptosis in the damaged cells. In human embryos aged 4 to 8 weeks, p53 is expressed in the mesonephric secretory canal epithelium and focally in the cells of the mesonephric glomeruli. The aim of this study was to examine p53 expression in developing and mature human kidney tissues. Material and Methods: We examined human fetal kidneys obtained from therapeutic abortions (N=5;9-12) and kidneys from autopsied cases (N=13;12-36 week) and mature kidneys (N=7) from nephrectomia for neoplasia. P53 expression was examined by immunocytochemical tecniques. Results In the early stages of the fetal kidney, no staining was seen in metanephric blastemal cells, S-shaped bodies and ureteric buds. In the maturation stage of the fetal and adult kidneys no staining was seen all tubule segments and visceral, parietal epithelial cells in the glomeruli. Conclusion: No, p53 expressions were seen in fetal and mature kidneys.

PP1-212
ATYPICAL TERATOID/RHABDOID TUMOUR OF THE CENTRAL NERVOUS SYSTEM: HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF 4 CASES

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Atypical teratoid/rhabdoid tumour (AT/RT) of the central nervous system is a rare aggressive infantile neoplasm of

uncertain origin. Here we are describing the clinicopathologic and immunohistochemical features of four cases diagnosed as atypical teratoid/rhabdoid tumour. Of the four cases, three were male, one was female whose ages were ranged from 1 to 8 years (mean age, 3.2). Tumours were located in posterior fossa (two cases), frontal lobe (one case) and third ventricule (one case). Histopathologically, rhabdoid cells were prominent at least focally in three of them, but one of them was characterized with mesencymal appearence through the tumor. Undifferantiated small cells mixed with epitheloid components were evident in two of them. Immunohistochemically, epithelial membran antigen (4/4), cytokeratin (3/4), smooth muscle actin (3/3), GFAP (4/4), S-100 (2/2) and synaptophysin (1/3) were positive in a varying proportion but desmin(0/2) was negative. One of the patients died of disease within five months after surgery, another one was alive 26 months after surgery. One of them has recently diagnosed and the other was alive with disease 6 months after the surgery. The unique immunohistochemical profile of AT/RT helps to distinguish it from a PNET/medulloblastoma. This is of clinical importance because the prognosis of a patient with AT/RT is worse than that of a PNET/medulloblastoma despite aggressive therapy.

PP1-213
CARDIAC MALFORMATION IN THORACOPHAGUS TWINS

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Background: Conjoined thoracophagus twins (CTT) have a very high incidence of congenital heart malformations, including joined and individual congenital heart diseases (CHD). The aim of our study was to investigate types of CHD in 5 autopsied, unoperated cases of CTT. Method: All twins were female, 2 of them were stillborns, 2 were 2 months old, and 1 was only 4 days old. All autopsies were performed using standard or modified Rokitansky technique, and wide accepted segmental diagnostic approach for CHD. Results: In case #1, hearts were completely separated, and in other 4 cases the twins had common pericardium and fusion at the atrial and/or ventricular level. In case #2, the only communication was at the ventricular level, in apical region. But each heart individually showed complex cardiovascular anomalies (complete AVSD in one, and TGA with single inlet and single outlet in the other heart. In cases #3 and #4, conjoined hearts had 4 chambers with connection at atrial and ventricular level, i.e. common atrium and common ventricle. In case #5, one of the twins had the heart with 4 chambers, and the other one with only 2. Connections were at the atrial and ventricular level too. In 4/5 cases TGA was present, and the right aortic arch was found in 3 cases. Conclusion: These findings indicates that in CTT are present major congenital heart malformations, and some individual CHD. Besides the case #1 (with 2 separated hearts), eventual surgical separation of CTT was possible only in case #2 (connection in apical region), but presence of associated complex anomalies in both hearts would make this separation aimless. In the remaining cases any surgical attempt would be impossible.

PP1-214
OSTEOPONTIN (OPN) IS EXPRESSED IN HUMAN ADRENAL TISSUES AND ENHANCES WITH ITS RECEPTOR INTEGRIN ß3 THE INVASION OF HUMAN ADRENOCORTICAL CARCINOMA CELL LINE NCI259R

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Background: Osteopontin (OPN) is a glycoprotein of the extracellular matrix and interacts with its receptor integrin ß3, which we previously found to be expressed in benign and malignant endocrine tissues of the utero-placental system, and which were shown to mediate cellular migration and invasion and to contribute to tumorigenesis in several types of cancers. Recently, expression profiling of adrenal neoplasms revealed OPN mRNA to be expressed in these tumors. The present study was designed to analyze the expression of OPN protein in normal and tumorous adrenal tissues. Method: Immunohistochemistry with specific anti-OPN and anti-integrin ß3 antibodies were performed in normal adrenal tissue as well as in benign and malignant adrenocortical tumors. In addition, we studied OPN and integrin ß3 protein expression using Western blot analysis. Invasion assays were used to study the functional role of OPN in regulating tumor cell invasion. Result: In the normal human adrenal gland, OPN and its receptor integrin ß3 were found to be expressed strictly in the cortex, with the staining intensity increasing in a centripetal fashion. Expression of OPN and integrin ß3 was more heterogenous in the analyzed adrenocortical adenomas and carcinomas with staining intensity being more accentuated in single tumor cells or nests. These results were confirmed by Western blot analysis. OPN protein expression could also be found in the human adrenocortical cell line NCI259R. Treatment of these cells with human OPN increased invasiveness dramatically; this increase was partially reversed by transfection of the cells with with integrin ß3, supporting the idea that OPN acts to facilitate tumor development and metastases. Conclusion: In this study we show for the first time, that OPN protein is expressed in the human adrenal gland. Furthermore, we demonstrate that OPN expression is strictly confined to the cortex, making it, thus, a potential diagnostic marker to distinguish between adrenal cortex and medulla. Our ongoing studies aim to clarify the role of OPN in adrenal tumorigenesis.

PP1-215
INTERRELATIONSHIP BETWEEN DIABETES MELLITUS AND ARTERIAL HYPERTENSION: MOLECULAR ENDOCRINOPATHOLOGIC ASPECTS

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Background. The aim of the study was clarification of interrelationships between hormonal, metabolic, electrolyte, renal factors and the activity of the renin-angiotensin-aldosterone system (RAAS) in the aspect of predisposition of patients (pts) to arterial hypertension (AH) and possibilities of its prevention. Methods. There were studied 176 patients with newly-diagnosed diabetes mellitus (DM) and 45 healthy controls. 82 pts had DM I,

94 pts had DM II, 53.40% of DM pts had borderline hypertension, 48.30% had microproteinuria. 57.92% had hyperlipoproteinemia (HLP). The main parameters of carbohydrate, lipid, lipoprotein and electrolyte metabolism, the condition of insulin-producing function of the pancreas, plasma renin activity (PRA) and contrainsulin hormones were studied. Insulin, C-peptide, STH, ACTH, cortisol, aldosterone, PRA were determined by radioimmunoassay. Total cholesterol, alpha-cholesterol, triglycerides, total lipids, beta-lipoproteins were analyzed by biochemical methods; including HLP phenotyping according to Fredrickson (1978). Results. The results were analyzed in pts, grouped according to presence of borderline AH, microproteinuria, HLP presence and type, with consideration of DM type I or II. The study has demonstrated that changes of the hormonal, lipoprotein, electrolyte, filtration-reabsorbtion renal function, RAAS activity regulation occur at the early stages of the evident DM, leading to dyslipoproteinaemias and HLPs with atherogenic character, endogenous and exogenous hyperinsulinism, RAAS elevation, to enhancement of the filtration of the kidneys, to electrolyte disbalance, creating a high risk of AH. A differentiated approach to determining the predisposition towards AH was used, including such parameters as HLP, HLP type, presence of microproteinuria, DM type. The study has revealed their high diagnostic value for determining the early stages of the disease. Conclusion. It has been found that the DM compensation leads to a lowering, up to normalization, of many hormonal, humoral, electrolyte and renal factors, to lowering of RAAS activity, becomes an early measure of preventing the risk of AH establishing in DM, and of the prophylactics of the progression of diabetic angiopathies.

PP1-216
CD10 AS A MARKER OF TUMORS FORMING MORULES WITH BIOTIN-RICH, OPTICALLY CLEAR NUCLEI THAT CAN APPEAR IN DIFFERENT ORGANS

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Background: It has been demonstrated that CD10 immunostaining represents a useful marker of morules in endometrioid lesions of the female genital tract, allowing us to identify various low grade lesions which are associated with these nodular structures. Morules with a characteristic nuclear clearing have been reported in endometria, ovarian endometrioid tumors, colorectal adenomas and carcinomas, gastric polyps, gallbladder adenomas, pulmonary blastomas, low-grade adenocarcinomas of fetal lung type, some papillary lung carcinomas, pancreatoblastomas, and finally, in the cribriform-morular variant (C-MV) of papillary thyroid carcinomas. Here we investigate the usefulness of CD10 in the diagnosis of morular metaplasia in neoplasms of extragenital locations. Method: We studied the immunoexpression of CD10 and beta-catenin in seven cases of tumors containing morules with cells displaying biotin-rich optically clear nuclei (BROCN) (BROCN-family tumors), corresponding to four instances of CM-V of papillary thyroid carcinoma (one of them in the setting of a familial adenomatous polyposis), one case of low-grade adenocarcinoma of fetal lung type, one case of pulmonary blastoma and one pancreatoblastoma in a child with Beckwith-Wiedemann syndrome. Immunohistochemical studies were performed on paraffin sections with a universal second antibody kit that used a peroxidase-conjugated labeled-dextran polymer (Dako EnVision Peroxidase/DAB; DAKO, Glostrup, Denmark). Results: For CD10 (clone 56C6, dilution 1:10, microwave oven, Tris-EDTA, Novocastra, Newcastle Upon Tyne, UK), all cases revealed strong membranous positive staining in morules, with some cytoplasmic staining probably due to diffusion. As a consequence, CD10 positivity allowed easy identification of morules at low power in the different samples. CD10 immunostaining also outlined the luminal borders of the neoplastic cells in the glandular formations of all tumors. Aberrant nuclear expression of beta-catenin (beta-catenin-1, 1:300, microwave oven, DAKO) was detected only in neoplastic cells of all samples examined. Conclusions: Confirming previous reports on the expression of CD10 in morules, our findings support the notion that CD10 can represent a useful tool in identifying morules in the BROCN-family tumors of various organs. Our results also support the concept of BROCN-family tumors as entities sharing morphological features and alterations in the APC/beta-catenin pathway. Supported by grant PI060209 from Instituto de Salud Carlos III, Spain.

PP1-217
THYROID CARCINOMA ASSOCIATED WITH FAMILIAL ADENOMATOUS POLYPOSIS - A CASE REPORT

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Thyroid carcinoma is an extracolonic manifestation that is present in 1% to 2% of patients with familial adenomatous polyposis (FAP). We report a case of a 26 year old female with familial adenomatous polyposis (6 years ago), who was admitted to hospital because of an incidental finding of two thyroid nodules. The fine-needle aspiration biopsy revealed thyroid papillary carcinoma. The patient treated by total thyroidectomy without lymph node dissection. Gross examination of the thyroid identified four whitish circumscribed solid nodules ranging from 0,5 cm to 2,8 cm. Histological examination of the nodules revealed the presence of tumours which showed a variety of architectural pattern of growth . The pathological findings identified nuclear clearing, trabecular, follicular, papillary pattern and morular formations. There were solid areas with spindle cells in short fascicles or whorls and cribriform areas composed of varying sized follicles. The neoplastic cells sometimes formed tubular structures lined by ampophilic to oxyphilic tall cells. Immunohistochemistry showed strong positivity for cytokeratin 19 and focal positivity for HBME-1 and galectin-3. The tumour was negative for thyroglobulin, calsitonin, NSE, CEA, synaptophysin and chromogranin. The morphological and the immunohistochemical profile of the tumour suggested the diagnosis of multicentral papillary thyroid carcinoma associated with familial adenomatous polyposis. The patient is well with no evidence of disease 6 months following resection of the tumour. It has been suggested that FAP associated thyroid carcinoma is significantly different morphologically from both papillary and follicular types and can be considered as a separate entity. In general these carcinomas show good prognosis. We present this case because of its rarity and we review the findings in the literature.

PP1-218

ASSESSMENT OF FIXATIVES, FIXATION AND TISSUE PROCESSING ON MORPHOLOGY, ANTIGENICITY, AND ACID NUCLEIC INTEGRITY FROM TUMORAL AND NON TUMORAL THYROID TISSUES

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Histotechnology arguably can be viewed as a discipline devoted to optimizing the microscopic morphology of formalin-fixed tissues. Unfortunately, for molecular biology techniques, formalin fixation may be too successful in its ability to prevent autolysis (the degradation of proteins into amino acids). So, formalin preserves tissues by cross-linking proteins; however RNA (and sometimes DNA) is fragmented, chemically altered and difficult to isolate in quantity from formalin-fixed, paraffin-embedded samples. In this regard, frozen tissues specimens are the gold standard for molecular analysis. However, snap freezing presents several challenges regarding collection and storage, and preservation of histological detail. The aim of this study was to evaluate different alternative preservation methods (RCL2, Excell plus, Glyofix, Finefix solutions) by analyzing morphology (after different stainings such as hematoxylin eosin safran, PAS, Gordon-Sweet), antigenicity (immunostaining with HBME1, CK19, Galectin 3, TTF1 antibodies) and RNA/DNA integrities (quantity and quality) on different thyroid tissues (normal tissue, adenoma, nodular hyperplasia, papillary thyroid carcinoma). These different analyses were performed after 6h, 12h and 24h of fixation and compared to 6h, 12h and 24h of fixation in formalin. Results showed that all alternative preservation methods used in this study preserved morphology, staining and antigenicity of the different used thyroid specimens. In each case, the fixed and embedded tissues produced results similar to that obtained from formalin-fixed specimens. Recovery of mRNA was reduced in both quantity and quality in the different alternative preservation methods comparatively to that obtained from snap-frozen tissues, but was strongly superior to that obtained from formalin-fixed samples and largely sufficient to perform reverse transcription polymerase chain reactions. Recovery of DNA from all the different alternative preservation methods was superior to formalin-fixed samples as determined by one-dimensional gel electrophoresis and polymerase chain reaction. In conclusion, thyroid specimens fixed in the different alternative preservation methods used in this study, and embedded in paraffin, produce good histology, and immunohistochemistry and permit recovery of DNA, and mRNA sufficient for several downstream molecular analyses.

PP1-219

RE-CLASSIFICATION OF NEUROENDOCRINE TUMOURS OF THE RECTUM - A STUDY OF 97 CASES

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Background: The WHO classification for neuroendocrine tumours (NETs) of the gastrointestinal tract was introduced in 2000. Before that, tumours, which showed neuroendocrine differentiation were classified as carcinoids or atypical carcinoid tumours or as neuroendocrine carcinomas. The WHO classification divides these tumours into three distinct groups: Group 1 includes well-differentiated neuroendocrine tumours, Group 2 well-differentiated neuroendocrine carcinomas and Group 3 poorly-differentiated neuroendocrine carcinomas. This classification is based on tumor size, invasive growth and proliferation index. Rectal tumours represent approximately 10% of all GI-NETs. According to Kang et al. the incidence rate in the U.S. was 6.8/million in 1992 and 10.6/million in 2000, suggesting an increase in incidence. The aim of this study was to re-classify all rectal NETs from the pathology database of Helsinki University Central Hospital and its affiliate hospitals in the Hospital District of Helsinki and Uusimaa in southern Finland from time periods 1980-2000 and 2001 - 2005. . Method: The total number of rectal NET cases found was 97. All tumours were re-analysed independently by two pathologists. In addition to HE stain, immunohistochemical chromogranin A stain was done to confirm the neuroendocrine diagnosis. MIB-1 stain was used to evaluate the proliferation index and CD31 to confirm or exclude angioinvasion. Re-classification of the tumours was then made according to the WHO criteria. Results: The first 20 years time period from 1980 to 2000 gave a total number of 46 cases. The following 5 year time period (2001-2005) consisted of an additional 51 cases. Out of the first 46 cases 38 (83%) were classified as WHO group 1, 8 (17 %) as WHO group 2, and none as group 3 tumour. Tumours from years 2000 to 2005 are still under investigation. Conclusions: 83 % of rectal NETs from 1980 - 2000 belong to WHO group 1 and 17% to group 2. Poorly differentiated neuroendocrine carcinomas were not found. The number of rectal NETS found from the database during the past five year (n=51) compared to previous 20 years (n=46) supports the studies reporting an increase in the incidence of rectal NETs. Increasing awareness of this tumour entity may also partly explain the increase in incidence. In further analyses we shall relate the WHO classification to tumour behaviour and patient survival.

PP1-220

ABSENCE OF BRAF MUTATIONS IN THE CRIBRIFORM-MORULAR VARIANT OF PAPILLARY THYROID CARCINOMA

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Background: The cribriform-morular variant (C-MV) of papillary thyroid carcinoma (PTC) is the term coined for a sporadic type of PTC morphologically indistinguishable from most of the thyroid carcinomas that arise in the setting of the familial adenomatous polyposis (FAP). Somatic or germline mutations in the APC-beta-catenin pathway are associated with this particular subtype of PTC that may also harbour RET/PTC gene rearrangements. The majority of studies on record have found that RET/PTC rearrangements and BRAF mutations are mutually exclusive PTC; there is, however, one study that advanced that BRAF mutations may cooperate with RET/PTC rearrangements in the development of PTC. Objective: To find whether or not BRAF mutation play a role in the setting of C-MV of PTC. Method: We examined two familial and three sporadic cases of C-MV of PTC, for BRAF mutations. DNA was isolated from paraffin-embedded tumor tissues and the exon 15 of the BRAF gene was sequenced directly. We also estudied the immunoexpression of beta-catenin (beta-catenin-1, 1:300, microwave oven, Dako) on paraffin sections with a universal second antibody kit that used a peroxidase-conjugated labeled-dextran polymer (Dako EnVision Peroxidase/DAB; Dako, Glostrup, Denmark). Results: Aberrant nuclear expression of beta-catenin was detected in all tumors. No

mutations were found in any tumor samples analyzed. Conclusions: BRAF mutations do not seem to play, at variance with RET/PTC and APC/beta-catenin mutations, a role in the etiopathogenesis of C-MV of PTC (neither the typical V600E mutation nor the mutation associated to the follicular variant of PTC-K600E, were detected in any case of the C-MV of PTC). Supported by grant PI060209 from Instituto de Salud Carlos III, Spain.

PP1-221
CD 56 IN INVASIVE OVARIAN CANCER AND BORDERLINE TUMORS

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Introduction: CD56 is a glycoprotein that plays a critical role as neuroadhesion molecule in cell binding, migration and differentiation. It is expressed on NK cells and neuroendocrine neoplasm. We investigated CD56 in ovarian carcinomas in a retrospective study of consecutive cases. Methods: 115 female patients with a history of ovarian cancer were analyzed in a retrospective study. Immunohistochemical study of CD56 Expression was undertaken for 107 invasive carcinomas and 8 borderline tumors. The two groups were compared in respect of the immunohistochemical expression of CD56. Results: 71cases (66,4%) out of 107 invasive carcinomas showed positive staining for CD56 whereas one out of eight (12,5%) borderline tumors was focally stained with CD56. The CD56 positive borderline tumor turned out to be a micropapillary type. Invasiveness of ovarian cancer was highly significant associated with CD56 expression in comparison with borderline ovarian neoplasm (p£0.05; chi-square tests). The expression of CD56 was not associated with overall survival. Conclusion: Our results of different staining of invasive carcinomas and borderline tumors of the ovary suggest that CD56 may play a role in ovarian epithelial tumor cells becoming invasive. Further studies will have to determine whether CD56 is a useful additional marker in borderline tumors of the ovary to select for adjuvant treatment.

PP1-222
C-KIT EXPRESSION IN NORMAL AND NEOPLASTIC, INFLAMMATORY, REACTIVE LESIONS OF THYROID

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Background: The c-kit proto-oncogene encodes a transmembrane receptor tyrosine kinase (KIT). The oncogenic mutations of c-kit gene or activation of KIT lead to signal transduction ascades activation which regulates cell proliferation, apoptosis, chemotaxis, and adhesion. The aim of this study is to investigate the expression of c-KIT in normal, inflammatory, neoplastic, and reactive lesions of thyroid. Methods: Immunohistochemical staining using a polyclonal anti-c-kit antibody was performed on tissue blocks from 92 papillary carcinomas, 19 follicular carcinomas, 31 follicular adenomas, 25 nodular goiters, 13 Hashimoto's thyroiditis, 5 Graves diseases, 1 De Quervain thyroiditis, 19 lymphocytic thyroiditis, and 25 normal adult thyroid tissues. Extend of staining was evaluated in a semi-quantitative method; staining ≤10% was accepted as negative regardless of the staining intensity. Staining between 11 to 50% was accepted as (1+) and staining more than 50% was accepted as (2+). Intensity of the staining was evaluated qualitatively. Weak staining (1+), obvious staining (2+), and heavy staining (3+). Results: Positive staining was determined in 65 of 92 papillary carcinoma cases. Staining intensity of papillary carcinoma cases were found as (3+), (2+), (1+) in 26, 22, and 17 cases, respectively. Positive staining was determined in 9 of 19 follicular carcinoma cases. Staining intensity of follicular carcinoma cases were found as (3+), and (2+) in 3, and 6 cases, respectively. Positive staining was determined in 13 of 31 follicular adenoma cases. Staining intensity of follicular adenoma cases were found as (3+), (2+), (1+) in 2, 4, and 7 cases, respectively. We found positive staining in only two out of 25 cases of normal thyroid tissue, and their staining intensity were evaluated as (+2). Positive staining was found in only two out of 25 cases of nodular colloidal goiters, and their staining intensity were evaluated as (+1). Positive staining was determined in two out of five Graves' disease cases. Their staining intensity were (3+), (1+) each. Positive staining was determined in two cases of 13 Hashimoto's thyroiditis cases. Their staining intensity were found as (3+), and (2+) each. Conclusion: Immunohistochemical c-KIT positivity was detected in a wide range of neoplastic and inflammatory thyroid diseases. Especially notable is the high rate positivity in papillary carcinomas which can lead to new diagnostic and therapeutic approaches. Our results need to be confirmed by other molecular and genetic studies.

PP1-223
ANALYSIS OF THE CYTOLOGICAL FEATURES OF 9054 FNA OF THYROID AND CORRELATION TO THE CYTOLOGICAL AND HISTOLOGICAL DIAGNOSES

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Background FNA has been widely accepted as the best tool in selecting thyroid nodules for surgery. We analyze the various cytologic features of 9054 FNA and correlate them to histologic diagnoses aiming to estimate the diagnostic accuracy of FNA and to identify possible sources of erroneous diagnosis as well. Method A total of 9054 patients were examined by FNA of the thyroid during the period 2000-2004. The cytologic reports were retrieved from our files and a data list comprising all reported cytologic features, final cytologic diagnosis, histologic verifications from 262 patients and the comparison between cytologic and histologic diagnoses was entered in a data base (MS SQL server,2005). Statistical analysis was performed using SPSS1/13. Results The diagnostic results of FNA were classified as benign (82.63%), malignant (2.77%), neoplastic (of unknown behavior) (1.38%), suspicious for malignancy (3.31%), and as indeterminate (borderline lesions between a benign follicular/oncocytic lesion and a follicular/oncocytic neoplasm), (1.19%). According to the cytologic diagnoses, 10% of our patients were expected to underwent subsequent surgery. A definite classification was achieved in 98.5% of malignancy, papillary carcinoma being the commonest diagnosis. Correlation of cytology and histology showed that 84.7% of FNA results correlated with the histologic diagosis (138 true negative and 89 true positive results) while there were 16 false-negative (5.97%), 1 false positive (0.3%) and 25 false-suspicious results (9.3%).Accordingly, the sensitivity of our method was 85% and the specificity 84.12% when both false-positive and false-suspicious were grouped together and 99.28% when false suspicious were not included in the false-positive group. The cytologic profile of false positive/suspicious FNAs was characterized by high cellularity (62.5%), scanty colloid (58.3%), presence of oxyphillic cells (41.6%) and many lymphocytes (50%). False-negative smears contained few epithelial cells (75%), scanty colloid (81.2%) and lymphocytes in various proportions (75%). True positive smears exhibited frequently high cellularity (74.1%), oxyphilic cells (20.2%), intranuclear inclusions (19.1%), multinucleated giant cells

(32.5%) and lymphocytes (16.8%). Conclusion: FNA consists a method of high diagnostic value in the management of thyroid nodules. Confusing features in the direction of false suspicious diagnosis are the high degree of cellularity and the presence of oxyphilic cells while lymphocytes proved to be confusing feature leading to either a false suspicious or false negative result.

PP1-224
ONCOMARKERS OF MITOGENETIC PATHWAYS IN DIFFERENT HISTOLOGICAL TYPES OF PAPILLAR THYROID CARCINOMA

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The aim of the study was to investigate oncomarkers of mitogenetic pathways in different histological types of papillar thyroid carcinoma (PTC). Surgical material was used of completely removed thyroids or their fragments from 65 patients operated in faculty surgical clinic of I.M.Setchenov's MMA (40 cases in 1998-1999), the Moscow munitipal hospital ¹ 61 (25 cases in 1998-2000) on account of central formations in thyroid. In 11 cases there has been executed a thyreoidectomy, in 27 - a subtotal resection of a thyroid and in 27 cases - hemithyroidectomy. The patients were observed till February 2006 during the period from 6 till 8 years after the surgical treatment. Ultrasonic thyroid investigation was performed 2 times per year in every patient. Key factors of the basic mitogenetic pathways in PTC (c-kit, c-erb B2, c-Met, caveolin-1), as well as the markers of proliferation (Ki-67), invasive growth (CD10) and malignancy (HBME-1) were studied by the method of tissue micro array (TMA) and immunohystochemistry. Results. In proliferation of the PTC tumour cells are activated the mitogenetic pathways with participation c-kit, c-erb B2, c Met, caveolin-1. In papillary variant of PTC the mitogenetic pathways with c-kit, c-Met, c-erb B2 is the most active, and in oxifillic variant of PTC - caveolin 1, c-erb B2 and c-Met; in follicular variant of PTC all mitogenetic pathways take part, but at lower activity level. CD10 increases in the sites of tumour invasion more than two fold and can be also used as a prognostic marker. Proliferation of tumoral cells at different histologic types of PTC is carried out due to work of various mitogenic pathways, which activity depends on a phenotype of a tumour. During tumoral progression of PTC, at development of the remote metastasises and relapsing of the tumours, the general level of the cancer cells does not change. The contribution in proliferation of tumoral cells of such mitogenetic stimuli as c-kit, c-erb B2 decreases, that is possibly compensated by other growth factors. Conclusion. Obtained results show that different types on PTC are characterized by particularities in mitogenetic pathways of growth stimulation that can be useful in target therapy.

PP1-225
THE VALUE OF TTF-1 EXPRESSION IN DIFFERENT THYROID NEOPLASMS

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Background: Currently studies show that TTF-1 immunohistochemical expression is usually present in many thyroid and lung neoplasm. Due to its restrict expression, TTF-1 is a very useful marker in the diagnosis of these lesions. However, there is a diversity of TTF-1expression when we analyze the different thyroid neoplasm subtypes. The aim of this study is to evaluate the TTF-1 patterns of expression in variable subtypes of thyroid cancer. Casuistics and Methods: Slides from 40 patients with thyroid neoplasm were selected for the immunohistochemical study with TTF-1 antibody (Novocastra, 1:100). Twenty-four cases of papillary carcinoma, nine of follicular carcinoma, four of Hurthe cell carcinoma, two medullar carcinoma and one poor differentiated carcinoma. Results : From the 40 cases, we observed the following pattern of positivity for TTF-1: 21/24 in papillary carcinoma (87.5%), 9/9 in follicular carcinoma (100%), 4/4 in Hurthe cell carcinoma (100%), 2/2 in medullar carcinoma (100%) and one poor differentiated carcinoma (100%). Conclusion: Although the small number of cases, all the Hurthe cell and medullar carcinomas were positive for TTF-1 and three cases of papillary carcinoma were negative. These values are different from the literature and demonstrate the need of further studies in this area.

PP1-226
GLUCAGON CELL ADENOMATOSIS (GCA): A NOVEL NEUROENDOCRINE PANCREATIC DISEASE UNRELATED TO KNOWN GENETIC SYNDROMES

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Background: Microadenomatosis of the endocrine pancreas is thought to be a hallmark of the multiple endocrine neoplasia type 1 syndrome (MEN1). Here we describe a new type of pancreatic microadenomatosis that was not associated with a hereditary syndrome. Methods: Pancreatic tissue specimens from five patients with multiple glucagon-producing microadenomas were examined by immunohistochemistry, double fluorescence microscopy and morphometric methods. The MEN1 and Von Hippel-Lindau (VHL) status was assessed on the basis of clinical criteria, PCR based mutational analysis and loss of heterozygosity (LOH) analysis using specific FISH probes. The clinical features were recorded. Results: All patients showed exclusively glucagon-expressing neoplasms, with a total number of 10 macrotumors (all well differentiated neuroendocrine tumors) and more than 700 microadenomas. None of the patients displayed evidence of MEN1 or VHL germline mutations. Allelic deletions of the MEN1 and VHL gene were not detected. Only one of the patients showed a glucagonoma syndrome. Conclusions: We observed a novel previously not described benign non-MEN1 and non-VHL-associated type of pancreatic endocrine microadenomatosis, which may be functionally silent or produce a glucagonoma syndrome. This disease entity is characterized by glucagon cell microadenomatosis on which occasionally macrotumors develop. The tumors show no signs of malignancy.

PP1-227
EXPRESSION OF CK-19, cErb B2, GALECTIN- 3 AND p53 IN PAPILLARY THYROID CARCINOMAS

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The purpose of our study is to determine the expression of CK-19, cErb B2 and Galectin-3 in papillary thyroid carcinomas. Besides, we tried to assess the potential staining of those tumors by p53. Immunohistochemistry was performed on 43 formalin fixed paraffin-embedded thyroid tumors (23 papillary carcinoma (PC) and 20 papillary microcarcinoma (PMC)). The extent of staining was scored semiquantitatively as 0 (no staining), 1+ (< 25 %), 2+ (25 %-75 %) and 3+ (>75 %) and the staining intensity was evaluated as faint or strong. Statistical analysis was

performed using Chi-square test. Of the PC cases 56,5 % and of the PMC cases 15,0 % were stained strongly by cErbB2. Although by means of staining intensity of cErbB2, the difference in PC and PMC cases was significant statistically (p= 0,019), the results in extent of staining were not (p= 0,195). Of the PC cases 47,8 % was scored as 2+ while only 10,0 % was scored as 2+ in PMC by Galectin-3 (p= 0,003). Strong immunoreaction for Galectin-3 was obtained in 52,2 % of PC and 15,0 % of PMC (p= 0,006). All PMC cases were stained by CK-19 (35,0 % 1+, 25,0 % 2+, 40,0 % 3+) and 21 cases of PC (17,4 % 1+, 21,7 % 2+, 52,2 % 3+) expressed positivity. CK-19 expression was strong in 73,9 % of PC and 65,0 % of PMC (p= 0,140). No nuclear immunostaining was detected by p53 in PC and PMC. However, 65,2 % of PC and 55,0 % of PMC cases showed cytoplasmic reactivity. Both PC and PMC cases showed similar staining extention and intensity by CK-19. This may conclusively help in identifying the papillary carcinoma cases. The majority of PCs stained diffusely and strong by Galectin-3 and expressed strong reaction by cErb B2 might suggest a relation with the size of the tumor as PMCs stained less diffusely and strong. As no nuclear staining was detected by p53 in totally 43 papillary carcinomas we support the idea that p53 expression is a late event in thyroid carcinogenesis.

PP1-228
TWO YEARS EXPERIENCES OF THE NATIONAL PROJECT OF A STANDARDIZED BIOPSY DIAGNOSIS OF NEUROENDOCRINE TUMORS OF THE GASTROINTESTINAL TRACT IN SLOVAKIA

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BACKGROUND: The neuroendocrine tumors (NETs) of the gastrointestinal tract (GIT) show unique biological behaviour and require a specialized diagnostic and therapeutic management. Because of limited experiences of clinicians and pathologists with these tumours, a national program (NP) of a standardized biopsy examination and interpretation of all the clinical and laboratory results for the patients with NETs in Slovakia was established in January 2005 and introduced at ESP congress in Paris in 2005. METHODS: The NP is based on a cooperation of pathologists and involved clinicians within the accepted regional centralisation system. It includes a second look opinion of the cases at 3 accepted biopsy centres in Slovakia, in relation to clinical data evaluation. In addition to the unified panel of morphologic and IHC methods of the NETs biopsy diagnosis, all hormonal and prognostic markers may be examined in situ at our department being in a position of a coordination centrum of the NP. Tumors were classified according to the WHO classification (2000). All the data are summarized by using standardized protocols. RESULTS: The benefit of the NP is increasing number of diagnosed NETs cases. From the January 2005 until the March 2007, 66 cases of primary NETs in GIT were diagnosed and registered in the centrum. Another 28 cases were excluded, because 18 cases represented metastatic NETs and in 10 cases the diagnosis of NET was not proved. Age of patients was in range from 9 to 84 years (median 55 y.), male (n=43) to female (n=23) ratio was almost 2:1. Functional status was known only in 12 patients. The most common localizations were appendix (n=16) and ileum (n=12) which represents 23% and 18%, respectively. Morphologically, well differentiated endocrine tumors predominated (n=30), followed by well differentiated endocrine carcinomas (n=26). Poorly differentiated endocrine carcinomas comprised only 10 cases. CONCLUSIONS: The NP starts to offer standardized results of NETs diagnosis for the clinical practice, representing a good basis for their management, including their targeted therapy. Moreover, centralisation of these rare tumors increases the diagnostic assurance as well as quality of the histopathological diagnosis.

PP1-229
UNEXPECTEDLY HIGH INCIDENCE OF PAPILLARY THYROID CARCINOMA IN CASES RESECTED FOR HYPERTHYROIDISM

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Objective: The coexistence of hyperthyroidism and thyroid malignancy is considered a rare occasion. However this association has been reported more frequently in recent literature. Papillary carcinoma is the most frequently seen, yet any type of thyroid cancer may co-exist with hyperthyroidism. The aim of the study was to evaluate the association of hyperthyroidism and papillary thyroid cancer and its features such as size, variants and multifocality. Method: One hundred nineteen patients who underwent a thyroidectomy for hypertiroidism between 06, 2005 and 03, 2007 were reviewed. Seventy-five (63 %) of the 119 patients had toxic nodular goiter, 36 (30 %) had toxic diffuse goiter and 8 (7%) had toxic adenoma. Results: Histological examination revealed the presence of papillary thyroid carcinoma in 35 (29%) of 119 patients with hyperthyroidism. Concurrent carcinoma was detected in 23(31%) patients with toxic nodular goiter, in 11(31%) patients with toxic diffuse goitre and in 1(13%) patient with toxic adenoma. The patients of papillary carcinoma consisted of 25 females and 10 males, ranging in age from 22 to 76 years (mean age 45). Twenty nine (83%) papillary carcinomas were microcarcinoma with a diameter less than 1cm. The papillary carcinoma in thirteen cases (37%) were seen multifocally and eight (23%) of them were bilobar, five (14%) were unilobar. Twenty-two (63%) of 35 papillary carcinoma were follicular variant, the remaining 13 cases were classical variant. Conclusion: The coincidence of hyperthyroidism and thyroid carcinoma is not a rare phenomenon. Furthermore, the rate of papillary thyroid carcinoma in resected cases of hyperthyroidism is unusually high in our series, which we believe is the result of thorough gross and histological examination of the specimen. Besides, regional factors may have influenced the rate of incidence. Microcarcinomas may be overlooked because nuclear features of hyperfonctioning follicles and papillary carcinoma are alike, especially in follicular variant of papillary microcarcinoma. Histopathologic examination of the hyperfunctioning thyroid should be done carefully because of its impact on the clinical management of the cases.

PP1-230
MIXED MEDULLARY-PAPILLARY CARCINOMA OF THE THYROID: A CASE REPORT WITH AN UNUSUAL PRESENTATION

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The term mixed medullary-follicular (or papillary, presenting follicular component) thyroid carcinoma denotes a tumor showing the morphological features of both medullary carcinoma with immunoreactive calcitonin, and follicular areas with immunoreactive thyreoglobulin. We report the case of 41-year-old female, presented with a 6 months history of right swelling in the neck, increasing in size for last several weeks. Local examination revealed a well-defined, non-tender nodule in right thyroid lobe, which was mobile upon deep tissues and with

deglutition. Fine needle-aspiration cytology suggested a diagnosis of medullary thyroid carcinoma. The preoperative value of serum calcitonin was 1140 ng/ml, while the level of thyreoglobulin was normal (7,1 □g/l). A total thyroidectomy, presenting a 3,1x2x1 cm whitish nodule in the right lobe, as well as bilateral radical cervical lymph nodes dissection and bilateral medistinal lymph nodes dissection, were performed. Microscopic examination, confirmed the presence of medullary carcinoma. In addition, a focus of papillary carcinoma intimely intermingled within the medullary component was observed, representing nearly 10% of the whole tumor. The mediastinal lymph nodes were free of tumor cells. Among numerous cervical lymph nodes, only one at the homo-lateral level III, present two micro-foci of medullary carcinoma tumor cells. No C-cell hyperplasia was found, nor nodular goiter or significant inflammation in the adjacent thyroid parenchyma. Serum calcitonin levels decreased to normal after operation and are currently stable. No clinical history suspected for familial medullary carcinoma was recorded for this patient, and RET proto-oncogene point mutations was absent by DNA analysis on peripheral leucocytes. The presence of microscopic metastasis in a single homo-lateral cervical lymph node, underlines the great importance of large cervico-mediastinal lymph nodes dissection in medullary thyroid carcinoma. Moreover, as for the majority of the reported cases of mixed medullary and follicular thyroid carcinomas, in the present case the medullary carcinoma component seemed to possess the more aggressive biological behaviour, although the therapeutic strategy in this particular type of thyroid cancer is still controversial.

PP1-231

UTILITY OF ECO-COLOUR DOPPLER SONOGRAPHY IN THE SELECTION OF THYROID NODULES FOR FINE-NEEDLE ASPIRATION CYTOLOGY

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BACKGROUND: Ultrasonography guidance has increased the sensitivity of fine-needle aspiration biopsy (FNAB). Nevertheless, in multinodular goiters the choice of nodule to submit to FNAB is still a problem. In a previous work, we have shown that thyroid nodules have a characteristic vessel distribution, that can be visualized on eco-colour doppler sonography (ECD). Purpose of the present study is to evaluate if the vascular pattern can be useful to select thyroid nodules for FNAB. METHOD: 263 thyroid nodules were analysed by ECD before FNAB and classified as follow: no vessels evident (type 1), only peripheral vessels evident (type 2), intranodular vessels (type 3). RESULTS: Type 1 nodules were 45 and were diagnosed on FNAB as negative for malignancy in 36 (80%) cases, follicular lesions (FL) in 5 (11,1%), papillary carcinomas (PC) in 2 (4,4%) and inadequate in 2 (4,4%) cases. Type 2 nodules were 57: 45 negative for malignancy (78,9%), 11 FL (19,3%) and 1 inadequate (1,7%). Type 3 nodules were 161: 93 negative for malignancy (57,8%), 56 FL (34,8%), 8 PC (5%), 1 medullary carcinoma (0,6%) and 3 inadequate (1,9%). CONCLUSION: Type III nodules show a highe rate of neoplastic lesions (40,4%). This suggests that vascular pattern may be useful in the selection of thyroid nodules for FNAB.

PP1-232

THE USEFULNESS OF CYTOKERATIN-19 IMMUNOHISTOCHEMISTRY IN THE DIFFERENTIAL DIAGNOSIS OF PAPILLARY THYROID CARCINOMAS

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Background: The gold standard for diagnosis of papillary thyroid carcinoma is conventional histology, the essential element of which is its characteristic nuclear features, regardless of whether papillary architectures are present or not. This study has been carried on to evaluate cytokeratin-19 immunohistochemical marker in the diagnosis and differential diagnosis of papillary thyroid carcinoma. Methods: Cytokeratin-19 has been tested on formalin-fixed, paraffin-embedded tissue from 380 surgically resected thyroid lesions including hyperplastic nodules (n: 243), granulomatous thyroiditis (n:3), lymphocytic (n:53) and Hashimoto's thyroiditis (n:11), follicular adenomas (n: 17), Hürthle cell adenomas (n:3) and neoplasia (n:1), well-differentiated thyroid tumor with follicular architecture of uncertain malignant potential (n:1), papillary carcinomas (n:45), follicular carcinoma (n:1), insular carcinoma (n:1) and medullary carcinoma (n:1). Using a monoclonal antibody to cytokeratin-19 and a standard avidin-biotin complex technique, immunostaining has been performed. The immunoreactivity has been scored as negative (less than 10% cells positive), 1+ (poor-focal cells positive), 2+ (strong-focal cells positive), 3+ (poor-diffuse cells positive), 4+ (strong-diffuse cells positive) based on the extent of the reaction regardless of previous diagnosis. Results: No cytokeratin-19 has been found in 100% of hyperplastic nodules, 1.9% of lymphocytic thyroiditis, 33.3% of granulomatous thyroiditis, 52.9% of follicular adenomas, 66.7% of Hürthle cell adenomas, 100% of Hürthle cell neoplasia and 100% of follicular carcinoma. All of the 45 cases of papillary carcinomas have positively reacted to cytokeratin-19, respectively; 57.8% 4+, 33.3% 3+ and 8.9% 2+. Here, to prevent confusion on, it can be said that the group with 2+ reaction is the micropapillary variant of papillary carcinomas. 100% of well-differentiated thyroid tumors with follicular architecture of uncertain malignant potential, 5.9% of follicular adenoma and 3.8% of lymphocytic thyroiditis have shown reaction 4+. 11.8% of follicular adenoma, 18.2% of Hashimoto's thyoiditis and 1.9% of lymphocytic thyroiditis have reacted 3+. Other cases have shown focal reactions. Conclusion: Papillary thyroid carcinomas have presented strong diffuse cytoplasmic reactivity with cytokeratin-19. Along with careful histologic evaluation, cytokeratin-19 provides a useful and objective means for the diagnosis of papillary thyroid carcinomas.

PP1-233

HASHIMOTO'S THYROIDITIS DOES NOT INCREASE THE RISK OF PAPILLARY THYROID CARCINOMA: A LARGE CLINICAL-PATHOLOGIC RETROSPECTIVE STUDY

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Background Several studies report a higher rate of papillary thyroid carcinoma (PTC) in patients with Hashimoto's thyroiditis (HT), suggesting (and vice-versa) a correlation between the two diseases. The occurrence in HT of foci of follicular cell proliferation showing striking nuclear clearing has been interpreted by some authors as a precancerous state leading to

subsequent increased incidence of PTC. This hypothesis is also supported by the detection of RET/PTC rearrangements in HT thyroid glands, although the relevance of this finding is still controversial. The aim of the present retrospective study is to re-evaluate the association of HT with PTC and other malignant or benign thyroid neoplasias in a large series of pathological specimens. Methods We retrieved 1350 consecutive cases of patients submitted to total thyroidectomy whose gland were analysed in the Pathology Department of the University of Cagliari in the period January 2001 - December 2005. Common histological criteria were employed for the diagnosis of thyroid neoplasm. The histological diagnosis of HT was made on the basis of histological parameters (diffuse lymphocytic infiltration with prominent germinal centers, oxyphilic metaplasia; cases with focal lymphocitic infiltration were not considered). Results Pathological evaluation showed: 491 cases of PTCs (36,4%), 202 cases of follicular adenomas (15%), 87 cases of follicular carcinomas (6,4%). HT has been found in 452/1350 cases (33,5%) and in association with PTC in 177/491 cases (36%) The proportion of PTC not associated with relevant lymphocitic infiltration was similar (314/898 = 35%, p= ns vs HT). In contrast , the proportion of malignant and benign follicular tumors associated with HT was significant lower than that found in the absence of HT. However we found a statistically significative negative correlation between the incidence of follicular carcinomas (12/452 in HT vs 75/898 without HT, p=<0.0001) and follicular adenomas (47 /452 vs 155 /898, p=0.0009) Conclusions Our results, suggest that, although the proportion of PTC associated with HT is higher than that found in follicular carcinomas and adenomas, an increased incidence of PTC in HT is unlikely. On the other hand, our data suggest that HT could be a protective factor against the development of these entities, but further studies are warranted to confirm this hypothesis.

PP1-234
IMMUNOHISTOCHEMICAL EXPRESSION OF THE APOPTOTIC AND ANTIAPOPTOTIC MARKERS FAS/FASL, Bcl-2, BAX AND P53 IN NEOPLASTIC AND NON NEOPLASTIC THYROID DISEASES

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Backround: The role of the apoptosis is considered crucial in the pathogenesis and development of the thyroid diseases. The most important proteins, involved in the mechanism of the apoptosis in thyroid tissues are the Fas/FasL system, p53, bax and Bcl-2. The Fas- protein functions as a cell-surface receptor against the factor FasL, which is mainly produced from the lymphocytes. The Fas/FasL system, which induces apoptosis, is regulated by p53 and bax proteins, which in turn also induce apoptosis and by bcl-2 protein which inhibits apoptosis. The purpose of this study is the examination of the apoptotic mechanisms, via immunohistochemical expression of the Fas, FasL, Bax, bcl-2 and p53 proteins in thyroid carcinoma. Methods: Our material consisted of 21 papillary, 6 follicular, 3 insular and 1 anaplastic carcinomas, 6 colloid nodules, 3 autoimmune thyroiditis and 5 normal thyroids. Immunohistochemical stains were performed on paraffin sections. The results were analysed statistically with the method x2. Results: Fas receptor was expressed in almost every carcinoma (94%), but also in a high percentage (78%) in non-neoplastic diseases (p=0.08). FasL factor was expressed in a lower percentage in carcinomas (35%), as well as in non-neoplastic diseases (50%), without important statistic significance. Bax protein expression was detected in 71% of the carcinoma-cases, but only in one non-neoplastic case (p=0.0004). Bcl-2 protein expression was also statistically significant (p=0.007) between carcinomas (35%) and non-neoplastic diseases (86%). Additionally, the correlation of bcl-2:Bax protein expression in carcinomas showed a significant statistic difference p<5. P53 protein was expressed only in the anaplastic carcinoma. Conclusion: Non-neoplastic thyroid, in this small sample, seems to resist to apoptosis. In the apoptotic mechanism of thyroid carcinoma, bax proteins seems to be mainly involved. The role of the Fas-FasL apoptotic pathway is not proved in our sample. However, the frequent expression of Fas- receptor in the neoplastic population might indicate its increased sensitivity in Fas-mediated apoptosis. This pathway needs more investigation for the possible involvement of other apoptotic or antiapoptotic signals.

PP1-235
MEDULLARY THYROID CARCINOMA PRESENTING AS A CYSTIC LESION

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Background: Ultrasonography has gained a more important role in the thyroid nodule evaluation. Medullary thyroid carcinoma (MTC) usually presents as a solid nodule, only three cases showing a cystic component have been reported in the literature. Purpose of the present study is to report on two further cases of MTC presenting as cystic lesions. Method and Result: Case 1: a 52 yr-old female presented with ultrasonographic 2 cm solid nodule with a cystic component. There was no family history of thyroid diseases. The patient had normal levels of TSH; T3; T4, and high levels of calcitonin (57,1 pg/ml). A fine needle aspiration (FNA) of the lesion was performed for cytologic examination. The intranodular level of calcitonin obtained by syringe's washing was 2000 pg/ml. Case 2: a 63 yr-old female, with no family history of thyroid diseases, showed at ultrasonography a 4 cm partially cystic nodule located in the left thyroid lobe. FNA led to the diagnosis of malignancy. The plasma level of calcitonin was high (6000 pg/ml). Both patients underwent total thyroidectomy with neck dissection. No lymph-node metastases were present. On histology both cases were composed of a central cystic area lined by neoplastic cells positive with anti-calcitonin antibody which confirmed the cytological and clinical diagnoses of MTC. No clinical evidence of recurrence has been noted in both patients. Conclusion: The risk of malignancy in thyroid cystic nodules is low, nevertheless the possibility of a cystic MTC should be taken in consideration. In this respect, very useful appears to be the evaluation of CT level in the syringe's washing, which is able to demonstrate the presence of intranodular CT-producing tissue.

PP1-236
CAVERNOUS HAEMANGIOMA OF THE THYROID GLAND

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Background: Haemangioma is a type of the commonly observed soft tissue tumours, composed of dilated, generally thin-walled blood vessels. At all sites, it is often difficult to determine whether benign vascular lesions are malformations or true neoplasms. The thyroid gland is an uncommon site for this type of neoplasm. Case description: We present a female patient 59 years old, who complaint of a thyroid mass. The x-ray showed a solid, heterogeneous structure at the right lobe of the thyroid

gland, and scintigraphy revealed a “cold” nodule at the right lobe of the thyroid gland. The results of biochemical examination, including thyroid hormone levels, were normal. The patient underwent thyroidectomy. Pathologic findings: Grossly a reddish nodule, 3 cm in size, spongy and of soft consistency, was found. Microscopically the lesion consisted of numerous proliferated vessels that enlarged to an irregular shape, and the lumen was full of red blood cells. Occasionally organized thrombi, were observed. The inner wall of vessels was covered with monolayered endothelial cells and the outer wall was fibrous. Stromal hyalinization, myxomatous degeneration and inflammatory infiltrates, were quite common. The findings mentioned above indicate the diagnosis of cavernous haemangioma, which as far as we know, is extremely rare in the thyroid gland. The patient is doing well, seven months after the surgical excision.

PP1-237
TWO UNUSUAL CASES OF ADRENAL HEMANGIOMAS

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BACKGROUND: Hemangiomas of the adrenal gland are very rare, benign, capillary or cavernous tumors with a tendency to thrombosis. Until 2006 only 50 cases have been published and none was associated with adrenal cortical hyperfunction. Papillary endothelial hyperplasia (PEH), an exuberant proliferation of vascular endothelial cells, very rarely develops in the adrenals. We present two cases of adrenal vascular lesions one with multifocal cavernous hemangioma and the other with a PEH arising at the periphery of a thrombosed, hyalinized, calcified cavernous hemangioma. METHOD: Case1: A 71 years old female with mild cortical hyperfunction underwent right adrenalectomy and the entire gland was removed weighing 16 gr and measuring 5,5X4,5X2,5cm. In between the solid yellow areas cystic hemorrhagic or myxoid foci up to 1 cm. Case 2: A 73 years old male with a suspected metastatic asymptomatic mass in the right adrenal gland during a follow-up for a resected colon adenocarcinoma two years previously. The tumor was 3 cm in diameter, well-circumscribed, white-yellowish, granular and chalcky and the entire specimen weighed 13 gr. Both cases were examined with H+E and immunohistochemically for CD34, CD31, Factor VIII, VEGF, Desmin, a-SMA. RESULTS: Case1: Multifocal cavernous hemangioma intermingled with hyperplastic adrenal cortex. Several vessels were thrombosed in different stages of organization. Case2: A non-encapsulated, well-circumscribed hemangioma thrombosed and hyalinized in most part with focal nodular calcifications. At the periphery there was a dilated sinus containing hyalinized papillary projections lined by a thin layer of endothelial cells without cytological atypia or mitotic activity. The mass was surrounded by residual islands of adrenal cortex. CONCLUSION: Multifocal adrenal hemangiomas are unusual and this is the first case associated with cortical hyperplasia. Despite their benign nature they should be removed for exclusion of malignancy. The etiology of PEH is not completely understood, is often associated with trauma and may require differential diagnosis from angiosarcoma. It usually occurs within the lumina of veins, hemangiomas, pyogenic granulomas and cystic lymphangiomas. In our case there was no history of post-colectomy vein thrombosis or septicaemia

PP1-238
ISLET 1 (Isl1) IS AN EXCELLENT MARKER FOR PANCREATIC ENDOCRINE TUMORS (PET) AND THEIR METASTASES

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Background: Metastases of pulmonary and gastrointestinal endocrine tumors can be diagnosed using the markers TTF1 and Cdx2, respectively. The identification of a pancreatic origin, however, is still an unsolved issue. Islet1 (Isl1) is a LIM homeodomain protein expressed in endocrine cells of normal pancreatic islets. The purpose of this study was to examine the diagnostic value of Isl1 as a marker for pancreatic endocrine tumors and their metastases. Study design: A tissue micro array (TMA), comprising 215 gastroenteropancreatic endocrine tumors and their metastases was constructed. Sections of the TMA were incubated with the antibodies directed against Isl1, Cdx2 and Ttf1. The stainings were scored semiquantatively. Results: Isl1 proved to be a highly specific marker for islet cells and PET. In primary PET its specificity was 92.1% (sensitivity 63.4%) and in metastases of PET the specificity reached 100% (sensitivity 72.4%). Depending on whether only a strong immunostaining or both a strong and a moderate immunoreaction were scored as positive, Cdx2 showed a specificity for gastrointestinal origin in primary tumors of 89.7% and 66% (sensitivity 75.7% and 79.3%) and in metastases of 100% and 93.3% (sensitivity 40% and 68%). Ttf-1 was not expressed by pancreatic or intestinal endocrine tumors. Conclusions: Isl1 is an excellent marker for identifying PET and their metastases. Moreover, our findings confirm that Cdx2 is a valuable marker for endocrine tumors of gastrointestinal origin.

PP1-239
AMYLOID IN PITUITARY ADENOMAS: INCIDENCE AND MORPHOLOGICAL ASPECTS

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Introduction:Amyloid deposits in tumoral hypophysis is found in 40% of pituitary adenomas and the amyloid protein precursor in these cases is a hormonal polipeptyde.Lactotroph and somatotroph adenoma series mention the presence of amyloid deposits in 50% and 65%, respectively. Amyloid may be interstitial with a fibrillary, starry sky pattern or rarely as spherules.The blood vessel walls are also compromised. Aim:1)Evaluate the incidence of amyloid deposits in pituitary adenomas through Congo-red stain and polarized light.2)Observe the morphology and distribution of amyloid material in positive cases.3)Correlate the occurrence of amyloid with hormonal production. Material and Methods: Forty eight pituitary adenomas operated by the Neurosurgery Service of UNICAMP Clinical Hospital and submitted to immunohistochmistry were distributed as such: 10 somatotroph adenomas, 5 lactotroph adenomas, 5 corticotroph adenomas and 28 non-functioning adenomas. The cases were stained by Congo-red and observed through polarized light. Results:The incidence of amyloid material in the total sample was 62,5%. The interstitial amyloid deposits showed a fibrillary and band like patterns specially in blood vessel wall. All the hormonal type adenomas showed amyloid deposits: somatotroph adenomas 30%, lactotroph adenomas 80%, corticotroph adenomas 60%, non-functioning adenomas 71,4%. Conclusion: Pituitary adenomas show a high incidence of amyloid deposits, no matter if it is a non-functioning or functioning adenoma. Among functioning adenomas, amyloid incidence was higher in lactotroph adenomas, followed by corticotroph and somatotroph adenomas.

Molecular Pathology

PP1-240
WHAT HAS CHANGED IN RUSSIAN PATHOLOGY SINCE THE FIFTIES?
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The question can be answered by the example of the Department of Pathological Anatomy of Moscow Medical Academy. This Department has been leading in our country. All main textbooks have been created here. Professor A. I. Strukov was Head of the Department in 1953-72. In spite of his support of Lysenko-affaire, Strukov was known as a highly educated pathologist and good practical specialist. Under his guidance much attention was given to the autopsy technique; trainees were taught to perform autopsy correctly and in detail. Histological laboratory was staffed with qualified technicians, numerous histological stains were performed routinely. Professor V.V. Serov, perfect lecturer and specialist in autopsy, was Head of the Department from 1973 to 1990. During this period, autopsy partly lost its academic profoundness. Post mortem diagnoses were sometimes adapted to a preconceived idea and formulated without sufficient evidence. Biopsy diagnostics was performed by experienced specialists individually. Discussions of biopsy cases at the departmental conferences were seldom; much more time was given to research and autopsy. Trainee pathologists were taught biopsy diagnostics only sporadically. Technique of gross examination remained primitive. For example, in case of mastectomy or gastrectomy, only 2-3 lymph nodes were usually embedded. Immunohistochemistry and other modern methods were available only for research and were not used on biopsy material. For making successful career, candidate's and doctoral theses and regular publications have been needed. Limited technical possibilities and access to foreign literature made global newness of research hardly possible. Nonetheless, global newness and practical significance were officially required from theses. This alone forced researchers towards strained interpretations and biased presentation of facts. Researchers often planned their results in advance and tried to formulate conclusions accordingly. Along with studies having value of review or compilation, numerous useless reports were published. Many scientists compiled their reference lists by means of Current Contents or reference lists from other articles. False quotation and incorrect statistical assessment could be encountered. Plagiarism from foreign sources was not infrequent. By the end of the eighties a new phenomenon became apparent: young functionaries, predestined for high positions, published and defended as theses largely fabricated papers, containing provable manipulations of statistics etc.

PP1-241
THE USE OF ARCHIVAL PATHOLOGY SAMPLES IN FORENSIC SCIENCES
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DNA identity testing has gained widespread in the field of forensic sciences since the first forensic use in 1985. The main fields of forensic DNA analysis are, linking biological evidence to suspects-victims-crime scene, paternity and maternity identification, missing persons and disaster victim identification. After disasters some special criterias are determined by police and multidisciplinary scientists. Evidences for identification provided by personal effects such as clothing, jewellery and pocket contents, external examination, internal examination, dental examination and genetic analysis. Human biological samples such as blood, saliva, semen,hair, bone, teeth are the main sources for DNA anaysis. Some other samples, which can be used in research for forensic cases although they were not created for that purpose. During more than a century of diagnostic practice, a large number of archival paraffin-embedded tissue banks have been established worldwide. When no other reference material is available, archival pathology specimens can be used as a source in forensic identification.

PP1-242
IMMUNOHISTOCHEMICAL DETECTION OF MAGE-3 PROTEIN IN COLORECTAL CARCINOMA & POLYPS
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BACKGROUND:The MAGE(melanoma antigen)gene family consists of 12 members and is expressed almost exclusively in cancer tissues and also encode tumor-specific antigens that are recognized by autologous cytotoxic T lymphocytes.The MAGE genes are activated in spermatozoa but silent in normal somatic cells.In cancers, these genes are re-activated and their encoding proteins are frequently expressed in various histological types of cancers.Because of this distribution characteristic,the MAGE proteins are termed as cancer-testes(CTAs)antigens.The human MAGE-gene family has been located to the X chromosome.The MAGE-3 is one of 12 closely related MAGE genes.It belongs to a growing clas of T-cell defined CTAs that are normally expressed only in the testes and ovary,yet are aberrantly expressed in a wide variety of primary tumors and cancer lines. This characteristic feature of CTAs makes them promising antigens for cancer-specific immunotherapy. METHODS:We examined the frequency of MAGE-3 immunopositivity within a group of 126 patients with primary colorectal adenocarcinoma and a group of 40patients with polyps.Paraffin blocks of all patients were examined immunohistochemically with monoclonal antibody 57mAb.MAGE-3 expression was compared with tumor differentiation degree, Dukes classification,age and gender. RESULTS:50 out of 126 colorectal adenocarcinoma were positive for MAGE-3 (39,68%)and 76 out of 126 colorectal adenocarcinoma werw negative (60,32%)($p<0.025$).According to the tumor grade,80.26% of patients with MAGE-3 negative tumor had tumors grade I and II. On the other hand, positive reaction on MAGE-3 protein was more often observed in poorly differentiated tumors ($p<0.05$).Also, more patients with Dukes A tumor had MAGE-3 negative tumor than the patients with Dukes B or C tumor ($p<0.05$).At the same time, patients with MAGE-3 positive tumors were slightly oldes comparing with the group of MAGE-3 negative tumors.There was no statistically significant difference in MAGE-3 expression between adenomatous polyps and villous adenomas. CONCLUSIONS: The results showed that MAGE-3 gene products could be identified by the 57mAb in colorectal adenocarcinoma and polyps. Also, the patients with advanced stage of the colorectal adenocarcinoma (Dukes B and C)could take advantage of active specific immunotherapy procedures targeting this antigen.

PP1-243
SIGNIFICANCE OF P21 AND C-myc EXPRESSION IN BLADDER UROTHELIAL LESIONS

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Abstract: Immunohistochemical tissue expression of the anti-proliferative marker P21 and the oncogenic marker C-myc were estimated in 40 cases with non-neoplastic and neoplastic urinary bladder lesions with or without schistosomal infection to assess the significance of their urothelial expression as a diagnostic tool in patients with higher risk of developing cancer bladder and as a prognostic indicator in malignant lesions. P21 expression was detected randomly in normal urothelial nuclei, while 50% of simple cystitis cases and all cases with premalignant changes were positive for P21 immuno-reactivity expressed into about 16% of urothelial cells. Eighty five percent of malignant cases expressed P21 in 48-55% of urothelial cells without significant variance between different histologic tumor types. Extent of P21 expression inversely correlated with bilharzial association, upgrading of malignancy and tumor invasiveness. C-myc was detected in 50% of normal urothelium (as cytoplasmic staining), in 80% of simple chronic cystitis cases (75% cytoplasmic, 25% cytoplasmic and nuclear expression), and in all cystitis cases with premalignant changes (as cytoplasmic and nuclear expression). Eighty nine percent of cancer cases were C-myc positive with predominance of nuclear expression to be seen in 16.7% and mixed with cytoplasmic expression in another 58.3% of positive cases. Malignancy upgrading and invasiveness raised C-myc positivity. In conclusion: P21 expression increases in an attempt to check cellular proliferation, but when the matter is out of hand, P21 is down- regulated, while the increase in the oncogen C-myc goes ahead. Loss of P21 and increased C-myc expression in a malignant lesion is a predictor of malignancy progress to higher grade or stage.

PP1-244
HEDGEHOG SIGNALING IN NEUROBLASTOMA

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The Hedgehog (Hh) signaling pathway is essential for embryonic patterning, cell proliferation and differentiation. Activation of the pathway has been shown in several human tumours and in neuroblastoma. Sonic (Shh), Indian (Ihh) and Desert Hedgehog (Dhh) are the three mammalian Hedgehog ligands. Hh binds to the receptor Patched (Ptch), leading to activation of the Gli transcription factors Gli1, Gli2 and Gli3. In the absence of the ligand, Ptch inhibits Smoothened (Smo), a downstream protein in the pathway. Nestin is expressed in many cells during the embryonic development and overexpression is linked to increased aggressiveness in several nervous system tumors. Routinely FFPE tissue from undifferentiated human neuroblastoma (n=6), well differentiated ganglioneuroblastoma (n=3), paraganglioma (n=2) were used. Immunohistochemistry was performed on cell blocks of the neuroblastoma cell lines SK-N-SH, CHP-212 and SH-SY5Y using a standard streptavidin-biotin-peroxidase technique (Dako). For in vitro differentiation of SH-SY5Y cells retinoic acid (RA) was applied. Immunohistochemistry was performed using antibodies against Gli1, Gli2, Gli3, Shh, Ihh, Dhh, Ptch, Smo, Hip, Ptch2. To investigate correlation of Hh signaling with other stem cell developing pathways b-Catenin, Notch and Nestin were determined. Transcriptional activity was analyzed in undifferentiated SH-SY5Y and differentiated SH-SY5Y-RA performing real time PCR on a Corbett Rotor-Gene 6000 platform. Immunohistochemical staining of the NB tissue showed expression of Hh signaling components in the well differentiated gangliocytes, whereas undifferentiated neuroblastoma cells did not show Hh activity. Nestin was overexpressed in well differentiated gangliocytes and in Schwann cells, but was not detected in ganglion cells. Double-immunofluorescence results showed Shh and Nestin co-expression, in contrast Ptch and Nestin were not expressed within the same gangliocytes. Immunohistochemical staining of SK-N-SH and CHP-212 show an expression of all Hedgehog components (Gli1, Gli2, Gli3, Shh, Ihh, Dhh, Ptch, Smo), whereas SH-SY5Y express only a few. Real time PCR of SH-SY5Y displays a overexpression of Ptch and Smo and a normal expression of Gli1 und Gli2. Real time PCR of SH-SY5Y-RA has been performed. Hedgehog pathway is active in human neuroblastoma and may be involved in neuronal cancer cell differentiation. As Nestin is present in Shh expressing cells and not in the recipient cells, this may indicate that Nestin and Hedgehog may crosstalk in the signaling of differentiation of neurogenic cells in NB.

PP1-245
MATCH PROJECT: IMPROVEMENT OF THERAPY SELECTION BY MATCHING MOLECULAR DATA FROM COLON CANCER PATIENTS

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The main focus of the MATCH project is the development of an automatic computer-based system supporting decision process in patients with colorectal cancer. The strategic foundation of the MATCH project addresses the challenge of the integration of clinical practice and molecular approach through establishment of a framework enabling efficient handling of diversified data sources. The framework is expected to improve adequacy of treatment options offered to colon cancer patients. Since the response to therapy is affected by genetic variability, one can assume that genetic fingerprinting could be one of the most relevant ways to assess the sensitivity of a patient with specific molecular profile to a particular therapeutic mode. Since Single Nucleotide Polymorphisms (SNPs) represent the molecular substrate of this variability, SNPs fingerprinting provides molecular snapshot of a patient profile with information highly relevant for anticipating the susceptibility to therapeutic agents or modes. The process of the MATCH system functionality development was planned to take place in three stages. In the first one, the system will be filled with quantitative and qualitative clinical and genetic (mainly SNPs information for tumour suppressor genes) data. Next, computational process will be carried out and finally, the results that concern more effectively colon cancer treatment will be obtained. The first stage crucial for the initiation of the process is provision of clinical and genetic data from medical facilities. Genetic data are represented mainly by SNPs information of TSGs from: patient normal tissues, patient primary tumour, lymph nodes and distant metastasis (when available). Additional genetic and molecular data may be also used. Clinical and genetic data of all patients are used to generate homogeneous clusters of patients with the longest subset of features (both clinical and genetic). It is expected that all members of a cluster will respond to specific therapeutic modes in similar way since their molecular profiles reveals high level of concordance. The automated decision support system is then used to match the clinical and genetic profile of a new patient with the clusters representing homogeneous groups of patients in MATCH data set. The new profile is assigned to the cluster that shows the smallest distance in its centroid profile. Statistical analysis of cluster population provides information on the best available therapy for the new patient based on the outcomes of all cluster members.

PP1-246
EXPRESSION OF COLLAGEN TYPE I AND III, MMP-1 AND TIMP-1 GENES IN MYOCARDIUM FROM PATIENTS WITH HYPERTROPHIC OBSTRUCTIVE CARDIOMYOPATHY AND PATIENTS WITH ESSENTIAL HYPERTENSION

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AIM: Studying gene expression for collagen type I and III, matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in myocardium of the right half of interventricular septum (IVS) from the children and adults with hypertrophic obstructive cardiomyopathy (HOCM). MATERIAL AND METHODS: Twenty-three patients with HOCM have been examined (9 children and 14 adults). Myocardial samples were taken from the right half of IVS (areas of maximal hypertrophy) upon surgery correction of HOCM (myotomy/myectomy), and used for RNA isolation with subsequent RT-PCR. We used autopsy myocardial samples from 8 patients with essential hypertension (EH) as controls. RESULTS: In children with HOCM, gene expression of type I collagen, as related to constitutive gene activities, was widely ranged from 0.37 to 1.07; (0.67±0.07), and in adults, from 0.53 to 1.31; (0.88±0.07; $p<0.09$). In children, the expression of type III collagen gene varied from 0.09 to 0.39; (0.26±0.04); in adults, from 0 to 0.39; (0.19±0.03; NS). MMP-1 gene expression was lower in children i.e., from 0 to 0.22; (0.11±0.04), as compared with adult group, from 0 to 0.45 (0.26±0.04; $p<0.008$). TIMP-1 gene expression in IVS was higher in children with HOCM, ranging from 0.21 to 2.18; (1.10±0.21), as compared to adults, i.e., 0 to 1.21; (0.70±0.11; $p=0.05$). In adult with HOCM gene expression of type I collagen varied from 0.53 до 1.31, (0.88±0.07) and was higher compared to patients with EH. Gene expression of type I collagen in patients with EH varied from 0.36 to 0.89; (0.60±0.057; $p<0.013$). In adult with HOCM gene expression of type III collagen varied from 0.00 до 0.39, (0.189±0.03) and was higher compared to patients with EH. Gene expression of type I collagen in patients with EH varied from 0.00 to 0.14; (0.08±0.014; $p<0.01$). In adult with HOCM TIMP-1 gene expression varied from 0.00 to 1.21, (0.70±0.11) and was higher compared to patients with EH. In patients with EH TIMP-1 gene expression varied from 0.00 to 0.42; (0.28±0.055; $p<0.01$). In adult with HOCM MMP-1 gene expression varied from от 0.00 to 0.45; (0,26±0,036), in patients with EH from 0.00 to 0.67; (0,22±0,067; NS). CONCLUSION: In myocardium of IVS children with HOCM, an imbalanced expression of genes encoding collagenolysis factors was demonstrated, due to decreased MMP-1 and increased TIMP-1. Increased expression of genes encoding production of TIMP-1, collagen of I and III types was detected in primary myocardial hypertrophy, compared to myocardial hypertrophy in patients with EH.

PP1-247
THE ROLE OF ANGIOGENESIS OF THROMBOXANE A2 SYNTHESIS PROTEIN

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The role of blood vessel formation within diseased blood vessels has become one of the outstanding puzzles in the biology of cardiovascular disease. The role of angiogenesis in atherosclerosis and other cardiovascular diseases has emerged as a major unresolved issue. Angiogenesis, by which new capillaries for from preexisting blood vessels, is essential embryonic development, normal physiological growth, tumor expansion, and wound healing. Wound healing is a complex processes involving cell proliferation and angiogenesis. Little is known about the role of coagulopathy in angiogenesis. Angiogenic cytokine therapy has been widely regarded as an attractive approach both for treating ischemic heart disease and for enhancing arterioprotective functions of the endothelium; conversely, a variety of studies suggest that neovascularization contributes to the growth of atherosclerotic lesions and is a key factor in plaque destabilization leading to rupture. The knockout mice model is a novel tool to assess the detailed pathophysiology of angiogenesis. Previous studies have shown that thromboxane A2 synthase (TXAS) is involved in lymphocyte differentiation, hemostasis, thrombosis, and thrombopoiesis. This study investigates in detail angiogenesis in TXAS knockout mice with corneal injury models. We find that TXAS is crucial to angiogenesis, and will help to understand the relationship between angiogenesis and coagulopathy.

PP1-248
PROTEINASE-ACTIVATED RECEPTOR – 2 EXPRESSION IN BREAST CANCER AND THE ROLE OF TRYPSIN ON GROWTH AND METABOLISM OF BREAST CANCER CELL LINE MDA MB-231

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OBJECTIVE: Proteinase-activated receptor – 2 (PAR-2) is a ubiquitous surface molecule participating in many biological processes. It belongs to the family of G protein-coupled receptors activated by the site-specific proteolysis of trypsin and similar proteases. Altered function of PAR-2 has been described in different malignant tumours. In the present study, we investigated the expression of PAR-2 in breast cancer surgical specimens and the role of trypsin in breast cancer cell line MDA MB-231 proliferation and metabolism. MATERIALS AND METHODS: A total of 40 surgical samples of infiltrative ductal breast cancer and breast cancer cell line were included in this study. We analysed PAR-2 expression by immunohistochemistry, RT-PCR and western blot. Activation of PAR-2 on cell line MDA MB – 231 was measured using calcium mobilisation assay determined by flow cytometry. MTT cell metabolism assay and cell count analysis were used to assess the trypsin influence on breast cancer cell line MDA MB – 231 proliferation. RESULTS: Immunohistochemical examination showed the expression of PAR-2 in all samples of breast cancer surgical specimens and cell lines in high level, this was confirmed by RT-PCR and western blot. Calcium mobilization assay corroborated the activation of PAR-2 on cell line MDA MB – 231 either by trypsin or by agonistic peptide. Cell metabolism assay and cell count analysis showed significant differences of proliferative activity of breast cancer cells dependent on presence or absence of trypsin and serum in culture medium. CONCLUSIONS: PAR-2 is expressed in a high level in infiltrative ductal breast cancer tissue specimens. PAR-2 is expressed in studied breast cancer cell lines in a high level. PAR-2 is activated by trypsin and by agonistic peptide as well in the model of breast cancer cell line MDA MB-231. Activation of PAR-2 in vitro influences proliferative and metabolic activity of breast cancer cell line MDA MB-231. The action of trypsin is modified by the presence of serum which is a potential source of protease inhibitors.

PP1-249
MUTATIONAL ANALYSIS OF THE BRAF GENE IN VARIOUS CONDITIONS OF THE PARATHYROID GLANDS

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BACKGROUND: The product of the BRAF gene is a cytoplasmic protein that plays a critical role in the mediation of signals from the cytoplasmic membrane to the nucleus via the RAS/RAF/MEK/ERK/MAPK pathway and it has been suggested to be implicated in many endocrine neoplastic disorders. The result is the activation of transcription factors which regulate gene expression, cell proliferation, apoptosis, and differentiation. In the present study we examined in total 34 specimens of enlarged parathyroid glands, including eleven cases of primary hyperplasia, twelve cases of secondary hyperplasia, ten of adenoma, and one case of carcinoma. The purpose of our study was the detection of BRAF mutations related to various conditions of the parathyroid glands since the pathogenesis of hyperplastic and neoplastic disorders is not well understood and the differential diagnosis between them is not always possible. METHODS: Our material constituted 34 paraffin-embedded parathyroid specimens from which genomic DNA was extracted by the method of phenol/chloroform. BRAF exons 11 and 15 were amplified by PCR using specific primers. The amplified products were purified by the QIAquick PCR Purification Kit (QIAGEN). Sequencing-PCR reactions were performed for both exons, using the BigDye Terminator Cycle Sequencing Kit (QIAGEN). Furthermore, a last purification of the Sequencing-PCR products was performed using the DyeExTM 2.0 Spin Kit (QIAGEN). Finally, the amplified products were sequenced on an ABI PRISM 310 automated capillary DNA Sequencer. RESULTS: Sequence analysis confirmed no mutations of the BRAF gene in all 34 cases used in this study. Even the common genetic alteration, the valine to glutamate substitution at residue 600 (V600E) was absent. CONCLUSIONS: These findings suggest that the BRAF gene -and particularly exons 11 and 15- do not appear to represent a frequent molecular event in the pathogenesis of various conditions of the parathyroid glands. Consequently, it is not possible to use the V600E substitution as a molecular marker for the diagnosis of hyperplasia, adenoma or carcinoma. Furthermore, these results enhance the suggestion that different biological pathways are activated in endocrine tumors of various localisations.

PP1-250
RET/PTC REARRANGEMENTS IN GREEK PATIENTS WITH PAPILLARY THYROID CARCINOMA

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Background Papillary thyroid carcinoma (PTC) is the most prevalent endocrine malignancy in humans. Chromosomal alterations that affect the RET tyrosine kinase receptor are associated with PTC. Three most common rearrangements, RET/PTC1, RET/PTC2 and RET/PTC3 account for the vast majority of all rearrangements found in the general population. Some geographical differences regarding frequencies of these alterations have been reported. Our aim was to evaluate the presence of RET/PTC rearrangements in Greek patients with papillary thyroid carcinoma. Method In this study, total RNA was isolated from 24 fresh papillary thyroid carcinomas. One μg RNA was reverse transcribed to cDNA, followed by polymerase chain reaction (PCR) using specific primers for RET/PTC 1, 2, 3. The efficiency of reverse transcription was verified by the amplification of the GAPDH gene. All PCR products were resolved on a 2% agarose gel containing ethidium bromide and visualized under ultraviolet light. Products indicating a RET/PTC rearrangement were direct sequenced to verify the result. Results Two rearrangements in two papillary thyroid carcinomas were detected by RT-PCR (8%). RET/PTC3 rearrangement was found in one carcinoma and RET/PTC2 in the second one. All rearrangements were confirmed by sequencing. Conclusion This study is the first regarding RET/PTC rearrangements in Greek patients with papillary thyroid carcinoma. The reported frequency of RET activation varies widely among different series from 0% to approximately 80% in post-Chernobyl papillary carcinomas. This variation reflects geographical distribution as well as influence of environmental factors such as ionizing radiation exposure. In our study, two RET/PTC rearrangements were detected among 24 samples (8%) by RT-PCR. One patient with papillary thyroid carcinoma exhibited RET/PTC3 rearrangement, while another patient exhibited RET/PTC2 rearrangement. These findings indicate that RET/PTC may not be of significant role in the pathogenesis of papillary thyroid carcinoma in the Greek population. Moreover, the relatively lower prevalence of chromosomal RET alterations in Greek patients compared to similar studies in neighboring countries (such as Italy, 33%) point toward to a minor effect due to exposure in I131 from the Chernobyl accident. Supported by the Community Support Framework 2000-2006, Operational Programme "COMPETITIVENESS", co-funded from the EU by 75% and from the Hellenic State by 25%.

PP1-251
HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF E-CADHERIN IN BREAST NEOPLASIA

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Background :.Although EC is emerging as an excellent marker to type breast carcinomas. The conflicting reports of EC loss as predictor of increased invasiveness. Metastatic potential ,and poor survival raise questions about its reliability for typing.Methods: Immunohistochemical staining was undertaken for E-cadherin (EC), estrogen and progesterone receptors; on tissue sections of normal,proliferative and malignant breast lesions. The EC positive expression manifested itself as sharp membranous staining and was assessed semi-quantitative into four categories:0;1+ were considered negative immunoreactivity, while 2+ and 3+ were scored as positive immunoreactivity. Results: Although 95% of non malignant proliferative breast lesions showed positive EC immunoreactivity , and reduced or lost EC expression in all Pre-invasive cases .None of invasive lobular cases express EC, in contrast to 30 % of invasive duct carcinoma cases, showed reduced or lost EC expression in high histological grades. Tubular Carcinoma showed a highly statistically significant difference between the groups ($P < 0.001$). Overall, negative staining of EC was specific for the diagnosis of invasive lobular cases However, positive staining did not exclude its diagnosis Conclusion: There was a significant relationship between E-Cadherin expression and different breast neoplastic histological types .The molecular "signature" of breast lobular carcinomas is the loss of E-cadherin protein expression as evidenced by immunohistochemistry, whereas ductal carcinomas are typically E-cadherin positive. Our study suggests that E-cadherin may be involved in the pathogenesis of this form of breast cancer.

PP1-252
HER 2/neu PROTEIN IMMUNOHISTOCHEMICAL EXPRESSION CORRELATED WITH ESTROGEN AND PROGESTERONE RECEPTORS IN HUMAN PAPILLOMA VIRUS-RELATED CERVICAL NEOPLASIA
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Background: The natural history of cervical neoplasias appears unpredictable with 1/3 to 1/2 of CIN lesions regressing without intervention; therapeutic targeting aids in screening and selecting patients at risk and elucidating treatment response. This study aimed at determining if a pattern of tumor suppressor, oncogene, and growth factor expression (specifically HER-2/neu), progesterone and estrogen receptors (ER/PR), exists in the progression of cervical cancer utilizing the widely applicable process of immunohistochemistry. Methods:17 Paraffin sections from were obtained from the surgical pathology department ,the national cancer institute Cairo University ,between the period of 2001 and 2002. Levels of expression in normal, low and high grade dysplasias, and invasive squamous carcinomas were measured via immunohistochemical analysis. Results: HER-2/neu staining occurred at greater levels in high grade lesions and invasive carcinomas being of prognostic significance. ER and PR expression generally remained low with a penchant for parabasal cells .Conclusions: While HPV infection immortalizes cervical cells the equivocal expression of pre-invasive and more aggressive lesions suggests the involvement of other. PR and ER play only a minor role in the development of carcinomas, indicating a lack of hormonal control in cervical tumor growth and proliferation. Her-2/neu, however, participates in the late events of cervical malignancy. Although HER-2/neu perhaps holds limited prognostic value in cervical carcinogenesis, similar molecular pathways independent of HPV need to be investigated.

PP1-253
CHROMOSOMAL ALTERATIONS IN LUNG METASTASES OF COLORECTAL CARCINOMAS: ASSOCIATIONS WITH TISSUE SPECIFIC TUMOR DISSEMINATION
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Comparative genomic hybridization was used to screen colorectal carcinomas for chromosomal aberrations that are associated with the metastatic phenotype of the lung. Thirteen lung metastases, six primary tumors, one lymph node metastasis, one liver metastasis, and one ovarian metastasis were investigated and added to our CGH colon cancer tumor collective, comprising 85 tumor specimens from 56 patients (see CGH online tumor database at http://amba.charite.de/cgh). Lung metastases showed more alterations than liver metastases, particularly more deletions at 1p, 3p, 9q, 12q, 17q, 19p and 22q and gains at 2q, 5p, and chromosome 6. Comparing lung metastases with their corresponding primary tumors, particularly more deletions at 3p, 8p, 12q, 17q, and 21q21 and gains at 5p were observed. Based on our results, we wish to suggest a metastatic progression model. Specific subpopulations of metastatic cells have a distinct metastatic potential which is reflected by a non-random accumulation of chromosomal alterations. Distinct alterations already exist within the primary tumor and this “ready to go package” gives the cells the metastatic potential to achieve the complex series of events needed for metastasis.

PP1-254
EXPRESSION ANALYSIS OF NDRG2 PROTEIN IN ADULT HUMAN TISSUES
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Background: NDRG2 (N-myc downstream regulated gene 2), a member of a new family of differentiation related gene, belongs to the NDRG family, which includes NDRG1-4. Although several functions of NDRG2 were studied, the exact molecular and cellular function remains to be illustrated. Design: To realize the more function of NDRG2 protein, we generated a mouse monoclonal antibody against NDRG2 to analyze the expression pattern of NDRG2 protein with immunohistochemistry in a large set of normal human tissues. Result: In human tissues, NDRG2 expression was seen in various epithelia of small intestine, large intestine, breast, bronchus, kidney, prostate, liver, salivary glands, endometrium, and pancreas. Dendritic cells of lymphoid tissue, skeletal muscles, cardiac muscle, peripheral nerve plexuses, Cajal cells of intestine, thymic Hassalls corpuscles, adrenal cortical cells, ovarian stromal cells, Leydig cells and astrocytes of brain were strongly expressed for NDRG2. The cellular localization of this protein was mostly in cytoplasm. Conclusion: In the present study, we demonstrate the expression pattern and cellular location of NDRG2 protein in a large set of human adult tissues. This is the first systemic demonstration of NDRG2 protein expression in adult human tissues. The multiple localization of this protein in normal human tissues indicates that NDRG2 protein might play multiple functions.

PP1-255
MUTATION FREQUENCY OF C-KIT AND PLATELET DERIVED GROWTH FACTOR RECEPTOR ALPHA (PDGFRA) TYROSINE KINASE GENE IN SAUDI GASTROINTESTINAL STROMAL TUMORS (GISTS)
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Background: Although gastrointestinal stromal tumors (GISTs) are rare, comprising about 1% of gastrointestinal cancer, they are the most common mesenchymal tumors of the gastrointestinal tract. The overwhelming majority of these tumors express mutations in the c-KIT gene that may produce gain-of-function effect on the tyrosine kinase binding pathway resulting in ligand-independent activation of the KIT receptor tyrosine kinase. Most frequent KIT mutations target exon 11 (66%), followed by exon 9 (10%), then exon 13 (1.2%) and exon 17 (0.6%). About 7% of GIST exhibit mutations of platelet derived growth factor receptor alpha (PDGFRA). GISTs is a good example of a neoplasm with an effective molecularly targeted therapy. Method: DNA extraction, polymerase chain reaction (PCR) and sequencing were performed on paraffin embedded tissue from 11 histologically confirmed GISTs that involve stomach and small bowel and show KIT (CD117) immunoreactivity. We analyzed the tumor samples for mutations in the exon 11, 9, 13 and 17 of the c-KIT gene and exon 12 and 18 of the PDGFRA. Results: A direct sequence analysis revealed c-KIT mutations in seven of the available tumor samples. Among the GISTs with c-KIT mutations, three had previously reported exon 11 mutations Valine to Aspartic Acid at codon 560, one had a novel mutation in which Valine changes into Alanine on the same codon 560 and three tumor samples had novel inframe deletion in the exon 11. DNA quality was not optimal for analysis in the other four samples. Conclusion: All mutations in the present study fall on the exon 11 of the c-KIT gene. Recent publications show the importance of mutational status of GISTs to predict response to target therapy. KIT tyrosine kinase inhibitor, imatinib mesylate, offers an effective treatment for most patients with advance GIST. The likelihood of clinical response appears to be significantly better for patients with exon 11 mutations than for patients with other mutations. Although the number of cases is small, this study provides the initial data for mutational status of c-KIT/PDGFRA oncoproteins in Saudi population that will be useful to predict the clinical response to therapy.

PP1-256
4EBP1, A KEY MOLECULAR "FUNNEL FACTOR" IN HUMAN CANCER WITH CLINICAL IMPLICATIONS
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BACKGROUND: in an attempt to identify molecules that clearly reflect the oncogenic role of cell signaling pathways in human tumors, we propose a concept we term "funnel" factor, a factor where several oncogenic signals converge and drive the proliferative signal downstream. MATERIAL AND METHODS: we studied the expression of several factors involved in cell signaling in 110 ovarian tumors, 105 breast carcinomas, 90 prostate tumors and 120 colon carcinomas by immunohistochemistry and Western blotting. The factors studied were: Her1 and Her2 growth factor receptors, the RAS-RAF-MAPK and the PI3K-AKT-mTOR pathways and the downstream factors p70S6K, S6, 4EBP1, and eIF4E. DISCUSSION: we found that phosphorylated 4EBP1 (eukaryotic translation initiation factor 4E binding protein 1) expression in breast, ovary, and prostate tumors was associated with malignant progression and an adverse prognosis, regardless of the upstream oncogenic alterations. Moreover, various cell lines were studied, including normal human fibroblasts (IMR90), kidney cells (293T) and breast cancer cell lines (MDA-MB-231 and MDA-MB-435). These cell lines were treated with rapamycin in order to investigate 4EBP1 hyperphosphorylation and its correlation with mTOR, and levels of p4EBP1 were evaluated by Western blot, as well as cell survival. Finally, a wild-type and a mutant 4EBP1 were both transfected into various cell lines. Rapamycin blocked phosphorylation of 4EBP1 in MDA-MB-231 and in 293T, but not in MDA-MB-435. However, all these cell lines were resistant to rapamycin, since cell numbers did not decrease. In addition, transfection in those cell lines of 4EBP1 with phosphorylation site mutations suppressed cell proliferation. CONCLUSION: p-4EBP1 seems to act as a funnel factor for an essential oncogenic capability of tumor cells, self-sufficiency in growth signals, and could be a highly relevant molecular marker of malignant potential.

PP1-257
GLYCOME ANALYSIS IN DISCOVERY OF NEW CANCER-ASSOCIATED ANTIGENS
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Human cells are covered by a layer of sugar moieties (glycans) that change in cancer and contribute to cancer progression and metastasis. Due to the lack of suitable analysis technology the cancer glycome – the whole spectrum of glycans in malignant tumors – has remained unknown. We combined MALDI-TOF mass spectrometry with specific enzymatic and chemical glycan modifications to identify tumor-associated changes in the protein-linked glycome. Material was collected from over 100 archival paraffin-embedded tumors (small cell lung adenocarcinoma, lobular and ductal breast carcinomas, pancreatic adenocarcinoma, colorectal adenocarcinoma, ovarian serous carcinoma, renal cell carcinoma) and related normal tissues. The analysis identified significant differences in each of the tumor/control tissue pairs. A number of distinct glycan signal groups were identified as the dominating tumor-associated protein-linked glycans in various carcinomas. These glycans were shown to be consistently upregulated in tumors, indicating that tumor progression is reflected in the expressed glycan structures. In lung carcinomas, the analysis led to identification of a tumor-associated glycan group characterized by abnormal terminal β-N-acetyl-D-glucosamine (GlcNAc) residues. Similar glycan antigens were also expressed in glycolipids extracted from the same tumors, suggesting a common biosynthetic background. The results suggest that global analysis of the cancer glycome is an effective tool for discovery of new molecular targets in cancer. The discovered glycan antigens have potential as diagnostic markers and as targets for novel therapeutic anti-neoplastic agents.

PP1-258
INVOLVEMENT OF VIP RECEPTORS IN SIGNALLING NETWORKS REGULATING CYCLOOXYGENASE-2 IN HUMAN PROSTATE CANCER TISSUE
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BACKGROUND: The vasoactive intestinal peptide (VIP)-$VPAC_1$ and $VPAC_2$ G protein-coupled receptors (GPCR) are related to prostate cancer progression to androgen-independent stage. Recently, we have demonstrated that VIP enhances the expression of the proinflammatory enzyme cyclooxygenase (COX)-2 in both human prostate non neoplastic and tumoral cell lines, acting through the nuclear factor-kappaB. The aim of this study was to detect the coexistence of $VPAC_1$ receptors and COX-2 enzyme in human prostate tissue as well as to compare the observations in healthy and malignant tissue from the same specimen. METHOD: Samples were obtained from patients undergoing radical prostatectomy (n=15, age range 51-73 years). Thereafter, tissue fragments were embedded in paraffin and the postoperative pathologic situation was staged pT1-pT4 (pN0M0, n=15) according to TNM classification. In order to quantify the expression of mRNAs for COX-2 and $VPAC_1$ receptor, RNA was isolated from paraffin sections and real time RT-PCR was performed. RESULTS: The results showed increases of COX-2 and $VPAC_1$ mRNAs in 14/15 and 10/15 samples, respectively, as compared to the corresponding normal tissue. The enhancement of expression for both molecules was coincident in 67% of cases. Immunohistochemical studies showed a similar coexistence of $VPAC_1$ receptor and COX-2 proteins in the same samples. CONCLUSION: Taken together, these results support VIP involvement in inflammatory signals. Such signals may initiate and promote prostatic oncogenesis, which gives a potential usefulness to $VPAC_1$ receptors and COX-2 as therapeutical targets in this disease.

PP1-259
EXPRESSION PROFILE OF NANOG USING TISSUE MICROARRAY
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Introduction: Nanog is a key regulator of embryonic stem cell (ESC) self-renewal and pluripotency. Aims: To analyse expression profile of Nanog in a multitumoral Tissue Micrroarray Material and methods: We analyse the exprresion of Nanog in at least 500 cases using tissue-microarrays containing melanomas,seminomas, embryonal carcinomas, colon, breast and lung cancers, as well as their corresponding normal tissues. Results: We observe two different patterns of expression. Nuclear

in normal testis and seminoma, and cytoplasmic in the other tumors analyzed in a percentage of 10%, 40%, 50% and 10%for breast, colon, lung and melanomas, respectively. Conclusions:1- TMA are a useful tool to identify expression profile.2-Nanog is highly expressed in the nucleus in normal testis, seminomas and embryonal carcinomas which could be used to differential diagnosis among testicular tumors. 3- Cytoplasmic expression must be analyzed in order to know its biologic significance and relation to prognosis.

PP1-260

MOLECULAR SCREENING IN PATIENTS WITH CLINICAL CRITERIA OF LYNCH SYNDROME BY COMBINATION OF IMMUNOHISTOCHEMISTRY AND MOLECULAR BIOLOGY

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Background. The Lynch Syndrome (LS) is the most prevalent genetic disease in hereditary non-poliposys colorectal cancer (HNPCC). It is characterized by germinal mutations in the Mismatch Repair (MMR) genes (hMLH1, hMSH2, hMSH6 and PMS2) and presence of microsatellite instability (MSI), although 15% of sporadic tumors present MSI without expression of hMLH1 because of promoter hypermethylation. MSI consists in DNA insertions and deletions in microsatellites sequences. In order to select patients for further genetic mutation studies, the techniques used in the daily practice are immunohistochemistry (IHC) of MMR proteins and MSI analysis by PCR using a panel recommended by NCI (Bethesda Panel). We decided value MSI by using a new pentaplex system in combination with IHC study. Methods. We collected 91 patients with colorectal cancer (CCR) selected by Bethesda clinical Criteria of LS. Haematoxylin-eosin stained slides obtained from formalin-fixed, paraffin-embedded tissues were reviewed and the areas with tumor and normal tissue were identified. We evaluated by IHC the nuclear expression of MLH1, MSH2 and MSH6 proteins in the neoplastic areas. Furthermore we extracted DNA from a paired normal-tumor samples for each patient and we evaluated the presence of MSI using a pentaplex method with BAT25, BAT26, MONO27, NR21 and NR24 microsatellites enclosed in the "MSI Analysis System" kit (Promega Corporation). Results. We detected loss of nuclear expression of MMR proteins in 19% (17/89) of the samples (MLH1: 12/17, MSH6: 3/17 and MSH2-MSH6: 2/17). 2 cases were not valuables because of formalin fixation problems. About MSI analysis by PCR we analysed 91 paired normal-tumor samples and we detected positivity for MSI in 14% (13/91) patients: 12/13 with High-MSI (instability in two or more microsatellites) and 1/13 with Low-MSI (instability only in BAT26 microsatellite). We found a complete concordance between the presence of MSI by PCR and loss of expression of any MMR protein by IHC (13 cases). 4 cases did not show expression of MLH1 protein by IHC without presenting MSI. Conclusion. We have demonstrated that the combination of MSI (pentaplex system) and IHC analysis allows us a better selection of the patients who will benefit of further germinal mutations studies. Both techniques are complementary in this screening, but others factors must be considerated in the future in order to improve the knowledge of the difference between hereditary and sporadic CCR.

PP1-261

STROMA IRRADIATION PROMOTES CYR61/AV INTEGRIN-DEPENDENT TUMOUR METASTASIS BY SUPPRESSING ANGIOGENESIS

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Radiotherapy is successfully used to treat human cancers. However, patients locally recurring after treatment are at increased risk of developing metastases. The clinical management of this condition is a challenge, and its underlying mechanisms remain largely unknown. Since radiotherapy modifies the tumour microenvironment, we hypothesized that radiation-induced modifications of the tumour microenvironment might favour the spreading of metastatic tumour cells. Here, we report that the irradiation of naïve stroma promotes tumour metastasis through the suppression of angiogenesis, increased of tumour hypoxia and selection of aggressive tumour cell populations that retain an invasive and metastatic phenotype even when they leave their hypoxic microenvironment. Through gene expression profiling and gain and loss of function experiments, we identified the matricellular protein CYR61 as a critical mediator of tumour cell survival during hypoxia, invasion and metastasis. Inhibition of aV integrin prevented tumour cell invasion and metastasis induced by stroma irradiation and CYR61 over-expression. We found high CYR61 metagene expression to correlate with tumour hypoxia and shorter relapse-free survival in human breast cancer. These results identify CYR61 and aV-integrins as proteins that co-operate to mediate metastasis of tumours growing in irradiated stroma and as potential therapeutic targets in patients at risk for post-radiation recurrences. They also illustrate the potential impact of therapy-induced microenvironmental hypoxia in determining tumour evolution.

Cytopathology

PP1-262
THE PRACTICE OF PATHOLOGY IN RUSSIA

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Pathologists at Ostroumovski Hospital, one of the leading clinical centers in Moscow, encounter problems, which are typical of those seen across Russia. Although the department has above-average equipment and is staffed by experienced specialists, problems with supplies and personnel management create impediments to quality care. Of about five available stains only hematoxylin-eosin is of acceptable quality; and only the Romanovsky-Giemsa stain is used for cytological smears. In the case of patients who had been previously hospitalized in other hospitals, histological slides of the biopsies performed in the past are requested and reviewed. While reviewing, diagnostic errors are sometimes found, but the original pathologist never receives any feedback, and opportunity for quality improvement is lost. Since cytologists and pathologists in Russia belong to different medical specialties, they are frequently located in different departments. Correlation of cytological and histological slides and diagnoses is not performed (regularly), and again this mechanism of quality control does not function. However, some cytologists do compare histological and cytological diagnoses on their own initiative. The gross examination of material in many departments is done without rinsing with water. The detritus and dirt are wiped with cloths or paper. For that reason and also because of inadequate washing of containers, there is increased risk of contamination by floaters. Previous slides are rarely pulled for comparison, and archives of blocks and slides are in bad condition even in the leading institutions such as the Cancer Research Center in Moscow. Biopsy reports are delivered to the clinic on the third to fifth day (or later) after submission of the specimen. It is one of the reasons why frozen sections are used very extensively. The mortician's offices are situated together with the departments of pathology on the hospital premises. Autopsy helpers are employed part-time in mortuary service. The request of a pathologist to an autopsy helper (for example to bring formalin) can be met with the reply that he has no time because he has to dress or shave a corpse. Difficulties are sometimes caused by poor discipline of ancillary personnel. All said, Russian pathology has many positive aspects. Pathologists work in close contact with clinicians, know their needs and requirements. There are medical technicians who consistently produce thin slides using old equipment. Despite remaining shortages, there are grounds for optimism.

PP1-263
HPV-DNA TESTING AND Ki-67 IMMUNOCYTOCHEMISTRY IN LIQUID BASED CERVICAL CYTOLOGY: IN PROSTITUTE WOMEN

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Aim HPV causes several changes in the function of host genes, and these interactions cause deregulation of the cell cycle manifested by abnormal expression of cell cycle associated proteins, such as Ki-67. The detection of Ki-67 can play a role in screening and diagnosis of HPV infection with risk of progression towards dysplasia and carcinoma. We aimed to show this relation in prostitute women. Materials and methods Cervical cells were collected in the PapSpin Collection Fluid (Thermo Shandon, Pittsburg, USA). A starting volume of 1000 µl for each sample, a 200 µl cell suspension was used to prepare each thin layer liquid based cytology (LBC) and stained by Papanicolaou. The cytological results were classified according to the Bethesda 2001 system. From the remaining cell suspension of 800 µl, 400µl was used for HPV DNA detection by PCR. From the remaining 400 µl cell suspension 50 µl was used to make thin layer preparations for immunocytochemistry. Single antigen staining was performed with Ki 67 protein, (Neomarkers-Biogen, Lab Vision Corp. USA). Cells were considered immunopositive if the nuclei were stained. All cells in one high power field (X400), which was considered to be representative of the whole cell area, were counted, and the fraction of positive cells on the slide was calculated. This fraction was expressed as the number of positive cell /1000 cells to compare results of samples. Results HPV types 6 and 32 in study group, HPV types 6 and 51 in control group were detected. The mean Ki-67 values were 2.7 ± 1.2 and 3.6 ± 4.1 in HPV positive and negative cases respectively. There was a positive correlation only with nuclear changes and HPV positivity (X^2=28.8 $p<0.001$). Our other results were not statistically meaningful. There was not a significant relation with HPV or Ki-67 and leucocytosis. There was not also a relation with HPV and contraseption, smoking, concurrent genital infection. Conclusions The prevelance of HPV types in different geographical locations and racial factors may indicate different etiologies of cervical cancer. Our results suggest that Ki-67 immunocytochemistry is not useful as a surrogate marker for 6, 32 and 51 types of HPV.

PP1-264
CYTOLOGIC FINDINGS IN PAP SMEARS WITH ACTINOMYCES-LIKE ORGANISMS: A RETROSPECTIVE STUDY

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Background: To detect whether there is a relationship between the presence of Actinomyces-like organisms (ALOs) and cytologic findings. Method:Cervico-vaginal smears obtained from 2490 women were stained with the Papanicolaou technique and were examined cytologically. Twenty-five (1.00 %) of the 2490 were diagnosed with ALOs and became the study group. Patients without infectious agents (n=1926) were the control group. Statistical analyses were conducted with the Chi-square test using the SPSS programme. The limit for statistical significance was $p=0.05$. Results: The study and control groups were compared statistically. There was a significant correlation between the presence of ALOs and other cytologic findings,such as Trichomonas vaginalis,cocci, endocervical cells, superficial cells, lactobacilli and polymorphonuclear leucocytes ($p<0.05$), but there was no statistically significant difference between the presence of ALOs and metaplastic cells or parabasal cells ($p>0.05$). Conclusions: ALOs in cervico-vaginal smears might provide a milieu for growing some infectious agents such as Trichomonas vaginalis and cocci. Lactobacilli were less plentiful in these cases. The exfoliation of superficial cells, more than of other type of cells such as parabasal cells, might be a clue to the presence of ALOs. Endocervical cells, might have been exfoliated because of the involvement of ALO cells with cervical tissue.

PP1-265
EVALUATION OF TZANCK SMEARS IN HERPES SIMPLEX VIRUS INFECTIONS EVALUATION OF TZANCK SMEARS IN HERPES
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Back ground: The diagnosis of herpes simplex virus may require virological confirmation. Tzanck smear is a old and inexpensive method but it is not specific. Objective: This study was conducted to compare for diagnosis of herpes simplex virus by Tzanck smears and viral culture .In patients who clinically suspected herpes simplex virus infection. Method: Cutaneous swab from vesicles fluid and base of vesicles on patients with herpes simplex infections were taken and then viral culture and Tzanck smears were preformed. Results: Of 56 samples 71.4% was culture positive and Tzanck smear was positive in 64.3% of cases. In all of cases in which Tzanck smear was positive ,viral culture was positive too. In 90% patients who culture was positive,Tzanck smear was also positive. Conclusions: These results demonstrate that Tzanck smear suitable for diagnosis of herpes simplex virus infections when culture or other virological method is not available.

PP1-266
MALIGNANT MESOTHELIOMA. OUR CLINICAL, CYTOLOGIC AND IMMUNOCYTOCHEMICAL FINDINGS
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Objective: Mesothelioma has an increased rate and contributed significantly to cancer-related morbidity and mortality in the last decades. The differdiagnosis mesothelioma from adenocarcinoma remains a major challenge for most cytopathologists. The early detection of mesothelioma in combination with appropriate therapy are of great significance for patients’ long term survival. Immunocytochemistry seems to be the key of diagnosis. The aim of the present study was the investigation of mesotheliomas with a panel of immunocytochemical markers and the response of new therapeutical schedule in these patients. Material and Methods: In this study included pleural and ascitic fluids collected by 15 cancer patients. The specimens were prepared with the use of Thin monolayer Cytology (ThinPrep technique) and stained by Papanicolaou method. Moreover, we used a panel of immunocytochemical markers (Calretinin, HBME-1, Cytokeratin 5 and Mesothelin) in order to distinguish mesotheliomas, especially epithelioid type, from adenocarcinomas. Immunocytochemistry was performed with the use of Ventana Nex Es Automated slide stainer and related Ventana reagents. Results: Diagnosis was based on clinical, cytologic and immunocytochemical findings. Calretinin had the best sensitivity and specificity for the mesothelioma followed by HBME-1, Mesothelin and CK 5. Furthermore, 10 out of 15 patients who were treated with chemotherapeutical agents (carboplatin and pemetrexed) had better survival rate (16-24 months). Conclusions: 1. The tumour markers Calretinin, HBME-1, Mesothelin and CK 5 seem to have the best sensitivity and specificity for the diagnosis of mesotheliomas. 2. The early detection of malignant mesothelial cells with appropriate immunocytochemistry contributes in prompt treatment procedure. 3. Consequently, this point is of high interest because the application of this new therapeutical schedule (carboplatin and pemetrexed) seems to improve the survival of these patients.

PP1-267
IS HPV TESTING NECESSARY FOR THE ADEQUATE EVALUATION OF THE ABNORMAL PAP SMEAR WITH A NEGATIVE BIOPSY? A CORRELATION STUDY WITH SURGICAL BIOPSIES
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Background: Several tests have been used as adjunct to the Pap smear for objective assessment of women at risk and guidelines developed for management and follow up of these women. The ALTS Study recommends colposcopy, cytologic follow up and triage by HPV DNA testing using Hybrid Capture II (HCII). Reported low HCII specificity poses a problem in the diagnosis of women with little/no immediate risk of disease. This retrospective study was conducted to estimate HCII s effectiveness to predict HSIL by correlation of Pap smears and available biopsy samples. Also addresses the question of the abnormal Pap with HPV results and negative biopsies. Design: 1,579 Pap smears in women with corresponding HR HCII were retrieved from LSUHSC files from January 2004 to October 2005. A total of 1413 (90.6%) had an ASCUS (ASC-US and ASC-H) on cytology and 981 (69.4%) were positive for HR HPV HCII. Available biopsies (n=438) were correlated with cytology and HPV HCII results, discordant results were reviewed by 2 authors. Results: When ASC-US was subcategorized into ASC-US, ASC-US suggestive of HPV and ASC-H, HPV HCII positivity was 65.56%, 81.69% and 81.51% respectively, $p<0.0001$. Total of 438 biopsies retrieved, majority cervical (n=373). Of the HR HCII positive cases: 82.3% LSIL and 92.5% HSIL. LSIL biopsy breakdown: 91%ASCUS (ASC-US and ASC-H), 0.9% LSIL,and 5.6% HSIL. HSIL biopsy breakdown: 56.98% ASC-US,16.0% HSIL, 0% LSIL . Of the 92 negative biopsies reviewed (3 cases unavailable), 15 % were ASC-US (combined ASC-US and ASC-H), 20.7% LSIL, 3 % HSIL. 59/92 (64 %) tested positive for HR HPV, one case had insufficient material. Conclusion: 1)The high number of abnormal Pap smears with positive HPV in women with abnormal biopsies confirms the usefulness of HCII. 2)A positive HPV test combined with atypical Pap should prompt review of negative biopsies. 3)The absence of squamo-columnar junction represents an important cause for false negative biopsies (30.0% in our study). 4)Endocervical curettage is not adequate to verify abnormal Pap results, justified by 18/22 (81.8%) negative cases after review. 5)ASCUS, suggestive of HPV with negative HPV DNA correlates with post-menopausal pseudokoilocytosis. 6)ASC-US, cannot rule out HSIL, with HPV negativity, could represent metaplastic epithelium with atypia.

PP1-268
ASSESSMENT OF PAP SMEAR QUALITY
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Background: Papanicolaou (Pap) smear is widely accepted as the important screening test for detection and prevention of cervical cancer which is one of the most common cancer worldwide. As smear quality may limit the interpretation, the Bethesda System recommends that evaluation of specimen adequacy should be included in Pap smear reports. Such feedback to clinicians is useful for improving the quality of sampling and smear preparation techniques, resulting in better specimen quality and

interpretation. This study aimed to evaluate quality of Pap smears in terms of specimen adequacy and contribution of factors affecting smear quality. Methods: A retrospective analysis of 377 Pap smears acquired from the OB-GYN out-patient department at Thammasat University Hospital was performed. Cervical smears were obtained by gynecologists and interns using Ayre spatula as sampling devices. Slides were processed according to standard Papanicolaou staining protocol. Adequacy of each smear was interpreted by a pathologist (D.S.) using the 2001 Bethesda System criteria. Results: Of 377 smears, 374 (99%) were classified as 'satisfactory for evaluation', and 3 (1%) as 'unsatisfactory for evaluation'. All of unsatisfactory samples were due to inadequate epithelial cell yield. In the group of satisfactory smears, 302 (81%) showed limited adequacy mainly caused by absence of endocervical cells (EC)/transformation zone (TZ) component (69%); thick areas (23%); obscuring inflammation or blood (22%); and airdry artifact (15%). Number of factors affecting adequacy of each smear varied from 1 to 4. 64% of smears with limited adequacy related to single factor, including absence of EC/TZ (62%); obscuring elements (13%); airdry (11%); and thick areas (8%). 32% of smears with limited adequacy were affected by 2 factors. Conclusion: In the present study, most Pap smears are satisfactory for evaluation. However, adequacy limitations of the smears are common, predominantly due to absence of EC/TZ component. All of unsatisfactory samples in this study relate to inadequate epithelial cell yield.

PP1-269
TRICHOMONAS VAGINALIS AND BACTERIAL VAGINOSIS

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Background: To determine whether there is a relationship between the presence of Trichomonas vaginalis (Tv) and bacterial vaginosis (BV) in cervicovaginal smears. Methods: Cervicovaginal smears taken from 800 women were stained with Papanicolaou technique and examined cytologically to determine bacterial vaginosis and Trichomonas vaginalis. Statistical analysis was performed with the chi-square test using the SPSS programme. The limit for statistical significance was p=0.05. Results: According to cytological examination, 51 of (6.37%) 800 women were diagnosed as having Trichomonas vaginalis [Tv(+)].Clue cells and free cocci without Döderlein bacilli were observed in 18 of (44.4%) 51 Tv (+) cases. These 18 Tv (+) BV(+) cases were evaluated as Group 1. Fourty of 749 Tv(-) smears (5.3%) were also positive for bacterial vaginosis and these 40 Tv (-) BV(+) cases were evaluated as Group 2. There was a statistically significant correlation between the two groups ($p<0.05$). Conclusion: It could be possible that Tv might create an anaerobic environment, thereby changing the vaginal flora and this flora becomes a more suitable milieu for growing of anaerobic microorganisms. It can be postulated that Tv might be the most important protozoan that contribute the overgrowth of BV-related microorganisms.

PP1-270
CYTO-HISTOLOGIC CORRELATION OF CERVICAL INTRAEPITHELILAL NEOPLASIA; OUR EXPERIENCE

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Background: Cytology screening interpretations define which women require focused clinical attention and histologic examination. Cyto-histologic correlation is an important determinant of quality control in cervical cancer screening. Methods: We compared cytologic interpretations in Pap smears with all representative cervical biopsy specimens (542 cases) which were processed at our department in the year 2006. We calculated the degree of agreement for negative cytologic interpretations, for low grade squamous intraepithelial lesions and for high grade squamous intraepithelial lesions. Results: We achieved 79% agreement for normal cytologic interpretations and 55% agreement for reactive changes. The cyto-histologic correlation of low grade squamous intraepithelial lesions was 60% and of high grade lesions 82%. The correlation between atypia/severe atyipa of glandular cells in Pap smears (15 cases) and pathologic findings on biopsy specimens was 73% if both, glandular (40%) and squamous (33) changes were considered. The average accuracy was 60% and accuracy within one degree was 93,5%. Conclusion: Our results showed that the cyto-histologic correlation was better for high grade squamous lesions than for low grade squamous lesions, which is in concordance with some previous studies.

PP1-271
CYTOLOGY IN FUNGAL KERATITIS

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BACKGROUND & OBJECTIVES: Fungal keratitis is a supurative and ulcerative infection of the cornea. The disease has a high ocular morbidity and is an important cause of monocular blindness. Rapid detection of the fungi plays a critical role to prevent important complications. The objectives of this study are to evaluate the cytomorphological findings and their contribution to the diagnosis in fungal keratitis. METHODS: A total of 25 corneal scraping and cytobrush smears of 19 (5 female and 14 male) patients were diagnosed as fungal keratitis between 2004 – 2006 at our institute and private clinics. Retrospective analyses of corneal cytology smears was carried out to investigate the cytomorphological findings in smears taken by corneal scraping or cytobrush. All the smears were alcholol fixed and stained with Papanicolaou and PAS stains. RESULTS: The hyphae and / or spores were seen in all 25 smears of 19 patients. However, in most cases hyphae could be seen within the inflamatory exudate or at the edges of the thick corneal epithelial sheets resulting difficulty to discriminate. Three smears revealed low numbers of corneal epithelium while 11 smears had scattered and groups of epithelial cells and 11 smears had sheets of epithelium. Sixteen (64 %) smears revealed inflamatory exudate, 7 smears had a small amount of neutrophils and 2 smears did not have any neutrophils. Amourphous groups of necrotic squamous cells (squame) were seen in 20 (80 %) smears. CONCLUSION: Although positive culture is accepted as the gold standart for diagnosing fungal keratitis, the method has its own limitations such as being expensive and time consuming. Antifungal therapy can be administered when fungal filaments are seen in the corneal smears stained with Papanicolaou and / or PAS. Hyphae intermingled with inflamatory exudate or thick sheets of corneal epithelium seems to be the major difficulty in microscopic examination. Corneal smears revealing squames are highly suggestive for fungal keratitis. However, further investigations to test this finding in other microbial keratitis are needed.

PP1-272
THE USEFULNESS OF CEREBROSPINAL FLUID ANALYSIS IN THE DIAGNOSIS OF PRIMARY PINEAL SEMINOMA PRODUCING BETA HCG

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The cytologic features of germ cell tumors such as seminoma are sufficiently characteristic to make specific cytologic diagnosis possible in technically high quality smears even when the biopsy is difficult to practice. We report a case of a primary intracranial seminoma producing beta HCG diagnosed by cytology cerebrospinal fluid in a 19 year-old-boy. The tumor was located in the pineal and the suprasellar ventricles. It was associated with a slight elevation of the beta-human chorionic gonadotropin (HCG) serum level (5, 2 mUI/ml) and a high cerebrospinal fluid level of beta-HCG (480 mUI/ml) indicating that trophoblasts cells were present in the tumor. Smears preparations were stained with May Grünwald Giemss (MGG) and hematoxylin and eosin stains. The tumor cells had large round nuclei with dispersed chromatin and one or multiple prominent nucleoli. The cytoplasm was usually scanty, often vacuolated and strongly positive to PAS-stain. Immunohistochemistry on cytologic smears was negative for placental alkaline phosphatase (PLAP). These cytologic features favourite the diagnosis of seminoma with syncytiotrophoblastic giant cells even though none were seen in cytologic preparation. Cytologic features of pineal seminoma are characteristic. So, we can conclude that cytology is performs and plays a great role in the therapeutic management of pineal seminoma particularly when histological verification can not be done.

PP1-273
KARYOCYTOMETRIC CHANGES OF ENDOTHELIUM AORTA AND LUNG ARTERY IN ATHEROSCLEROSIS

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Objective: We analyzed karyometric parameters 5 heterogeneous types of endothelial cells (EC) of lung artery (LA) and aorta (AO) in view of severity of atherosclerosis. Methods: It was received the imprints fatty streaks, plaques and unaffected surfaces of LA and AO from 5 died patients (age of 35-85 yrs). The samples were stained on Romanovsky-Gimsa. It were measured the area and volume of 2434 EC nuclei of LA and 2627 EC nuclei of AO. Results: Histograms of parameters showed skewing of values aside large and giant nuclei. Over fatty streaks of LA area and volume of nuclei of giant mononuc1ear EC averaged 332 mkm^2 and 4847 mkm^3, binuclear-331 mkm^2 and 5147 mkm^3, trinuclear-426 mkm^2 and 8037 mkm^3, giant multinuclear- 766 mkm^2 and 20347 mkm^3. The area and volume of usual nuclei EC over unaffected LA surface was equaled 101 mkm^2 and 762 mkm^3, respectively. Over LA plaques the average area and volume of nuclei of giant mononuclear EC amounted 283 mkm^2 and 3730 mkm^3, binuclear - 285 mkm^2 and 4286 mkm^3, trinuclear- 351 mkm^2 and 6149 mkm^3, giant multinuclear - 686 mkm^2 and 18133 mkm^3, horseshoe-shape nuclei - 319 mkm^2 and 4391 mkm^3. Over fatty streaks AO the average area and volume of nuclei giant mononuclear EC amounted 304 mkm^2 and 4229 mkm^3, binuclear - 288 mkm^2 and 4509 mkm^3, trinuclear- 403 mkm^2 and 8091 mkm^3, giant multinuclear - 709 mkm^2 and 19359 mkm^3, horseshoe-shape nuclei - 248 mkm^2 and 3042 mkm^3. The EC area and volume over unaffected surface of AO was equaled 103 mkm^2 and 793 mkm^3. Over AO plaques area and volume of nuclei giant mononuclear EC averaged 312 mkm^2 and 4248 mkm^3, binuclear - 332 mkm^2 and 5781 mkm^3, trinuclear - 387 mkm^2 and 7515 mkm^3, giant multinuclear- 676 mkm^2 and 18543 mkm^3, horseshoe-shape nuclei - 239 mkm^2 and 3172 mkm^3. Parameter values of heterogeneous and usual EC nuclei were statistically different. Conclusion: the average area and volume of heterogeneous types of EC nuclei over fatty streaks and plaques is higher than usual EC nuclei over unaffected arterial surfaces. The area excess reached 3-7 times and the volume excess - 5-27 times. The karyometric analysis showed increasing dimensional attributes of EC nuclei as the atherosclerosis progresses. [GTEQ]

PP1-274
TREATMENT ASPECTS OF PLEURAL MESOTHELIOMA IN RELATION TO PATIENT SURVIVAL

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The median survival of patients diagnosed with pleural malignant mesothelioma does not exceed 24 months. This is a retrospective study of all the cases presented and treated in our hospital during the last 10 years. Materials and methods: We examined the follow-up of 32 patients diagnosed with pleural malignant mesothelioma. A fraction of these patients were treated conservatively and the rest were subjected to various surgical procedures. Results: a) Surgical biopsy was performed on 18 patients, which ultimately were treated with chemotherapy after diagnosis was established. The median survival of these patients was 28 months. b) 10 patients were treated with local excision of tumor involved pleura, removal of the adjacent pneumonic parenchyma and adjuvant chemotherapy and radiotherapy. The mean survival in this group of patients was prolonged to 30 months. c) 4 patients under-went external pleural excision of the lung with accompanying excision of the diaphragm and adjuvant chemotherapy. The median survival was 20 months. Results: Even though the biological behaviour varies among histological subtypes of malignant mesothelioma, it is evident that limited surgical excisions accompanied with chemo/radiotherapy cause lower morbidity, such as visceral pain and excessive dyspnoea. More importantly, the survival rate of these patients is raised by 33.3% compared to the survival rate of patients treated with more radical surgical procedures.

PP1-276
FINE NEEDLE ASPIRATION CYTOLOGY OF THE PANCREATIC LESIONS – REVIEW OF THREE YEAR EXPERIENCE

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Background: Fine needle aspiration (FNA) cytology represent an increasingly used diagnostic tool in diagnosing the pancreatic lesions, mainly of the pancreatic tumors. In our study, we introduce our experiences with that method in correlation with subsequent histopathological findings. Further, we discuss an unusual case of a cytological diagnosis of a solid pseudopapillary tumor. Methods: Specimen for cytological analysis was obtained from 43 patients using Linear Endoscopic Ultrasound, Hitachi Pentax. Cytologic smears stained by standard Giemsa stain were analyzed in each case. Results: From the total 43 specimens, in 23 cases (53.5%) the tumor, suspected from MRI examination, was not present in the smear. From them, however, pancreatic cancer was disclosed in three cases in the surgical specimen by histopathological analysis. In the 19 cases (44.2%) the pancreatic cancer was suspected by FNA and it was verified by the subsequent histopathological examination in 8 cases. In one case, the suspected pancreatic tumor was not confirmed by histopathology and the final diagnosis was chronic pancreatitis. One case was cytologically suspected as a solid pseudopapillary tumor of pancreas, diagnosis of which was verified also by histology and immunohistochemical analysis. Conclusion: Fine needle aspiration (FNA) cytology represents a powerful tool helpful in differential diagnosis of pancreatic lesions, even in case of uncommon disorders. Supported by the Grant of Ministry of Health of the Czech Republic IGA 1A/8611-3 and VZ FNM 00064203.

PP1-277
SPINDLE CELL LIPOMA OF THE PAROTID GLAND
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Back ground: Spindle cell / Pleomorphic lipoma is a circumscribed subcutaneous lesion, occurring typically on the neck and back,usually of males. The parotid gland is an uncommon site for this distinct histological variant of lipoma. Report of the case: A 74 years old man presented in December 2006, with a long history of an asymptomatic swelling of his left chick. The computed tomographic scan revealed a tumor with fatty density located in the parotid gland. Superficial parotidectomy was performed. The specimen received for pathologic analysis, was a 7X6X3 cm resection of the parotid gland. A well circumscribed, encapsulated soft, fatty tumor, 2.5 cm in diameter, was found in the gland. Microscopic examination revealed lobules of mature adipocytes and bland, mitotically inactive spindle cells arranged in parallel registers between them. Among them "floret like" multinucleated giant cells were also found as well as large numbers of mast cells. There was also prominent myxoid change of the stroma. Immunohistochemically spindle cells were positive for CD34 antigen and S-100 protein. Based on the above mentioned pathologic and immunohistochemical findings, the diagnosis was spindle cell / pleomorphic lipoma. The patient is doing well, four months after the surgical excision.

PP1-278
CARBOWAX THIRTEEN YEARS OF EXPERIENCE
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CARBOWAX Thirteen years of experience in our profession we have to count on with an excellent preservation sample material. In our cytological archives we collected random samples, dated between 1993 - 2005. The selected material were sputum 16, bronchial lavage 27, pleural fluid 7, saliva gland secretion 1. We made the cytology diagnosis and compare it with the previous diagnosis and cellular material. We found identical cellular material and a 100% match in diagnosis in all samples. Since 1980 we have used Saccomano's fixative as a preservation media solution for all our cytological samples. Carbowax is capable to conserve cellular elements and microorganism thought many years. It is easy to manufacture, inexpensive and it does not require refrigeration.

PP1-279
ALCOHOLIC FIXATIVE AND QUALITY OF NUCLEIC ACIDS
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Background: In surgical pathology, the large majority of tissues are formalin fixed and paraffin embedded (PET) as a routine procedure. This traditional method of tissue preservation retains tissues integrity for future analyses, such as immunocytochemistry (ICC), but it compromises the quality and integrity of nucleic acids essential for biomolecular analyses. In routine practice with the advent of new diagnostic tools and therapeutical strategies, the clinicians, the pathologists and the researchers feel the urgent need of a practical fixative in order to simultaneously maintain good morphological qualities of tissues for light microscopy and ICC, along with satisfactory length of nucleic acids. We present the comparison of an alcoholic-based fixative (FineFix, FF, Milestone, Italy) to formalin for cytological material obtained by fine-needle aspiration (FNA) and biopsies. Methods: 50 samples from true-cut biopsies or FNA were divided into two parts: a part was fixed in FF (group A) and the other in formalin (group B) and both of them, subsequently, were paraffin embedded. ICC with a panel of 10 commonly employed antibodies was conducted under the same conditions for the group A and B. DNA and RNA were obtained from A and B tissues by phenol/chloroform extraction, resuspended in deionised water and loaded on agarose gel to check the quality of nucleic acids and RT-PCR and PCR analyses were performed to assay their integrity , using beta-actin as house-keeping gene. Results: DNA extraction resulted more efficient in group A samples compared to group B tissues. In particular, beta-actin fragment amplification of 815 bp was obtained from group A DNA, while no amplification resulted from group B DNA. DNA and RNA yield and purity improved at least 10-folds in group A compared to group B. ICC results did not demonstrate significant differences, as well as for routine stains. Conclusions: This formalin-free fixative FF demonstrated to be suitable not only for tissue preservation, but also for DNA and RNA extraction for molecular purposes. DNA, RNA and proteins are well preserved, with a quality comparable to fresh tissues and the use of this fixative allows extensive molecular analysis of biological samples, than from formalin. Moreover, laboratory safety is better achieved with formalin free procedures.

PP1-280
HYBRID CAPTURE (CH-2) VS. PCR (KIT CLINICAL ARRAYS PVH)
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Background: HPV infection is common in general female population. Though only some types of HPV seem to correlate with cervical cancerigenesis. Different methods are available to typify HPV. Aim: To compare Hybrid Capture (CH2, Digene) with CLINICAL ARRAYS Papillomavirus Human kit (kCA da ALFAGENE) in thin prep cervical cytology HPV tipification. Material and methods: HPV was typified, both by CH2 and kCA, in 181/744 cervical cytology specimens, selected according to our hospital protocol. We analysed a) High risk (hr), probably high risk (phr), undetermined risk (ur) and low risk (lr) HPV types; b) co infection viral types; c) cytological diagnosis (CD) and HPV types. When there was a discrepancy DNA was sequenced. Results: 1. Out of 181 cases 37% where positive by CH-2 and 60.7% by kCA: 41,4% - one type of HPV (27 HPVhr, 6 HPVphr; 8 HPVlr e 5 HPVur; 57,6% - co infections of 2 to 7 types, 79.6% had one or more HPVhr types. The more frequent types were: HPVhr [16 (31), 51 (21), 33 (20) e 58 (11)], HPVphr 53 (25); HPVlr [70 (21), 61 (17) e 81 (11)]. 2. In 92 cases that had no cytological lesion, 9 had one HPVhr type, 2 one HPVphr and 21 co infections (HPVhr, HPVphr, HPVur e HPVlr) by kCA. In 74 cases that had CH-2 negative and no cytological lesion 26 had HPV infection by kCA (10 HPVhr, 6 HPVphr with one or more types). 3. All CD with ASC-H and HSIL had HPVhr and /or co infections. 4. In 24 cases DNA was sequenced: only one was discordant (kCA(-)/seq. HPV16). Conclusions: kCA is a more sensitive method than CH-2 in the detection of HPVhr, HPVphr infections and co infections in cytological specimens.

PP1-281
IS THE CYTOLOGICAL DIAGNOSIS OF CHROMOPHOBE RENAL CELL CARCINOMA POSSIBLE? A REPORT OF EIGHT CASES

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BACKGROUND: There is an increasing tendency to perform conservative surgical procedures and preoperative chemotherapy for selected cases of renal neoplasms. Such therapeutic approaches demand a precise preoperative diagnosis. Both, fine needle aspiration and intraoperative cytology are increasingly used to evaluate renal tumors, preoperatively or during surgery. Due to their different therapeutic management, the cytologic recognition of chromophobe renal cell carcinoma (CRCC) and its distinction from conventional clear cell carcinoma and oncocytoma is particularly important. The purpose of this study is to review our experience with eight cases of CRCC. The cytologic descriptions of this entity are still few, and information concerning the diagnostic capacity of cytology is needed. METHOD: Eight cases of CRCC evaluated preoperatively using FNA cytology (five cases) or scrapping cytology during intraoperative studies (three cases) were selected. The cytologic samples were air-dried or alcohol fixed and stained with Diff-Quik and Papanicolaou, respectively. The expression of vimentin was evaluated in four cases using immunocytochemistry that was performed in alcohol-fixed material. In all cases a complete histopathologic and immunohistochemical study was available. RESULTS: Neoplastic cells were arranged mainly in small, discohesive monolayered groups and single cells. These cells were heterogeneous creating a pleomorphic image. A transition from small to large cells was seen. All of them exhibited large amounts of ill-defined cytoplasm. Its quality was granular with clear spaces that in many cases resembled perinuclear halos. Microvacuolizated or dense, homogeneous cytoplasms were uncommon. Nuclei was pleomorphic but no intense atypia was seen. Binucleation and marginal nuclear location was a common event. Some cells showed a very large amount of cytoplasm and relatively small nuclei, determining a balloon-like image. Necrosis, basal membrane or other stromal material were not present. Vimentin was not expressed in the four cases analysed. A precise cytologic recognition was possible in our last five cases. CONCLUSION: There is increasing evidence that a cytologic diagnosis of CRCC is possible. In our experience, histopathologic features of CRCC were well reflected in cytologic samples allowing in most cases a specific recognition. In our cases the only differential diagnosis considered was clear cell carcinoma, since differentiation from oncocytoma was always possible. Cytology can be specially helpful in the evaluation of intraoperative samples.

PP1-282
ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION FOR MEDIASTINAL STAGING OF LUNG CANCER.

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Background: Staging of the mediastinum in lung cancer is essential for patient management. Mediastinoscopy is an invasive method with a certain level of morbidity. Endobronchial ultrasound (EBUS) is a new minimally invasive method in which a flexible ultrasound miniprobe is inserted through the working channel of the flexible bronchoscope, which helps to localize mediastinal lymph nodes and allows a transbronchial needle aspiration (TBNA). Methods: The aim of the current study was to evaluate the usefulness of EBUS-guided TBNA in the staging of lung cancer in the 50 first cases done at our institution from March 2006 to February 2007. EBUS-guided TBNA was performed using the 20 MHz ultrasound miniprobe (UM-BS 20-26 R) connected to an ultrasound unit (EU-M60 Olympus). The method, needle features, and cytopathologist were the same throughout the study. The aspirated material from 3-4 passes was smeared onto glass slides, fixed in alcohol and stained with the Papanicolaou method. Specimens were categorized as positive (presence of tumor cells), negative (lymphocytes but no tumor cells), or inconclusive (poor cellularity or only bronchial cells). Final diagnosis was based on mediastinal biopsy results, surgery and/or clinical follow-up. Results: EBUS-guided TBNA was used to sample 50 lymph nodes from 41 patients with mediastinal lymph nodes that were suspicious by CT scan or PET. A positive cytological diagnosis was achieved in 7 cases (14%). Of these, 5 showed non-small cell carcinoma, 1 case contained atypical cells and 1 metastatic adenocarcinoma. Specimens from 18 patients (36%) were recorded as negative (one of them was an unsuspected granulomatous lesion) and 25 (50%) as inconclusive. Conclusions: EBUS-guided TBNA is a safe and useful method for lymph node staging in patients with lung cancer. A positive diagnosis avoids more invasive staging procedures. To improve the inconclusive cases (in our study 50%) immediate interpretation by an on site cytopathologist would be necessary to confirm adequate material.

PP1-283
DIAGNOSIS OF CYSTIC LESIONS OF THE JAW BY FINE NEEDLE ASPIRATION CITOLOGY

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Background: The phenotypic diversity of primary intraosseous jaw cystic lesions poses some clinic, radiological and histopathologic diagnostic difficulties. Cytodiagnosis by fine needle aspiration (FNA) technique can be an evaluation method of the tumours vs tumor-like conditions of intraosseous jaw lesions. Method: Our study included 27 FNA of intraosseous jaw cystic lesions performed at the oral and maxillo-facial clinic from Timisoara, between 2005-2006. Cytodiagnosis was correlated with histodiagnosis. Results. Aspirated samples were adequate for evaluation and allowed citodiagnosis in 24 of 27 cases. In 10 of 24 cases malignant cells were found. Cytodiagnosis was confirmed by histodiagnosis in all these 10 cases (100% accuracy). Cytodiagnosis of benign lesions was confirmed in 9 of 14 cases (65%). Difficulties in distinguishing between benign vs malign were met in odontogenic tumors. Conclusion. Differentiation is a prerequisite to treatment becouse the management differs in each lesion.

PP1-284
IS IT POSSIBLE TO START THE TREATMENT BASED ON IMMEDIATE CYTOLOGIC EVALUATION (IMPRINT AND/OR SMEAR) OF CORE NEEDLE BIOPSY OF THE SPINAL LESIONS?

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BACKGROUND: Core needle biopsy are widely used to diagnose spinal lesions. Final histopathological report of the specimen usually could be given after several days. In some patients these several days delay hamper the acute treatment. The aim of this study was to determine the value of immediate

cytological evaluation of the core needle biopsy regarding to provide a reliable immediate diagnosis which guide the correct treatment strategies in spinal lesions. METHOD: The cytological materials (imprint and/or smear) of the 213 core needle spinal biopsies which were prepared immediately after biopsy, in the Izmir Ataturk Training and Research Hospital during the period January 2003 - November 2006 were analyzed retrospectively. Cytological assessments were compared with both histopathological analysis and final clinical diagnoses obtained by open surgery or clinical follow up. RESULTS: Of 213 cases, a final diagnosis could be reached in 176 patients. 101 of these had both histological and cytological material that formed the backbone of current study. According to final diagnoses 40 patients had infections, 59 patients had neoplasms and 2 patients had no pathology. The positive predictive values of diagnoses of malignancy and infection on cytology were 1 and 0.93 respectively while the values for histology were as follows 1 and 0.97 respectively. The sensitivity of diagnoses of malignancy and infection on cytology were 0.8 and 0.68 respectively while the values for histology were as follows 0.78 and 0.75 respectively. In the spinal metastases positive predictive value was 0.97 for cytological and 1 for histological assessments. In the subgroup of tuberculosis infection, lymphoma and plasmositoma positive predictive values of both cytological and histological assessments were 1. CONCLUSIONS: Immediate cytological evaluation of core needle biopsy is a useful and reliable tool to assess the spinal lesions that necessitates urgent treatment. In case cytological specimen disclose the diagnose of spinal metastasis, lymphoma, plasmositoma or tuberculosis, the treatment of the patient may be started without waiting the result of the histological examination.

PP1-285
DIAGNOSTIC VALUE OF FNA APPLICATION IN THE CYTOLOGICAL EXAMINATION OF THE THYROID GOITER

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Introduction: FNA is very useful method in cytological diagnosing of different lesion in the thyroid gland, among them is benign goiter. In consideration of the goiter; it is the most frequent lesion in the thyroid tissue, significance of FNA as a diagnostic method become greater. Material and methods: In the period of 2 years citologically was analyzed 600 aspirates from the thyroid lesions. Aspirates was stained with: hematoxylin-eosin (HE), May-Grunwald-Giemsa (MGG) and Prussian blue. In patients with cytological diagnosis goiter we analyzed: 1) sex distribution, 2) age distribution, 3) correlation between clinical and cytological diagnoses and 4) goiter classification in accordance with cytological characteristics. Results: From 600 analyzed aspirates 414 cases (69%) were diagnosed as goiter. This lesion was five times frequent in females then males (348 women, 66 men). Statistically by X^2 test was confirmed high significance for arising goiter (X^2=192,08; p<0,005) in females. In our examination the most patients were old between 41 and 50 years. In the opposite, the least patients (only 6) were younger then 20. The youngest patient was 13, and the oldest was 78 years old (the average years were 46,5). Statistically by X^2 test was confirmed high significance for arising goiter (X^2=494,30; p<0,005) according to the ages of life. From 414 cytological confirmed goiters 222 had clinical diagnosis “nodular goiter”, 127 “diffuse goiter”, Our conclusions are: 84% of cytological diagnosed goiters had the some clinical diagnosis, in only 1,5% of all patients clinically tumor was suspected. We confirmed high correlation between clinical and cytological diagnosis. According to the cytological characteristics all goiters was classified in 2 categories: goiters with and without degenerative changes. From all cases in 174 (42%) we found degenerative changes-hemorrhage. Without hemorrhage were 240 goiters (58%). X^2 test confirmed statistical significance in appearing such goiter, but it is not high statistical significance (X^2=10,4;p<0.01). Conclusion: FNA permits a substantial reduction or even elimination of other diagnostic procedure, such as imaging, with consequent saving in time and money. Furthermore, benign cystic lesion may be cured by FNA (a diagnostic and unexpected therapeutic value of FNA).

PP1-286
THE RELATION BETWEEN QUALITY OF THE CYTOLOGICAL SMEARS AND STATUS OF THE MEDICAL CARE UNIT TAKING A CERVICAL SPECIMEN FOR CYTOLOGY. THE STUDY OF 33842 SMEARS EVALUATED IN 2006 DURING PROPHYLACTIC SCREENING PROGRAM “CERVICAL CARCINOMA”

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Background. The quality of the cytological smears is critical for proper evaluation according to Bethesda system. It seems that worse smears quality has a direct influence on quantity of ambiguous diagnosis (ASCUS). The objective of this study was the assessment of the correlation between the quality of the smears and medical unit experience in screening program examinations (the quantity of smears taken) or the type of the management (public v. private). Verification of the thesis about correlation of the quality of the smears and the percentage of ASC-US diagnosis was an addition aim. Methods and results: 33842 cytological smears were evaluated in prophylactic program “ Cervical Cancer” in PTD OC between January and December 2006. The optimal quality (AI) was noted in 15091 (44,59%) cases. 790 (2,33%) smears were evaluated as insufficient for diagnosis, the percentage ranged from 0% to 19% depending on medical unit. 447 (1,32%) ASC-US and 115 precancerous lesions and cancers were diagnosed. The lowest medium percentage of insufficient smears for diagnosis (1,6%) was observed in medical units taking between 100 and 400 smears. Over twice more frequently (3,31%) insufficient smears for diagnosis were evaluated in material from medical units preparing more than 400 smears. Higher percentage of insufficient for diagnosis smears was connected with a decrease of the percentage of ASC-US diagnosis. Good quality of the smears correlated with positive diagnosis (HSIL, carcinoma). Over twice lower percentage of insufficient for diagnosis smears and slight higher percentage of positive diagnoses (HSIL, carcinoma) was observed in material from public medical units comparing the private one. Conclusions: The quality of the smears is higher in medical units with low and medium quantity of the smears contracted and in public medical units. Worse smears’ quality is connected with lower percentage of neoplastic lesions and paradoxically with lower percentage of ASCUS.

PP1-287
FINE-NEEDLE ASPIRATION BIOPSY FINDINGS IN SCLEROSING POLYCYSTIC ADENOSIS OF THE PAROTID GLAND

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Introduction: Sclerosing polycystic adenosis (SPA) is a recently described, rare lesion of the salivary gland analogous to fibrocystic disease of the breast. Recognition of this benign entity is important since the differential diagnosis includes other more common benign and malignant salivary gland neoplasms, particularly mucoepidermoid carcinoma and tumors with cystic

and oncocytic features. While the histomorphology of SPA is well documented, there is only one other cytologic description of SPA in the English literature. Case: We describe the fine-needle aspiration biopsy (FNAB) findings in a case of SPA of the parotid gland in an 84 year-old woman. The aspirate was characterized by flat cohesive sheets of epithelial cells with moderate amounts of finely granular oncocytic cytoplasm and enlarged round nuclei with indistinct nucleoli. Some epithelial groups formed glandular structures with lumens, and the background contained small amounts of delicate mucoproteinaceous material. Occasional markedly vacuolated cells were present as well as many cells with apocrine change manifested by well-defined apical snouting. Familiarity with the cytomorphologic features of SPA, including its characteristic apocrine changes, is important for distinguishing it from other more clinically significant salivary gland lesions.

PP1-288
STRONGYLOIDES STERCORALIS IN EBRO DELTA: PATHOLOGICAL DIAGNOSIS.

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INTRODUCTION: Strongyloides stercoralis (Ss) is an endemic nematode of the Mediterranean countries. The main risk factors for infection in humans are being a farmer of advanced age (79%) and having worked barefoot in contact with soil of irrigated areas. 13% of patients develop severe forms of disease and 4% die. In the Spanish Mediterranean coast, the delta of Ebro River is a wet zone with similar environmental conditions to the area of Gandia in the Valencian community, where Ss has been found to be endemic. AIM: To identify the number, type of diagnostic samples and clinical context of cases of Ss diagnosed at the Pathology Service of the Hospital de Tortosa Verge de la Cinta which is the referral hospital of Ebro Delta. METHODS: We identified all cases diagnosed of Ss at surgical pathology department from 1990 up to now using the SNOMED code (E 45770, E45780). After identifying the cases, we revised the slides and the clinical records. RESULTS: 10 patients, 9 men and 1 woman with a mean age of 70 years (range: 63-80 y.) were identified. In 4 patients, the Ss identification was made by cytology (2 patients in a sputum, 1 patient in a bronchial washing (BW) and a sputum, and 1 patient in a pleural fluid and a BW), in 5 patients by biopsy (2 from stomach, 2 from duodenum and 1 from colon) and in one case of hyperinfection, the Ss was discovered in the necropsy. Subjacent diseases were pulmonary carcinoma (n=3), lymphoma (n=1), advanced chronic obstructive pulmonary disease (COPD) (n=2), Helicobacter pylori gastritis (n=3) and hypertension and mental retardation (n=1). No patient was affected by human immunodeficiency virus infection. CONCLUSIONS: In our hospital, the pathological diagnosis of Ss has been mainly made in men of advanced age who were affected by a neoplasm or an advanced COPD. In 40% of the cases, the parasites were identified in a cytological study, mostly from respiratory secretions and in, one case, in a pleural fluid. In 50% of the cases, it was identified in a biopsy of duodenum, stomach or colon. Death directly related with a Ss hyperinfection was only observed in a case (10%). (PI 04/1440, 04/1467, 05/1527)

PP1-289
WHICH HEMATOXYLIN FORMULA IS THE BEST FOR LIQUID-BASED CYTOLOGY?

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BACKGROUND: The most important element of a perfect Papanicolaou stain is hematoxylin. Especially for malignant serous effusions, diagnosis depends heavily on the malignant nuclear features. Many studies in the literature evaluated the best nuclear stains for conventional smear techniques. In the last decade, liquid-based cytology (LBC) emerged as an alternative to conventional cytopreparatory methods. However, in contrast to conventional techniques, studies on the applicability of hematoxylin formulas suitable for LBC are absent. We aimed to examine and compare the performance of various hematoxylin formulas for LBC of body cavity fluids. METHOD: Total of 168 LBC slides were prepared from two different malignant effusions. The slides were coded and stained with 7 different alum hematoxylin formulas(Harris hematoxylin, Mayer hematoxylin, Carazzi hematoxylin ,Lillie hematoxylin,Gill I hematoxylin, Gill II hematoxylin and Gill III hematoxylin) followed by standard classical Papanicolaou stain. All slides from were evaluated semiquantitatively for the visibility of nuclear membrane, chromatin structure, nucleoli, cytoplasmic staining, and background staining. The slides were reviewed independently by two pathologists and one student of cytotechnology. General Linear Model Univariate Analysis of Variance is used to analyze data among hematoxylin types and between graders. RESULTS: For mono-layered smears, hematoxylin Carazzi and Mayer were found to be significantly better than the other formulas ($p<0,001$). In the presence of 3-dimensional configurations seen in metastatic adenocarcinomas, there was no statistically significant difference among hematoxylin types. There was a statistically significant difference among graders in the presence of such clusters. Studies have suggested that Gill hematoxylins are the best for conventional cytology while in our study, they fared among the worst for LBC. Slides stained with Carazzi hematoxylin had the highest quality nuclear membrane and chromatin patern staining, with visible nucleoli, transparent cytoplasm and clean background. An additional advantage is that, it requires a shorter staining time compared to the other hematoxylins. CONCLUSION: Our findings indicate that for the LBC of serous effusions, application of Carazzi hematoxylin gives the best results.

PP1-290
A COMPARATIVE STUDY WITH CONVENTIONAL SMEARS AND LIQUID-BASED CYTOLOGY FOR DIAGNOSTIC PERFORMANCE

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Objectives: For over 30 years, screening for cervical cancer has used the conventional Papanicolaou smear. Despite limited accuracy of the test the incidence of cervical cancer has fallen substantially. Liquid-based cytology (LBC) has since been developed as an alternative to conventional cytology. The objective of this study is to compare the diagnostic performance of cervical smears prepared by conventional methods and liquid based cytology procedure. Methods: Cervical smear samples from 540 randomly selected cases from women which admitted to the Gynecology outpatient clinics of the Cukurova University Hospital were examined at the Pathology Department of the same hospital. Conventional Papanicolaou smear (CP) and liquid-based cytology (Papspin-PS) were taken from same woman

simultaneously. Before gynecologists prepared the LBC procedure they spreaded the CP with one side of the cervical brush tool of the PS equipment. Then the remainder of the cells on the brush were put in to the container of the PS. Two pathologists evaluated the smears. The results were evaluated in terms of the Bethesda III classification. Statistical analysis was done according to the CP results as a golden standart. Results: From the standpoint of epithelial cell abnormalities, sensitivity and specificity rates of the PS were 42.8% and 99.8% respectively. Positive predictive value (PPV) was 75.0% and negative predictive value was 99.2%. According to the adequacy, the PS sensitivity rate was 33.3% and specificity was 95.9%. The PPV and NPV of the endocervical and/or metaplastic cells were calculated as 99.2% and 9.3% respectively in PS. Conclusion: Liquid-based cytology is reported to increase the sensitivity of cervical cytology and the proportion of slides that are satisfactory for assessment, in comparison with conventional cytology. Although some countries have changed to liquid-based cytology for cervical screening, controversy remains. Some meta analysis on this subject is claimed that new technology will not be the answer to the remaining incidence and mortality rates of cervical cancer. At this point, despite the confident results of larger studies and the preparing techniques that may have affected the PS; the results of our study do not show an improvement concerning increased rate of specimen adequacy and higher sensitivity to detect epithelial cell abnormalities in LBC.

PP1-291
ENDOSCOPIC BILIARY TRACT BRUSH CYTOLOGY

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Objective: Endoscopic bile duct brushing (EBDB) is performed in patients who have pancreaticobiliary disease to differentiate benign from malignant biliary strictures. Study Design:The study included 40 patients with bile duct obstruction or pancreatic mass who underwent EBDB and follow-up surgery or biopsy between 2006 to 2007 years. Results: Seventeen (42.5%) of these cases were positive for malignancy in both EBDB and follow-up surgical biopsy; nine cases (22.5%) were negative in both EBDB and follow-up surgical biopsy specimens. Seven cases 17.5%)were negative in EBDB cytology but were positive in the surgical or biopsy specimens (false-negative). Our sensitivity rate was 70.83% and specificity rate 100%. Conclusion: Endoscopic brushing is a safe diagnostic procedure for the evaluation of biliary tree lesions. Small, three-dimensional epithelial clusters with marked atypia signify malignancy and warrant the diagnosis of a malignant neoplasm even when only one or two such clusters are seen in the smears. Single cells, cytoplasmic vacuoles and prominent nucleoli are not essential for a diagnosis of malignancy.

PP1-292
HOW MANY WOMEN WITH CARCINOMA OF THE CERVIX UTERI HAVE NOT BEEN PROTECTED BY THE PROGRAMMED SCREENING IN THE NETHERLANDS? AN AUDIT OVER 2005

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Aim An audit of cervical carcinoma is important for quality control in screening programmes to gain insight into the relation between having been screened and incident carcinomas. Previously, others showed that in women aged 30-65 797 (55%) women had never been screened at all prior to the diagnosis of cancer. The other 661 (45%) did have a smear (either opportunistic of programmed) any time prior to the diagnosis of cancer. From these 661 women, only 285 (19% of all the 1458 cases) had been screened in a programme within one round prior to their diagnosis. We addressed the nationwide databases to measure whether this small fraction of 19% was still applicable over the year 2005. Method Query nr 506 into the national database PALGA counted 629 squamous- or adenocarcinoma cases of the cervix uterus in women 28-67. Prior history of all these cases going back to 6 years (coinciding with screening interval in the Netherlands) was obtained based on matching records. Screen smears could be identified and further divided into invitational versus non-invitational smears and further subdivided on the basis of their conclusion/ advice. Smears obtained with 2 weeks of the cancer diagnoses were excluded, being very probably part of the diagnostic work for carcinoma, i.e. not a lead-smear. Results. Of the 629 women with carcinoma of the cervix, 220 (35%) had never been screened at all prior to the diagnosis of cancer in 2005. 409 women had had a cervical smear among whom 289 (46%) an invitational screening smear and 120 (19%) an opportunistic smear. The ‘advice profile’ of the 289 women with proper screening history was as follows: 113 (40% of 289) had a regular advices (within normal limits), while 45 (16%) had had a repeat smear advice based on ASCUS/mild dyskaryosis and the residual 127 (44%) had a colposcopy advice. Conclusion. The percentage of women that had not been protected by the program has been lowered from 55% to 34%, suggesting an improved coverage in the programmed cervical screening in the Netherlands in 2005 as compared to the situation in 1994-1998. Further queries are needed to confirm whether this constitutes a trend or not.

PP1-293
IS THE REST OF THE BLOOD IN THE NEEDLE FOR THE LIVER CORE BIOPSY USEFUL FOR DIAGNOSIS LIVER TUMOR?

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BACKGROUND/AIMS: Although fine needle aspiration biopsy is a useful tools in the diagnosis of primary malignancies and metastatic lesions of the liver the core biopsy more common use for diagnostic procedure. Histologic and cytologic preparations from the rest of the material in the needle of the liver core biopsy were examined. The aim of this study was to determine efficiency of disease and tumors diagnosed by this method. METHODS: The liver core biopsy and cytologic material were obtained from 45 patients with a variety of liver diseases. The results were compared each other. RESULTS: There were 10 cases (22%) including malign lesion and 30 cases (66%) non-malign diseases. The inadequate materials rate was 11%. Malignant neoplasms were detected by cytology alone in whole of the patients (metastatic carcinoma 9/1 patients and 1/10 hepatocellular carcinoma). CONCLUSIONS: The cytologic examination of the rest of the material in the needle of the liver core biopsy could use for a diagnostic tool for liver tumors.

PP1-294
BILATERAL SEROUS PSAMMOCARCINOMA OF THE OVARIES IN A 28 YEARS OLD FEMALE. REPORT OF A CASE

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Introduction: Psammocarcinoma is a very rare variant of serous neoplasm that appears to arise as often in the peritoneum as in the ovaries. The mean age of patients with ovarian psammocarcinoma is 59 years. The most common presentation is with abdominal or pelvic pain and pelvic mass. Description of the case: We present the case of a 28 years old female with a cyst in left ovary (9cm in diameter) and a papillary tumor in the surface of the left ovary. The tumours consisted of papillae or nests with 2 or 3 neoplastic cells in diameter with no areas of solid epithelial proliferation. There was mild cytological atypia and foci of hobnail differentiation. Mitotic figures were rare. Psammoma bodies were numerous and replaced over 75% of the papillae. There was destructive invasion of ovarian stroma and extraovarian tumor implants were invasive consisted of psammoma bodies surrounded by neoplastic cells in omentum and fallopian tubes. Sample of peritoneal washing revealed by cytological analysis a large number of psammoma bodies and small to medium size tumor cells with fine cytoplasmic vacuolisation, nuclear abnormalities and increased nuclear to cytoplasmic ratio. Conclusion: Serous psammocarcinoma is a rare form of ovarian carcinoma with only 14 cases reported in literature and only one case of them concerned an adolescent. The clinical behaviour of psammocarcinoma more closely resembles that of borderline serous tumors than of serous carcinomas of the usual type, following a protracted course and being associated with a relatively favourable prognosis.

PP1-295
HPV-DNA TYPING AND CERVICAL CYTOLOGY AND PERSISTENCE MUCOPURULENT ENDOCERVICAL DISCHARGE

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Background:N Gonorrhoeae and C Trachomatis are responsible for mucopurulent endocervisitis (MPC). Treatment of MPC consists of treatment patient and all sexual partners with some antibiotic regimen for Gonococcal and Chlamydial infections. Treatment of bacterial vaginosis aids this regimen. HPV infection is a sexually transmitted disease and coexist other infections. We detected HPV –DNA and cervical cytologic abnormalities in patients who had persistent mucopurulent endocervistis. Methods: We screen 66 women aged 20-58years. On this screening, we has taken two sample using cone –shaped cervical sampler brush, the first for conventional cytology and the second for HPV DNA testing. We have used a PCR-based assay to detect Human Papillomaviruses (HPV) in samples and determine genotype. For the detection of HPV, nested-PCR was applied to amplify consensus MY09/11 region of HPV with MY09/11 and GP5+/6+ primers. For the HPV genotyping, after amplification of E6/E7 oncogene region of HPV using consensus E6/E7 primers, nested multiplex PCR with type specific primers were used to genotype each 13 high risk HPV (HPV-16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-66, and -68). The amplification products were visualized in EtBr stained agarose gel electrophoresis. We assesed individuals who had HPV –DNA positive result and abnormal cytological findings. Findings: We found high rate HPV-DNA positive results (56 % n= 37) in this study. Abnormal cytology was 30,158 % of all patients (n=18). HPV positive test with abnormal cytology was 73,68 % (n= 14 of 19). The mean age, duration of sexual intercouse, mean number of birth and abortion, oral contraceptive usage were unrelated HPV-DNA positive test. Smoking, educational level, usage IUD and condom were found with high ratio in HPV-DNA positive people. Also, in this group abnormal cervical biopsy results were high. High oncogenic HPV types that we detected in our study group were HPV 16, 18,35, 45,56, 58 . Conclusion:We found high HPV-DNA prevalence in patients with MPC. The risk factors determined in this study were smoking, educatonal level, usage IUD and condom. Finally, HPV-DNA detection is very effective sceening method. But it is expensive for our population; so, cytology is kept value as common screening test.

PP1-296
THE ROLE OF NUCLEAR MORPHOMETRY IN EQUIVOCAL BREAST FINE NEEDLE ASPIRATION CYTOLOGY

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INTRODUCTION: The most common breast lesions that result in equivocal diagnosis, hence form the gray zone in fine needle aspiration cytology (FNAC), are fibrocyctic changes, fibroadenomas and low grade ductal carcinomas. In case of equivocal FNAC, a multidisciplinary approach is required and usually the case necessitates histopathological confirmation. Current study aims to determine value of nuclear morphometric analysis (NMA) in cases with equivocal FNAC results for further categorization. MATERIAL AND METHOD: 45 equivocal breast FNAC cases that were accessed between 2001-2006 in our department (Izmir Ataturk Training and Research Hospital) and evaluated by same cytopathologist, have been analyzed retrospectively. All cases had histopathological confirmation. NMA has been performed on alcohol fixed H&E stained archival FNAC slides. We utilized computerized image analysis software to obtain mean nuclear diameter (MND), mean nuclear perimeter (MNP) and mean nuclear area (MNA) from measuring 30-50 nuclei in a microscope with 40X objectives. These values were compared with histopathological diagnosis. RESULTS: Of 45 cases, 12 were benign (7 fibroadenomas, 3 fibrocystic changes, 1 tubuler adenoma, 1 sclerozing adenosis). In benign group, MNP was 9,9 µ (7.2-11.34); MNA was 29,26µ (21.57-35.15) and MNA was 68,19µ2 (37.18-96.02). Malignant group were composed of 21 infiltrative ductal carcinomas, 5 intraductal carcinomas, 3 lobuler carcinomas, 2 mixed invasive ductal and lobular carcinomas, 1 invasive papillary carcinoma and 1 tubuler carcinoma. MND was 10µ (6.86-13,72); MNP was 30,28µ (20,94-55,67) and MNA was 70,03µ2 (34,35-121,98) in malignant group. In infiltrative ductal carcinoma subgroup MNP was 10,41µ, MNP was 27,42µ and MNA was 72,79µ2. No statistical differences of MNA, MNP and MND were found between malignant and benign groups with nonparametric tests. CONCLUSION: Limiting the number of equivocal FNAC diagnosis would decrease the number of unnecessary biopsies in patients with benign breast lesions. Our results showed that lesions leading to equivocal FNAC diagnosis (such as fibradenomas and low grade invasive ductal carcinomas) display similar nuclear morphometric values. NMA based on image analysis did not help to distinguish the borderline lesion for further categorization of FNAC groups.

PP1-297

THE VALUE OF THYROID TRANSCRIPTION FACTOR-1 (TTF-1) IN DIFFERENTIAL DIAGNOSIS OF METASTATIC PULMONARY AND EXTRAPULMONARY CARCINOMAS IN PLEURAL EFFUSIONS

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Metastasis of unknown primary site in serous pleural effusion is common and cytopathological analysis may have important therapeutic implications. Primary lung and metastatic tumors may share similar cytopathological features with difficulties in differential diagnosis especially in routine cytology practice. TTF-1 is highly specific and sensitive in the diagnosis of certain types of primary lung carcinomas, especially adenocarcinoma and small cell carcinoma in histological sections. In this study, we determined the usefulness of TTF-1 in pleural fluids for the differential diagnosis of carcinomas of pulmonary and extrapulmonary origin. Cytological material from 36 patients with malign pleural effusion were evaluated. The diagnosis of primary site was identified for all cases by clinical, radiological, cytological and/or histological correlation. Twenty-five out of 36 cases were primary lung carcinomas and 11 were metastatic. Immunohistochemical study was performed on the cell block sections or unstained effusion cytology specimens. Among 25 metastatic pulmonary carcinomas, 19 cases showed strong nuclear positivity with TTF-1 (15 adenocarcinoma, 3 nonsmall cell carcinoma and 1 small cell carcinoma) (sensitivity %76) and 6 cases were negative (2 nonsmall cell carcinoma, 1 adenocarcinoma, 1 squamous cell carcinoma, 1 large cell carcinoma, 1 small cell carcinoma). None of the 11 metastatic extrapulmonary carcinomas stained for TTF-1 (6 breast, 2 gastric, 1 kidney, 1 ovary, 1 oesophagus) (specifity %100). Our results show that, TTF-1 is sensitive and also highly specific marker in discriminating between metastatic pulmonary and extrapulmonary carcinomas in effusion cytology specimens.

PP1-298

CLINICAL SIGNIFICANCE OF STAGE-DEPENDENT QUALITATIVE DIFFERENCES OF AMYLOID A DEPOSITS IN RHEUMATOID ARTHRITIS

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The histochemical characteristics of amyloid deposits are different and depend on (a) the type of amyloidosis, and (b) the stage of amyloidosis (i.e. on the extent and quantity of amyloid eposition) (1). Aim of the study was to determine the qualitative differences between fresh and old amyloid A deposits in RA. Patients and Methods A randomized autopsy population of 161 in-patients with rheumatoid arthritis was studied. Amyloidosis was diagnosed histologically, according to Romhányi (1971), and the type of amyloid deposits were determined histochemically according to Romhányi (1979), and Wright (1977). The quantitative differences of amyloid A deposits were evaluated by semi-quantitative, visual estimation of amyloid deposits on a 0 to 3 plus scale. The solubility and resistance of amyloid deposits were analyzed according to Bély (2006). In selected cases the structural arrangement of amyloid A deposits was investigated by electron microscopic methods. Results The early stage of amyloidosis is characterized by minimal loose amyloid A deposits, involving only a few tissue structures in some organs. Advanced stages of amyloidosis are characterized by extensive, dense amyloid A deposits, involving most of the tissue structures of investigated organs. Relatively fresh amyloid A deposits are more sensitive to KMnO4 oxidation-induced proteolysis: they are resistant for 30 sec- 1 min, and soluble within 2-3 min. Old amyloid deposits are more resistant to KMnO4 oxidation-induced degradation: they are resistant for 30 sec - 4 min, and soluble in 5 min, or longer. All amyloid A deposits are sensitive to performate pre-treatment in early as well as late stages of the disease. By electron microscope early amyloid A deposits are smaller in amount and looser in structure; in advanced stages the amyloid A filaments are present in larger amounts and more compactly arranged within the vessel walls or around the capillaries. Conclusions In early stages, amyloid A deposits are fewer in number and smaller in size, as well as structurally looser and more soluble. Therapy is more likely to be successful in early stages of amyloidosis (when deposits are structurally loose), than in advanced stages (when massive and dense amyloid A deposits are present). For this reason early diagnosis of AAa is important. Biopsy is suggested in all hospitalised RA patients, with or without clinical signs of amyloidosis.

PP1-299

THE ULTRASTRUCTURAL INVESTIGATION OF MITOCHONDRIA IN B – CLL CELLS DURING PROCESS APOPTOSIS

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B - Chronic lymphocytic leukemia (B-CLL) is an example of human malignancy caused by alternations in the pathways of apoptosis. Mitochondria play a critical role in the regulation of this process. The B-CLL cells dying in apoptosis showed typical morphological characteristics: the reduction of the nuclear volume is accompanied with the reduction of the cytoplasmatic volume, while many of organelles remain intact. The aim of our study is ultrastructural investigation of mitochondrial morphology in apoptotic B- CLL cells. Our study included peripheral blood samples from 40 B-CLL patients. The samples were fixed in 4% glutar-aldehyde buffered in 0.1 cacodylate

buffer and post fixed in 1% osmium-tetroxide in the same buffer. The specimens were dehydrated in a graded series of alcohol and embedded in EPON 812. The ultra-thin sections stained with uranyl acetate and lead citrate. Ultrastructural analysis of sections was performed on Philips electron microscope 208S at 80kV. The most frequent mitochondrial abnormalities in apoptotic B-CLL cells were a reduction of size with a hyperdensity of their matrix (mitochondrial pyknosis), and markedly swollen with peripherally placed, disorientated and disintegrate cristae. In some apoptotic cells, we also detected close association of mitochondria with loops of rough endoplasmatic reticulum. The results of our study showed the numerous of mitochondria damages in B – CLL cells during apoptotic process. The correlation between ultrastructural damage and functional activity of mitochondria in apoptotic B-CLL cells is still not clear and requires further investigation.

PP1-300

MORPHOLOGICAL EVALUATION OF RESPIRATORY TRACT MUCOSA IN PATIENTS WITH CLINICALLY SUSPECTED CILIARY DYSKINESIA SYNDROME

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Background. Primary ciliary dyskinesia is a rare syndrome with a genetic background. The structural ciliary abnormalities lead to dysfunction of ciliary motility and in turn caused impairment of muco-ciliary escalator. The aim of the presented work was to define ultrastructural abnormalities of cilia as well as lesions in the respiratory mucosa in the patients with a clinical suspicion of primary ciliary dyskinesia. The indications for the biopsy of respiratory mucosa, were recurrent untreatable chronic bronchitis (with short or very short remission). Method. The material consisted of 114 biopsies of the bronchial and nasal mucosa. The age of the patients ranged from 2 months to 62 years. All samples were processed for epoxy resin blocks. Tissue sections were studied using light and transmission electron microscopy. Results. In all examined material abnormalities in the ciliary structure were found. The most common was lack of inner dynein arms (68% cases). There were also cases with lack of both dynein arms (12%) or mixed forms (20%). Additionally, there were other ultrastructural abnormalities such as: aberrant arrangement of microtubules (peripheral and central) and presence of mega cilia. Furthermore, between cilia there were present microvillus cytoplasmic outpouching (single, in groups or branching). On the free surface of ciliated cells decreased number or complete absence of cilia was observed. In such cells rete ridges was mis-shaped. In respiratory epithelium changes in proportion between goblet cells and ciliated cells was reported. Goblet cells appeared in higher numbers and focally formed clusters. In the lamina propria of the bronchial mucosa features of fibrosis were present (numerous fibrocytes and fibroblasts, and collagen fibbers which focally formed bundles). Moreover there were changes in number, placement and structure of vessels. In patients with clinically diagnosed exacerbation of bronchitis numerous inflammatory cells between epithelial cells were found. In some patients squamous metaplasia and/or dysplasia were diagnosed. Conclusion. The results of the present work reflect cause-effect relationship between primary ciliary dyskinesia and following alterations. The observed broad fibrosis of the lamina propria of the mucosa can be treated as an effect of recurrent inflammatory events resulting from inappropriate in ciliary transport and mucus retention. Moreover, long term inflammatory processes causing frequent regeneration of epithelium can lead to irregularities in the structure epithelium.

PP2 - Displayed on Monday, September 10

Breast Pathology

PP2-1
SILVER IN SITU HYBRIDISATION (SISH) – A NOVEL TECHNIQUE FOR THE ROUTINE ASSESSMENT OF HER2 STATUS IN BREAST CANCER

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Background: The accurate assessment of HER2 status is essential for the correct management of breast cancer patients. Until very recently immunohistochemistry (IHC) and reflex fluorescent in situ hybridisation (FISH) analysis has been the accepted standard procedure for this assessment despite the sometimes inaccurate data obtained by IHC and the time –consuming nature of the two test strategy. The aim of this study was to evaluate a novel technique for use as a single frontline test for HER2 status. Design: We assessed the accuracy, reproducibility and practicality of a new-to-the-market, FDA-approved, fully automated brightfield ISH technique. Silver in situ hybridisation (SISH) uses the process of reducing the hybridised silver ions to metallic silver atoms in order to visualise the bound HER2 probe. We tested 49 breast cancer cases on tissue microarrays (TMA) using SISH and compared the results to those obtained when tested with dual colour FISH. Results: 35 cases were not amplified, 13 cases were amplified and one case was classed as borderline by both techniques. The results were identical in every core taken from the same tumour sample. There was 100% concordance between the results for the two ISH techniques. Conclusion: Brightfield ISH allows for clear-cut correlation between tumour morphology (e.g. invasive tumour versus DCIS) and gene copy number. With SISH there was excellent preservation of nuclear detail and the hybridised signals were distinct and easily enumerated within the nuclei. In addition the use of TMA for HER2 analysis will make routine assessment significantly more affordable.

PP2-2
THE EXPRESSION OF CD44 IN THE DIFFERENTIATION OF BENIGN AND MALIGNANT PAPILLARY LESIONS OF THE BREAST

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Background: CD44 is a family of transmembrane glycoproteins that play an important role in the malignant transformation of breast epithelium. Recently, it has been reported that CD44 expression was significantly higher in normal breast epithelium and breast papilloma than in papillary carcinoma. The diagnosis of carcinoma within papillary lesions of the breast and the differentiation from papilloma is often difficult. In these cases immunohistochemistry plays an important role in assisting such differentiation, considering the fact that CD44 expression is different between benign and malignant papillary lesions of breast. The purpose of this study was to evaluate the diagnostic value of CD44 in distinguishing between benign and malignant papillary lesions of the breast which may be difficult morphologically. Material-Methods: In this preliminary announcement, 27 patients with papillary tumors of the breast treated surgically were examined retrospectively. All patients were female with an age range of 34 to 80 years (mean age 57 years). The tumors were 8 papillomas and 19 invasive papillary carcinomas. The surgical tumor specimens were formalin fixed, paraffin embedded and immunostained to CD44 (clone DF1485 Dako dilution 1:50). The positivity of CD44 was based on percentage of stained epithelial cells and was scored and categorized as ≤10%, 10-70%, >70%. The normal breast tissues surrounding the lesions served as control. Results: The staining was membranous and generally restricted to the basal and basolateral epithelial cells. Myoepithelial cells were also positive. Normal breast epithelial cells and 4 of papillomas expressed CD44 in a high proportion of cells (10-70%). In contrast the majority of papillary carcinoma cases (14 of 19) expressed this marker in <10% of the cells. Immunostaining for CD44 was positive (10-70%) in 4 of 8 (50%) papillomas and in 5 of the 19 papillary carcinomas (26.3%). The difference in CD44 staining between these two groups in our study is lower than that which reported previously. Conclusions: We conclude that CD44 detection by immunohistochemistry is useful in assisting the differentiation between benign and malignant papillary lesion of the breast.

PP2-3
THROMBOSPONDIN 1 EXPRESSION AND ANGIOGENESIS IN PATIENTS WITH BREAST CARCINOMA AND THEIR RELATION WITH PLATELET ACTIVITY

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Background: This study investigates angiogenesis and the expression of TSP-1 in invasive ductal carcinoma of the breast and their possible relation to platelet counts and platelet activity in breast carcinoma patients. Method: The study included 20 cases of invasive ductal carcinoma. Platelet count was measured in Coulter counter T890. Platelet activity was evaluated by determining thromboxane B2 and cGMP levels by EIA. The expression of TSP-1 was studied in formalin fixed, paraffin embedded tumour tissues. Endothelial cells were labeled immunohistochemically using CD34. Microvessel counts were determined by using the method of Weidner et al. Results: Platelet counts were within normal ranges in both groups, but mean platelet count of the patient group was significantly greater than the mean platelet count of the control group ($p=0.05$). The platelet counts were positively-correlated with tumour size ($r=0.61$; $p=0.004$) and MVD grade ($r=0.46$; $p=0.05$). The mean basal platelet cGMP level in the patient group was significantly lower than it is in the control group ($p=0.02$). There was no significant difference between TxB2 levels of the controls and the patients. Focal TSP immunoreactivity was detectable in 5 (20%) of the tumours in the tumour cells, and in 9 (45%) of the tumours in the stroma. TSP-positive cell zone composed of macrophages was observed in 7 (%35) of the tumours. We did not observe TSP staining in adjacent normal breast tissue. Microvessels were noted to be most numerous at the periphery of the infiltrating tumour cells. We did not find any correlation between TSP-1 staining and angiogenesis, platelet counts, platelet activity, and the histological prognostic parameters. Conclusion: The platelet counts were positively-correlated with tumour size and MVD grade. This confirms the essential role of platelets in angiogenesis and tumour growth. We found increased platelet counts and decreased cGMP levels in the patient group. Decreased levels of cGMP may cause platelet hyperreactivity, but there was no correlation between cGMP levels and angiogenesis. Since cGMP level is accepted as an index of NO production, we thought that further studies are needed to explain the role NO-cGMP pathway in carcinogenesis and tumour-induced platelet aggregation. Although TSP-1 may be up-regulated in malignant breast tissue, this is not sufficient for tumour growth and dissemination according to our results.

PP2-4

THE CORRELATION AMONG EGFR AND MMP-9 LEVELS AND PROGNOSTIC FACTORS IN THE EARLY AND LOCALLY ADVANCED BREAST CANCER

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Background: We aimed to investigate the correlation among EGFR and MMP-9 and classical prognostic factors in patients with breast cancer and the effects of neoadjuvant chemotherapy to these prognostic factors our patients with stage III breast cancer. Method: In the study, 20 patients with the diagnosis of fibroadenoma were assigned as the control group (Group 1). Twenty patients who were diagnosed as stage I, II breast cancer (Group 2). 20 patients found as stage III breast cancer according to evaluation of the pathological specimens (Group 3), neoadjuvant chemotherapy receiving 16 patients whom diseases were accepted as stage III after physical examination and imaging studies (Group 4), 10 of the group 4 patients who underwent surgical treatment after neoadjuvant chemotherapy (Group 5). Modified radical mastectomy was performed in the study groups and excisional biopsy for the control group. Results: Statistical significance was found for EGFR positivity ($p<0.05$) but not MMP-9 ($p>0.05$) among the groups. Estrogen and progesteron receptor levels and axillary lymph node involvement were not found different ($p>0.05$) in EGFR positive patients. Weak and opposing correlation was found between MMP-9 level and axillary involvement and hystologic grade ($p>0.05$). Between the group 4 and 5 which treated with neoadjuvant chemotherapy statistical significancy was found in terms tumor diameter and MMP-9 levels ($p<0.05$) but not EGFR status ($p>0.05$). Conclusion: Besides the classical prognostic factors, EGFR and MMP-9 levels may predict the prognosis and response to the treatment. Tumor diameter can be reduced with neoadjuvant chemotherapy in patients with stage III breast cancer and this allows better surgical outcome.

PP2-5

NATIONAL PROGRAM OF HER2 BREAST CARCINOMA TESTING IN SLOVAKIA: EXPERIENCES WITH EXAMINATION OF 5746 CASES IN THE PERIOD 2003-2006

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Background: A standardized testing of HER2 status represents an essential component of the invasive breast carcinoma diagnosis. The aim was to develope a national system of a standardized HER2 protein and gene testing of all diagnosed breast carcinomas and its quality control based on the principles of a cooperation of pathology departments in the Slovak Republic. Methods: A national network system of a breast carcinoma biopsy diagnosis provided by regional centralization of biopsies in 11 departments of pathology in Slovakia using a standardized biopsy diagnostic approach, incl. immunohistochemical HER2 protein testing was introduced in 01/2003. For cases showing 2+ protein positivity, HER2 gene testing is since 01/2006 provided centrally in the coordination center of the project first by CISH technique and for cases showing low amplification also by FISH. The quality of HER2 protein testing was controlled in 2003 and 2006 by using blind testing cases being examined in the participating departments and subsequently evaluated centrally by a panel of experts. Results: On the basis of quality controls and numbers of the examined cases (2003: 973, 2004: 1287, 2005: 1655 and 2006: 1831 cases), the examinations of the biopsies were centralized to 9 regional centers. Technical and interpretational problems related to HER2 testing and all the experiences are regularly evaluated and discussed by the educational conferences and joint meetings of the involved participants. Altogether biopsy examinations of 5746 cases diagnosed in 01/2003-12/2006 proved HER2 protein positivity in 1481 cases (25.8 %): 3+ positivity in 768 (13.4 %) and 2+ in 713 (12.4 %) cases. From 213 cases tested in 2006 centrally by in situ hybridization techniques, 31 were found to show amplification of HER2 gene: 25 of 167 cases with HER2 protein positivity 2+ and 6 cases with HER2 protein posivity 3+. Correlation of HER2 status with other parameters seems to be significant. Conclusions: The described program offers standardized results representing a good basis for the therapeutical considerations and for further optimization of HER2 testing and studies of all the parameters of the disease. The number of the tested cases in the last evaluated year seems to correspond to the expected incidence of the cases in Slovakia.

PP2-6

THE PROGNOSTIC VALUE OF THE DUCTAL AND LOBULAR IN SITU CARCINOMA OF THE BREAST

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Introduction. The carcinoma in situ is considered a heterogeneous neoplasia with clinic, pathologic and biologic variability. It is the most important risk factor for developing invasive carcinoma. Material and Methods. We reviewed 69 cases that have been operated between 1995 and 2005. Among these, 54 were ductal carcinoma in situ (DCIS), 5 were lobular carcinoma in situ (LIN) and 2 were mixt carcinoma in situ (ductal and lobular). The diagnosis of carcinoma in situ was established on frozen section examination (the clear margins were also assessed in order to perform conservative surgery) or during histopathologic examination for another condition. Special atention also was payed to the evaluation of multifocal lesions and of the integrity of the basal membrane (microinvasion assessment). The Van Nuys prognostic index was evaluated in DCIS cases according to the usual parameters: tumor size, cytonuclear grade, comedonecrosis and histological margin status. Most of the cases (69%) had a moderate prognostic index and 21% had an unfavorable score. All recurrences (6 cases) occurred in high grade DCIS. Conclusions. The results of this study confirmed the value of the conventional histologic parameters. The evaluation of the Van Nuys prognostic index has an important role in the local recurrences control for conservatively treated cases. Margins status remains the most important factor for the success of conservative treatment for carcinoma in situ.

PP2-7

BENIGN MAMMARY LESIONS IN MENOPAUSE

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Breast benign pathology is characteristic for young age women but can also be identified in postmenopausal patients. In this group diagnostic errors may occur. Clinical examination is of low value, imagistic investigation may be confuse, pathological diagnosis is essential. We analyse 891 patients operated in our clinic for benign mammary lesions between January 1993-December 2007. 82 women (9,8 %) were postmenopausal. Risk factors for breast cancer after the diagnosis of benign breast disease include the histologic classification of a benign breast lesion and a family history of breast cancer. Histopathology certified the diagnosis and excluded in situ lesions or invasive carcinoma. Surgical treatment consisted in wide local excisions considering the possibility of unexpected malignancy.

PP2-8

CHANGES IN THE EXPRESSION OF STROMAL PROTEINS FOLLOWING PREOPERATIVE SYSTEMIC THERAPY IN BREAST CANCER

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Introduction. Tumors of similar histological appearance may have different response to preoperative systemic therapy (PST). Stromal component of the cancer tissue has a proven effect on the regulation of cancer cells. Significant stromal alterations are being observed following PST in breast cancer. Aims. To analyze the qualitative and quantitative changes in the tumor stroma in the context of the response rate of breast cancer patients undergoing PST. Patients and Methods. Breast cancer samples of 50 patients receiving PST were evaluated for clinical and pathological parameters. To evaluate the pathological response to PST, samples were classified according to Chevallier's (classes I-IV) and Sataloff's classification (classes A-D). Using immunohistochemistry we analyzed the expression of representative extracellular matrix (ECM) proteins: adhesion molecule (tenascin-C), proteoglycan (syndecan-1) and structural proteins (collagen-IV, smooth muscle actin /SMA/). The expression levels of the above mentioned proteins were compared to 10 breast cancer samples of patients previously not treated with PST. The results of the immunohistochemical reactions were evaluated by the Leica QWin V3.1.0 software. Statistical analysis with Mann-Whitney method was performed. Real-time PCR procedure was also accomplished to evaluate the mRNA expression level of the syndecan-1 and tenascin-C proteins. The PCR data were analysed with REST software. Results. The median age was 51 years (range, 27 to 69). Among the structural proteins the expression of the collagen-IV was significantly up-regulated in all four groups both in Chevallier's and Sataloff's classification ($p = 0,025$, $p = 001$; respectively) compared to the not treated group. Syndecan-1 levels were down-regulated in all four groups ($p = 0,015$, $p = 0,015$; respectively). Tenascin-C was significantly up-regulated in the Chevallier IV class of patients ($p = 0,008$) and the according Sataloff D class of patients ($p=0,003$). The results of immunohistochemistry were in concordance with the real-time PCR data. Conclusion. The role of cell-ECM interactions to modulate the cellular phenotype and gene expression of tumor cells is well known. According to our observations the stromal component of breast carcinomas developed significant changes following PST. Up-regulation of structural protein levels is presumed. Possible role of tenascin-C and syndecan-1 is implicated in stromal changes, which suggests their indirect effect on tumor cells during PST. Supported by the Hungarian Society of Clinical Oncology in 2006.

PP2-9

HISTOCHEMICAL CHARACTERISTICS OF THE STROMA IN INVASIVE AND IN SITU LESIONS OF THE BREAST: AN HYPOTHESIS OF DESMOPLASTIC-LIKE CHANGES IN THE STROMA SURROUNDING IN SITU CARCINOMAS

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BACKGROUND - The importance of the interactions between epithelial structures, basal membrane and stroma in breast carcinonogenesis has been widely demonstrated in literature. We observed a strong Alcian Blue stain of desmoplastic stain in a single case of invasive breast carcinoma. We couldn't find previous reports about these findings in literature. The aim of the study was to investigate the occurrence of the same staining features in other invasive carcinomas and in in situ carcinomas, in order to evaluate the histochemical properties of the stroma in neoplastic breast lesion. MATERIALS - We stained with Alcian Blue dye 20 ductal invasive carcinomas, 5 lobular invasive carcinomas , 5 non high grade in situ ductal carcinomas, 5 high grade in situ ductal carcinomas, 5 mixed in situ and invasive carcinomas 2 lobular in situ carcinomas, and 2 cases of adenosis. The nuclei was counterstained with Feulgen stain. RESULTS – The desmoplastic stroma was strongly stained with alcian in all invasive carcinomas and in all invasive foci in mixed invasive – in situ lesions, and the stain was specific, since the stroma of normal parenchyma and benign lesions was negative, and we observed the same staining properties exclusively in easily distinguishable lesion, as in mucous accumulations and in a peritumoral fibradenoma with hyaline change. We observed the same staining in defined areas of the stroma surrounding high grade in situ carcinomas and around the foci of microinvasion. A series of double staining with actin, S-100 and laminin proved that the alcian-stained spots were present around glandular structures where no invasion or microinvasion could be demonstrated. In lobular in situ carcinomas we obsesved some foci around in situ lesions were stained with alcian, and in one of these areas we could demonstrate a corresponding small focus of invasive lobular carcinoma. CONCLUSION Alcian Blue stains performed in a series of in situ and invasive breast lesions show that the desmoplastic tissue and the stroma surrounding in situ breast carcinomas with high risk of invasion, (as high grade intraductal lesions and lobular lesions) share the same histochemical properties. Notwithstanding the relatively small number of cases and the restrictions of histochemistry, these evidences suggest that the stroma around in situ foci could get the biochemical structure of the desmoplastic tissue when the basal membrane damage in not still evident. Further studies are mandatory to strengthen these preliminary observations with immunohistochemical evidences.

PP2-10

MOLECULAR ANALYSIS OF C-erb-B2/HER2/neu GENE AMPLIFICATION IN INVASIVE PRIMARY BREAST ADENOCARCINOMAS WITH BORDERLINE (2+) C-erb-B2/HER2/neu PROTEIN IMMUNOREACTIVITY

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BACKGROUND-OBJECTIVE: c-erb-B2/HER2/neu oncogene encodes a 185 kDa membrane glycoprotein with tyrosine kinase activity involved in signal transduction pathways. Dysregulation of HER2 expression through gene amplification and protein overexpression has been observed in various neoplasms including 15-30% of breast carcinomas and leads to chemoresistance and poor prognosis, independently of lymph nodes status and/or hormonal receptors (ERα/PgR) expression levels. Patients with increased HER2 protein expression respond well to targeted therapy with monoclonal antibodies.Targeted therapy is effective only in patients whose breast tumors exhibit HER2 gene amplification and/or protein overexpression (3+ in immunohistochemical evaluation). Breast carcinomas with borderline (2+) HER2 immunoreactivity should be assessed by in situ hybridization in order to detect the HER2 gene copy number and evaluate the efficacy of targeted therapy.The objective of this study was the assessement of HER2 gene status by in situ chromogenic hybridization (CISH) in sporadic primary breast carcinomas with borderline (2+) HER2 protein immunoreactivity and to correlate gene copy number with tumor type and histological grade.MATERIALS AND METHODS:Retrospective study of 239 cases of sporadic primary invasive breast carcinomas (201 ductal, 20 lobular, 10 mixed, 8 other types) with borderline (2+) HER2 protein immunoreactivity evaluated by immunohistochemistry using monoclonal antibodies (Hercept test) and according to the accepted guidelines. Semiquantitative assessement of HER gene copy number by chromogenic in situ hybridization (CISH). RESULTS AND CONCLUSIONS:A. CISH revealed: 185 (77.4%) of the carcinomas, independently of tumor type, showed no gene amplification (2 copies/nucleus) or putative polysomy of chromosome 17 (3-5 copies/nucleus, not evaluated by Chr 17 centromeric probe), 24 (10%) with low gene amplification (LGA, 6-10 copies/nucleus) and 30 (12.6%) with high gene amplification (HGA, >10 copies/nucleus and gene clusters).B. Correlation with tumor type revealed: Among HGA tumors (N=30) 27 were ductal (90%), 1 lobular(3.3%),others 1 (3.3%).LGA tumors (N=24): 21 ductal (87.5%) and 3 lobular (12.5%), no gene amplification carcinomas (N=185) :153 ductal (82.1%), 16 lobular (8.6%), 9 mixed (4.8%) and others 7 (3.8%). C. Correlation with histological grade demonstrated: Grade I:LGA 8 (3.8%), HGA 0, no gene amplification 0, Grade II: LGA 5 (2.4%), HGA 11 (5.3%), no gene amplification 74 (35.9%), Grade III: LGA 13 (6.3%), HGA 21 (10.2%), no gene amplification 74 (35.9%).

PP2-11

IMMUNOHISTOCHEMICAL STUDY OF TUMOR SUPPRESSOR GENE PTEN EXPRESSION IN SPORADIC PRIMARY BREAST ADENOCARCINOMAS. CORRELATION WITH C-erb-B2/HER2/neu EXPRESSION STATUS

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BACKGROUND-OBJECTIVE: The tumor suppressor gene PTEN encodes a dual-specificity phosphatase antagonizing PIK3 kinase activity through dephosphorylation of cell membrane lipid PIP3 to its inactive form PIP2. PIK3 mediates growth factors induced activation of receptor tyrosine kinases (HER2, IGF-R1) via phosphorylation of PDK and Akt kinases. Activated PTEN prevents the mitogenic signal transduction. Genetic and/or epigenetic alterations of PTEN gene results in inactive protein or loss/reduction of PTEN expression and eventualy in carcinogenesis. PTEN mutations have been implicated in syndromes with high risk of cancer development (Cowden, Bannayan-Riley-Ruvalcaba) and in sporadic cancer (melanoma, glioblastoma, prostate Ca, endometrial carcinoma). Loss of heterozygosity (LOH) or gene deletion of PTEN has been observed in breast and prostate cancer. Recently, it has been demonstrated that decrease or loss of PTEN expression with parallel high expression or amplification of HER2 gene is associated with resistance to HER2 targeted therapy.Thus, information on the expression status of PTEN could be a valuable predictive factor for HER2 targeted therapy of breast cancer.In this context we investigated PTEN expression in invasive breast carcinomas and correlated its expression loss with tumor type, histological grade, lymph nodes status and HER2 expression levels. MATERIALS AND METHODS:Retrospective study of 345 cases of sporadic primary breast adenocarcinomas. IHC detection of PTEN and HER2 expression using monoclonal antibodies. Semiquantitative protein expression assessement according to the accepted guidelines. RESULTS AND CONCLUSIONS:A. Overall and independently of tumor type 54.8% of adenocarcinomas demonstrated high expression, 27.8% weakly positive and 17.4% negative. B. In 60 tumors with loss of PTEN expression, 70% were ductal, 20% lobular and 10% mixed type. C. Correlation of PTEN loss of expression with tumor type and histological grade revealed: Ductal (N=40, ND:2): Grade I 5%, II 37.5%, III 57.5%, Lobular (N=10, ND:2): Grade I 10%, II 50%, III 40%, Mixed (N=5, ND:1): Grade I 40%, II 40%, III 20%. D. In 40 PTEN (-) breast carcinomas with resected axillary lymph nodes, 39% were positive for metastasis while 61% were negative. E. Correlation of PTEN loss of expression with HER2 expression: In 75 HER2 (3+) breast cancers, 21.3% were weakly positive for PTEN and 14.6% were negative.

PP2-12

SYNOVIAL METAPLASIA OF BREAST IMPLANTS CAPSULES

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BACKGROUND: Synovial metaplasia is developing in tissues surrounding joint prosthesis, in skin and soft tissues in healing or in surgical wounds. The latest years synovial metaplasia has been described in periprosthetic breast capsules, after silicone leakage. We recorded an increased incidence of cases with synovial metaplasia due to the expansion of breast augmentation for cosmetic reasons and therapeutics treatments after breast cancer. MATERIALS AND METHODS: We examined 12 cases with silicone implants after breast augmentation . The patients were female, from 46 to 65 years old and have been operated for breast cancer and breast restoration. Sections have been obtained from the adjacent the silicone capsule tissue and microscopic, histochemical and immunohistochemical examination followed. RESULTS AND CONCLUSIONS: In 6 cases, from totally 12 cases of periprosthetic capsular removal, the microscopic examination revealed the presence of synovial metaplasia in the inner surface of the capsules (50%). The capsule consisted of dense fibrocollagenous connective tissue. The adjacent tissue was infiltrated by lymphocytes,plasma cells, histiocytes and foreign body giant cells. In addition to this foreing body reaction, a significant feature was the presence of synovial type cells. Histochemically, these cells were positive for glycogen and

mucin with PAS and Alcian Blue. Immunohistochemically, they were vimentin positive and focally positive for a1-antitrypsin, a1-anti-chymotrypsin, lysozyme and CD68. No immunoreactivity was observed for cytokeratin AE1-AE3 and S100. In conclusion, synovial metaplasia in breast implants capsules is developing in 50% of periprosthetic capsules cases and although is a benign histological feature the unknown -so far- clinical significance and the increased occurence during the last years demands for the attention of surgical pathologist in order to recognise this entity.

PP2-13
INVESTIGATION OF MICROORGANISMS IN CASES OF MAMMARY DUCT ECTASIA BY HISTOCHEMICAL METHODS

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Background: We investigated the presence of microorganisms in cases of mammary duct ectasia by histochemical stainings in histological sections. Methods: Seventeen cases diagnosed as ductal ectasia at Departments of Pathology of Inonu and Mersin Universities Faculties of Medicine were included in this study. Histochemically, Periodic Acid Schiff, Grocott's methanamine silver, gram and Erlich-Ziehl-Nelsen stainings were performed. The intensity of periductal and interstitial inflammation, presence of granulomatous inflammation and rupture of ducts were also evaluated. Results: Rupture of ducts was seen in ten cases. Granulomatous inflammation was observed in five cases. While mild inflammation was present in seven cases, six cases showed moderate inflammation. Severe inflammation was seen in four cases. In ten cases, a few gram positive microorganisms were detected in the lumen, especially in the cytoplasm of macrophages. No organisms were seen by the other stainings. Conclusion: In the literature, the culture media studies made of nipple discharge of patients with duct ectasia exist. Some authors reported a low frequency of bacteria in duct ectasia, others were not showed. There are controversies about the microorganisms present in duct ectasia. In our study, while a few gram positive microorganisms in the lumen were observed in tissue sections, no organisms were detected by Periodic Acid Schiff, Grocott's methanamine silver, Erlich-Ziehl-Nelsen stainings. Sensitivity of histochemical stainings might be correlated with the number of bacteria. To our knowledge, this is the first study to investigate the presence of microorganisms in duct ectasia by histochemical stainings in tissue sections.

PP2-14
PSEUDOANGIOMATOUS STROMAL HYPERPLASIA WITH JUVENILE FIBROADENOMA FOCI (CASE REPORT)

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BACKGROUND: Pseudoangiomatous stromal hyperplasia (PASH) of the breast is a rare, benign proliferative mesenchymal lesion which may present with rapid growth. Capillary-like clefts that are formed due to separation of collagen fibers and myofibroblasts as a result of stromal hyperplasia and endotelium-like spindle cells surrounding these clefts are charasterics of the lesion. Differentiation of this entity from especially low grade angiosarcoma is crucial. CASE: The case was a 16 years old female patient complaining of left breast enlargement. At physical examination, a tumoral mass involving almost all of the left breast and thinning the nipple and breast skin was found. At mammography, a 145x 100 mm tumoral mass localized at left breast was observed. Ultrasonography (USG) revealed edema at left breast epidermal and subepidermal regions. At tru-cut biopsy, at intralobular and interlobular regions, anastomosing capillary-like spaces and spindle myofibroblast-like cells lining those spaces were seen in increased collagenous dense stroma. Occasionally, branching ducts were found which were increased in number and frequency and lined by hyperplastic epitel without atypia. The case was determined to be pseudoangiomatous stromal hyperplasia with juvenil fibroadenoma foci. Subcutaneous mastectomy was performed. At the specimen, a 22x12x9 cm white nodular lesion involving almost all of the breast was seen. At histopathological examination, morphological findings similar to tru-cut biopsy was observed. Low grade angiosarcoma was taken into account at differential diagnosis. Immunohistochemical examination (IHC) revealed cells layering capillary clefts that showed positive staining with CD34 and actin and negative staining wih CD31, F8 and cytokeratin. By the contibution of IHC and histopathology, the diagnosis was established as PASH with juvenile fibroadenoma foci. CONCLUSION: Although PSAH is a rare entity, it should be included in the differential diagnosis of benign and malign mass forming breast lesions. Results should be supported by IHC.

PP2-15
THE EVALUATION OF CONCORDANCE BETWEEN THE INTRAOPERATIVE PATHOLOGY CONSULTATION (FROZEN SECTION AND IMPRINT CYTOLOGY) AND THE FINAL PATHOLOGY OF SENTINEL LYMPH NODES IN PATIENTS WITH BREAST CANCER

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The techniques for intraoperative evaluation of sentinel lymph nodes (SLNs) vary. We used the most common techniques in the world which include the frozen section, imprint cytology / touch preparation cytology, and scrape cytology (SC). The purpose of this study was to evaluate whether there is concordance between the result of intraoperative pathology consultation and the final pathology of SLNs in patients with breast cancers. From June 2003 to April 2007 sentinel lymph node biopsies were attempted in 67 patients with breast cancer using a blue dye. From the sample of 67 sentinel lymph node materials, intraoperative and postoperative lymph nodes were not obtained in 5 cases (93% success rate). Of 62 sentinel lymph nodes, 18 sentinel lymph nodes have metastatic tumor infiltration. Two of these 18 sentinel lymph nodes have micrometastas(<2 mm). 42 patients had negative for metastatic tumor. Two false negative cases occurred, one of them with micrometastasis (90% sensitivity rate). There was no false positive case in our study (100% spesivity rate). As a result, intraoperative pathology consultation is safe choice with high diagnostic accuracy rate for evaluation of SLNs.

PP2-16
EVALUATION OF HER-2/neu AND EGFR IN PURE MUCINOUS BREAST CARCINOMA

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Background: HER-2/neu and EGFR are two important growth factor receptors for oncogenesis. They are now potential therapeutical target in case of overexpression. They are poorly investigated in breast mucinous carcinoma. Aims: to assess the expression of both HER-2/Neu and EGFR in mucinous breast carcinoma. Material and methods: 24 formalin-fixed paraffin-embedded pure breast mucinous carcinoma were tested for both HER-2/Neu and EGFR using Dako® Kits. The intensity of reactivity was scored by using a 4-tier system (negative, 1+, 2+, and 3+) and the site of immunolabelling was noticed (membranous and/or cytoplasmic. The Prognostic factors (age,

tumor grade, node status) and cytological type (with or without intracytoplasmic mucine) were also studied. Results: All cases showed no overexpression for both proteins. Immunohistochemical study of HER-2/Neu showed either absence of labelling (18 cases) or incomplete weak staining (1+) in 6 cases. Immunohistochemical study of EGFR showed either absence of labelling (21 cases) or weak cytoplasmic staining (1+) in 3 cases. Conclusion: The low expression of HER-2/Neu and EGFR in pure mucinous breast carcinoma could be related to their good prognosis. Testing these markers fails in identifying a sub-group with less good prognosis in our study but merits analyse of a larger number of cases.

PP2-17
COMPARATIVE STUDY OF THE MORPHOLOGICAL PECULIARITIES OF HORMONE-DEPENDENT BREAST CANCER

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In defining the prognosis and the treatment strategies of breast cancers a constantly increasing number of morphological criteria are applied, such as more accurate gross characteristics, histological features, hormonal status of cancers cells, and the involvement of axillary lymph nodes. The aim of the present study is to assess the morphological peculiarities and the immunohistochemical profile of estrogen, progesterone and HER2 receptor positive tumors. These findings were correlated with patient and tumor characteristics at the time of surgery. In total, 120 cases of breast cancer derived from two diagnostic centers along the Bulgarian Black sea coast were examined. In all of them hematoxylin-eosin-stained paraffin sections of the primary tumor and from all the axillary lymph nodes removed during surgery were studied. Besides grading and staging, several other microscopic parameters, including lymphoid stroma, pushing margin, comedo-type necrosis, vascular cellular emboli were evaluated. The hormonal status (estrogen, progesterone and HER2 receptors) was determined with primary antibodies and LSAB Kit (DAKO). Estrogen and progesterone-receptor negative cancers constituted the major part of tumors showing more aggressive criteria – low grade, invasive edge, positive HER2 status, and most of them presented with axillary lymph node metastases at time of surgery. On the other pole, estrogen and progesterone-receptor positive cancers showed greater variability in regard to the distribution of the other microscopic criteria. The interrelationships of these pathologic correlates and their relative value as prognostic indicators were studied.

PP2-18
CYTOKERATIN 5/14 EXPRESSION IN INVASIVE DUCTAL CARCINOMAS OF THE BREAST: AN IMMUNOHISTOCHEMICAL STUDY

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Breast ducts contain two types of epithelial cells, inner luminal cells and outer basal/myoepithelial cells. These cells can be distinguished by their immunophenotypes. Cytokeratins (CKs) 8 and 18 are expressed in the luminal layer, whereas CK5 and CK14 characterize the basal epithelial layer. For this reason, tumors expressing these CKs have been named 'basal-like subtype' breast cancers. Retrospectively expression of CK5 and CK14 was studied in 92 invasive ductal carcinoma using immunohistochemistry. The CK5 and CK14 expression were compared with histologic grade, existence of lymph node metastasis, tumor size, immunohistochemical findings for estrogen receptor (ER), progesterone receptor (PR), and c-erb B-2. The two basal cell CKs (CK5 and CK14) were not identified exactly in the same tumor when used alone. Among the 92 tumors, there were twenty one positive for CK5 (22,8%) and ten positive for CK14(10.9%). Our results showed that CK5 and CK14 are coexpressed in most tumors, but the tumors expressing CK5 only also exist. In basal-like tumor ER was negative in 19 cases (91%)(p [LTEQ]0.001), and PR was negative in 18 cases (86%)(p [LTEQ]0.001), and c-erb B-2 was negative in 16 cases (76%). All CK14 positive basal-like tumor were negative for c-ErbB2 (0/10, 100%); while in CK5 positive basal-like tumor, c-erb B-2 negativity were seen sixteen cases of 21(76%) The rate of grade 3 histology seen in the basal-like tumors was %86. (p [LTEQ] 0.001). CK5 positive breast cancers represent about 22% and CK14 positive breast cancers %11 of sporadic invasive ductal breast cancers. These tumors were mostly aggressive grade III, steroid hormone receptor-negative breast cancers and they were inversely associated. Our results showed that there was no statistically significant association between CK5/14 positivity and stage of the tumor.

PP2-19
IMMUNOHISTOCHEMICAL STUDY OF ANDROGEN RECEPTOR IN BREAST CARCINOMAS

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Androgen and androgen receptor (AR) are involved in the pathogenesis of breast cancer. Epidemiologic studies have shown a significant association between the risk of breast cancer and androgens. The present study was set up to investigate the prevalence of a AR in a series of consecutive invasive breast carcinomas. Among the 110 consecutive invasive breast carcinomas (consisting of 92 ductal, 14 lobular, 1 medullar, and 3 musinous neoplasms), AR immunoreactivity was observed in 77/110 (70 %) cases, being expressed in 61/92 (66 %) ductal, 13/14 (93 %) lobular, 1/1 (100%) medullar, and 2/3 (67 %) musinous carcinomas. AR expression was frequently associated with estrogen receptor (ER) (78%), PR positivity (82 %) (p[LTEQ] 0.001), and c-erb B-2 negativity (86 %) (p[LTEQ] 0.05). Most AR positive cases showed a low or intermediate histological grade (G1-G2) (88 %). No association between AR expression and tumor size, and lymph node metastasis was found. The potential role and the impact of ARs in breast carcinoma needs further study; however, ARs are a potential new target for hormonal therapies and they may be clinically useful predictors.

PP2-20
THE VALUE OF CORES WITH MICROCALCIFICATIONS EXCISED BY VACUUM-ASSISTED BREAST BIOPSY

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BACKGROUND: To assess cores with microcalcifications and cores without microcalcifications obtained from Vacuum-assisted breast biopsy (VABB). METHOD: The study included: 12 atypical ductal hyperplasias (ADH), 37 ductal carcinomas in situ (DCIS), and 7 invasive ductal carcinomas (IDC), diagnosed by VABB (11G) on the stereotactic Fischer's table. More than 24 cores were excised. For cores with and without microcalcifications, a separate pathology report was given. Open surgery followed, and underestimation was calculated. The discrepancy of cores with and without microcalcifications was evaluated (superiority, identity, inferiority). RESULTS: Cores without microcalcifications failed to make the diagnosis in 8.3% and 35.1% of ADH and DCIS, respectively. In 28.6% of IDC,

diagnosis was made through cores without microcalcifications. The volume of cores with microcalcifications was 1.2±0.3 cc for the two IDCs missed by cores with microcalcifications, 1.0±0.4 cc for the 40 cases of identical diagnoses and 1.4±0.5 cc for the 14 cases of superiority of cores with microcalcifications (p=0.048, Kruskal-Wallis test). The volume of cores without microcalcifications was 6.3±1.8 cc the two IDCs missed by cores with microcalcifications, 2.6±1.8 cc for cases with identical diagnoses and 3.4±1.6 cc for cases of CM superiority (p=0.018, Kruskal-Wallis test). The underestimation rate was 8.3% in ADH, and 10.8% in DCIS. CONCLUSION: Cores with microcalcifications are superior for DCIS/ADH diagnosis. However, cores without microcalcifications may be valuable for the diagnosis of the invasive component.

PP2-21

IMMUNOHISTOCHEMICAL ASSESSMENT OF C-erbB2 AND p-53 PROTEINS IN VACUUM-ASSISTED BREAST BIOPSY SPECIMENS OF BREAST CARCINOMA

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BACKGROUND: Vacuum-assisted breast biopsy (VABB) is a minimally invasive technique for the evaluation of mammographically detected, non-palpable breast lesions. This study examines whether VABB specimens provide an accurate immunohistochemical assessment of c-erbB-2 and p53 proteins. METHOD: Fifty-one consecutive cases with a VABB diagnosis of ductal carcinoma in situ (DCIS, n=34) or invasive ductal carcinoma (IDC, n=17) were included in the study. VABB (11G) was performed on the Fischer's table, and 24 or more cores were obtained in all cases. A preoperative and a postoperative diagnosis were established. Only when the two pathological diagnoses were concordant, their immunohistochemical profiles were comparatively evaluated. RESULTS: Among the 34 DCIS cases, three (8.8%) were postoperatively revealed to be IDC, i.e. were underestimated. In 12/34 DCIS cases (35.3%) and 5/17 (29.4%) IDC cases, no malignant tissue was found in the postoperative pathologic examination. Concerning c-erbB-2, there was 100% concordance for both DCIS (one-sided CI: 82.4%-100%), and IDC (one-sided CI: 71.5%-100%). As far as p53 is concerned, the observed concordance rate was 11/12 for IDC (91.7%, 95% CI: 61.5-99.8%) and 100% for DCIS. CONCLUSION: Specimens derived by VABB provide an accurate immunohistochemical evaluation of p53 and c-erbB-2 proteins. This is of great importance since no malignant tissue is found frequently in the postoperative pathologic examination, and thus VABB specimens are the only material available.

PP2-22

ESTROGEN AND PROGESTERONE RECEPTORS IN VACUUM-ASSISTED BREAST BIOPSY SPECIMENS OF BREAST CARCINOMA: IMMUNOHISTOCHEMICAL ASSESSMENT

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BACKGROUND: Vacuum-assisted breast biopsy (VABB) is a minimally invasive technique for the evaluation of mammographically detected, non-palpable breast lesions. This study examines whether VABB specimens provide an accurate immunohistochemical assessment of estrogen receptors (ER) and progesterone receptors (PR). METHOD: Fifty-one consecutive cases with a VABB diagnosis of ductal carcinoma in situ (DCIS, n=34) or invasive ductal carcinoma (IDC, n=17) were included in the study. VABB (11G) was performed on the Fischer's table, and 24 or more cores were obtained in all cases. A preoperative and a postoperative diagnosis were established. Only when the two pathological diagnoses were concordant, their immunohistochemical profiles were comparatively evaluated. RESULTS: Among the 34 DCIS cases, three (8.8%) were postoperatively revealed to be IDC, i.e. were underestimated. In 12/34 DCIS cases (35.3%) and 5/17 (29.4%) IDC cases, no malignant tissue was found in the postoperative pathologic examination. Regarding ER, the concordance rate was 100% in DCIS (one-sided CI: 82.4%-100%), and 11/12 in IDC (91.7%, 95% CI: 61.5-99.8%). For PR, the concordance rate was 18/19 (94.7%, 95% CI: 74.0%-99.9%) for DCIS and 11/12 in IDC (91.7%, 95% CI: 61.5-99.8%). CONCLUSION: Specimens derived by VABB provide an accurate immunohistochemical evaluation of ER and PR receptors. This is of great importance since no malignant tissue is found frequently in the postoperative pathologic examination, and thus VABB specimens are the only material available.

PP2-23

THE IMMUNOHISTOCHEMICAL EXPRESSION OF ESTROGEN RECEPTOR BETA IN BREAST CANCER, ITS CORRELATION WITH SURVIVAL AND RESPONSE TO TAMOXIFEN

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Background: The role of estrogen (ER) and progesterone receptors (PgR) in breast cancer is well established. Less is known about the recently discovered another type of estrogen receptor, termed ERβ, and its potential role as a prognostic or predictive factor is still disputed. Recent clinical evidence suggests that high levels of estrogen receptor beta predict a better overall, and better disease-free survival of patients treated with adjuvant tamoxifen. The purpose of this work was to determine the extent of ERβ and ERa, PgR expression in breast cancer and to determine if the ERβ expression is associated with clinical outcome. Methods: Formalin-fixed, paraffin embedded breast cancer tissues used in our study came from 120 women who had undergone surgery at our department between 1998-1999. None of the patients had been treated pre-operatively with endocrine therapy. Immunostaining for ERα, ERβ and PgR was performed using monoclonal antibodies against ERα, PgR (DakoCytomation), and against ERβ (CHEMICON). The EnVision detection system was applied. The data were analyzed using a nonparametric Fisher-Freeman-Halton test and log-rang test for disease-free survival (DFS) and overall survival (OS). The statistical significance was considered when p<0,05. Results: 55% of tumors were ERβ positive, 57.5% were ERα positive and 64% were PgR positive. As many as 14% of ERβ positive tumours had no expression of ERα. The expression of ERβ protein correlated significantly with ERα and PgR, and ERβ positive tumours were of a lower histological grade. There was no correlation between ERβ expression and tumour size axillary node involvement and age. Patients with tumours expressing ERβ had better DFS (8 years follow-up) and better OS. Seventy three patients were treated with adjuvant tamoxifen.. In that group DFS and OS were also better in patients with ERβ positive tumours. Conclusions: Expression of ERb was found in a significant proportion of breast cancers. It was also present in a noticeable proportion of ERα negative tumors which in the past would have been considered hormonal receptor negative. Further, the improved survival of ERβ positive patients treated with tamoxifen suggests that ERβ may be an independent predictor of response to hormonal therapy.

PP2-24

CD10, Ki67/MIB1, p53 EXPRESSION IN DIFFERENTIAL DIAGNOSIS OF BREAST CANCERS WITH TUBULAR COMPONENT

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CD10-positive cells have been reported in the stroma of various carcinomas such as prostate, breast, colorectal, and lung ones. CD10 positivity has also been reported in stromal myoepithelial cells from normal tissue and benign myoepithelial tumors of breast. Some reports indicated that stromal CD10 expression is associated with biological aggressiveness in various epithelial malignancies. Several reports denoted that the tubular cancer is a part of mixed types of breast neoplasms. AIM: The aim of presented study is evaluation of usefullness of CD10, p53 and Ki67/MIB1 expression in differential diagnosis of breasts cancers with tubular component and morphological evaluation of mixed tumors with that histological pattern. METHODS: In the years 1996 to 2006 in the 656 total mastectomy specimens, 41 (6,25%) cases with different forms of tubular component have been diagnosed. It should be stressed that in this group there were 1,1% „pure" tubular cancers. All these selected cases have been reevaluated. An immunohistochemistry evaluations with CD10, p53 and MIB1 have been also performed. RESULTS: These evaluated cases of cancers with tubular component include 22,8% „pure" tubular, 25,7% tubular mixed with cribriform intraductal cancer, 31,4% ductal infiltrative cancer with tubular component and 19,9% of other forms of mixed patterns . Only about 30% cases in this group were positive for Estrogen and Progesteron receptors. Stromal expression of CD10 has been observed in 80% of mixed type of cancer in contrast to „pure" form (about 50% positive for that antibody) and it was correlated with low expression of p53 (70% cases has less than 1% of p53 positive cells) and a low MIB1 positive cells rate. Positivity for CD10 of stromal cells correlates with tumor size, but there were no correlation with nodal status. CONCLUSIONS: Tubular cancer of the breast presents mixed pattern in majority of cases. Stromal expression of CD10 is rare in "pure" tubular cancer of the breast and does not correlate with p53 status. In 30% cases of tubular cancer expressions of Estrogen and Progesteron receptors have been noted.

PP2-25

CYCLIN D1 AND Ki-67 EXPRESSION IN COLUMNAR CELL LESIONS OF THE BREAST

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Introduction: Columnar cell lesions (CCLs) encompass a heterogeneous disorder that might progress to some forms of low grade invasive breast carcinomas. The aim of this study was to explore the biological potential of CCLs measuring its proliferative activity (via Ki-67) and cell cycle regulation (via cyclin D1). Material and methods: Forty four breast samples, diagnosed in period 2005-2007 at the Department of Pathology, Clinical Center of the University of Sarajevo, were randomly selected and retrospectively analyzed. All CCLs were categorized into: Columnar change disease (CCD), columnar cell hyperplasia (CCH) and flat epithelial atypia (FEA) as earlier proposed. For immunohistochemical purposes, monoclonal antibodies for cyclin D1 and Ki-67 were applied. All immunohistochemical staining were captured by Olympus Digital Camera and semiquantitatively measured as a percentage of positive cells. All samples were measured independently by three researchers and mean value was taken as a final result. Statistical Package for Social Sciences (SPSS, version 11.5, Chicago, IL, USA) was used for statistical analysis. Results: The lesions were distributed as follow: Columnar cell change (21 cases, 47.7%), columnar cell hyperplasia (20 cases, 45.5%) and flat epithelial atypia (3 cases or 6.8%) as earlier proposed. We found significant association between CCH and FEA and low grade breast carcinomas (p=0.008, Chi-Square test). Significant positive correlation was found between CCH and FEA and carcinomas (p=0.001, Spearman's rank Correlation coefficient, rs=0.474). Cyclin D1 expression was generally low in CCLs of the breast with mean value of 9.86% positive cells. Significantly higher expression of cyclin D1 was detected in CCH and FEA compared with CCD (p=0.013, Chi-Square test). Ki-67 expression was also very low with an average of 3.48% positive cells. Ki-67 had a higher positivity in CCH and CEA in comparison with CCD (p=0.012, Chi-Square test). There was also a trend toward positive correlation between cyclin D1 and Ki-67 expression (p=0.066, Spearman's Correlation Test). Conclusion: Significant positive correlation was detected between CCH and FEA and low grade invasive breast carcinomas. Cyclin D1 and Ki-67 expressions were generally low in CCLs of the breast. Both markers showed significantly higher expressions in CCH and FEA in relation to CCD. Therefore, attention has to be paid on CCH and FEA as potential precursors of low grade breast carcinomas.

PP2-26

GHRELIN EXPRESSION IN INVASIVE DUCTAL CARCINOMA OF THE BREAST

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BACKGROUND: Ghrelin, the endogenous ligand for the GH secretagogue receptor (GHS-R), has been primarily linked to the central neuroendocrine regulation of GH secretion and food intake, although additional peripheral actions of ghrelin have also been reported. Ghrelin is expressed in some malignant tumors, but little is known about the contributions of Ghrelin in breast carcinogenesis. The aims of this study were to investigate the expression of the Ghrelin in breast carcinomas and to compare the results with several established prognostic markers of breast cancer. METHODS: Formalin-fixed, paraffin-embedded tissue sections from 48 invasive ductal carcinomas were immunostained for Ghrelin, ER, PR and HER2/neu. Immunohistochemical staining was performed using the avidin-biotin complex with microwave antigen retrieval. The cytoplasmic immunostains for Ghrelin were scored using a four-scale (0 to 3+) system based on the percentage of cells stained and the intensity of staining. Ghrelin staining was compared to ER, PR, HER2 status, tumor size, histologic grade, and nodal status. The relationship between the clinical and histopathological markers was analyzed by chi-square test. RESULTS: All the patients were female, with a median age of 53 (24-77). The histological type in all cases was invasive ductal carcinoma, not otherwise specified. The majority of cases were graded as G2 (54%), 18% were G1 and 27% were G3. 14.6 % of the tumors were [LTEQ]2 cm in size, 60.4% were between 2 and 5 cm and 25% were more than 5 cm in size. Ghrelin was expressed in 37 (77%) of 48 breast carcinomas and demonstrated a diffuse cytoplasmic pattern of staining. Concerning the size of the tumor, Ghrelin positive were 81.8% of the tumors [LTEQ]2 cm, 45.5% of the tumors between 2 and 5 cm and 72.7% of the tumors more than 5 cm in size. Ghrelin positive were 63.6% of G1 carcinomas, 72.7% of G2 and 63.6% of the G3 breast carcinomas. 75% of the lymph node positive tumors were Ghrelin positive. Regarding the ER and PR

immunohistochemical expression, 72.9% (35/48) were concomitant Ghrelin and ER positive, 81.3% (39/48) were Ghrelin and PR positive and 54.2% (26/48) were Ghrelin and HER2/neu positive cases. CONCLUSION: The present data suggest that Ghrelin was expressed in invasive ductal carcinomas. The majority of our Ghrelin positive carcinomas were well and moderately differentiate, 72.9% of ER positive, 81.3% of PR positive carcinomas. Our results suggest that increased expression of Ghrelin may be involved in the carcinogenesis of the breast and may be a potential therapeutic target.

PP2-27
THE PREVALENCE OF BREAST CANCER IN UNIVERSITY HOSPITALS IN AHVAZ FROM 2001-2002
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Breast cancer is the most common organic malignancy in women and is the second cause of death after lung cancer. Early diagnosis for breast cancer is important because if the stage is lower when cancer was diagnosed, prognosis will better. The propose of this study is demonstrating of breast cancer stage and determine it's relation with age and histological type of tumor. Method: This study was performed cross-sectional on pathology Result of Mastectomy in pathology laboratory in teaching centers of Ahvaz University of medical science at 2001 and 2002. We used Fisher's test and chi-square test and spss software for statistical analysis. Results: According to this study, the most common type of carcinoma is undifferentiated carcinoma (84/4% of cases). The most common stages are III a (%30 of cases), III b (%27/3 of cases) and the rarest stage is stage I (%3 of cases). In undifferentiated carcinoma, the most common stages are III a,II a and III b respectively. In papillary carcinoma, stage II a and III a each include 50% of cases. There is one case of medullary carcinoma with stage II a, on case of Mucinous carcinoma with stage III b and one case of Puget's disease and undifferentiated carcinoma with stage III b and one case of Puget's disease and undifferentiated carcinoma with stage I. In age group of 50 and younger than 50 years, late stage (%68/8) is more than early stage (%33/3) and also in older them 50 years: the late stage (%58/8) is more common than early stage (%41/2) and there is no significant correlation between these age group with two stage groups (p.v>0/05). There is significant correlation between the early stage and late stage with skin metastasis (p.v=0/02) but there is no significant relation with vascular metastasis (p.v>0/05). Conclusion According to this study, In breast cancer high grade stages are common is young ages, There fore, screening programs must be consider and instruct women about the important of breast examine and related symptoms must be instructed and should be perform mammography from 40 years old.

PP2-28
HISTOLOGICAL PROFILE, IMMUNOHISTOCHEMICAL EXPRESSION AND NOTTINGHAM PROGNOSTICATION INDEX (NPI) OF BREAST CARCINOMAS
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Background: To evaluate the histological profile, the immunohistochemical expression, the Nottingam Prognostication Index and their usefulness for the prognosis of female breast carcinomas. Method: 62 archival cases of breast carcinomas from our hospital, from the period 1997-2007 were reviewed. Clinical information were available for 37 cases. All cases were stained for ER, PR, c-erbB2, p53, ki-67, PS2, bax, bcl2, MDR and EGFR. The Nottimgham Prognostication Index of each case was evaluated from the grade, stage and the tumor diameter. Results: 15 carcinomas were located at the right and 22 at the left breast. The age of the patients ranged 23-86 years (median 56.7). 33/37 cases underwent mastectomy and lymph node dissectomy. 18 cases had lymph node metastases. The tumor diameter ranged 0.6- 7.5 cm. 34 cases were ductal carcinomas, 1 case was lobular carcinoma and 2 cases were mixed type. The ductular carcinomas were graded as grade I (15 cases), grade II (11 cases) and grade III (8 cases). The majority of cases were positive for ER or /and PR, bcl2 and MDR and negative for c-erbB2. Few cases showed overexpression of p53 and very high proliferating index and rare cases were positive for EGFR. PS2 and bax were variably positive. NPI ranged 2,2-7,5. According to NPI 13 cases were classified as having good prognosis, 14 cases as moderate and 6 cases as poor prognosis. The rest 4 cases did not get a NPI because there was no lymphadenectomy. All patients received adjuvant chemotherapy including anthracycline containing regimens. Node negative patients also received FEC while node positive ones received dose dense sequential chemotherapy with Epirubicin, Cyclophosfamide and Taxanes. One patient with a high NPI died of the disease 4 years after initial appearance. Two patients, one with a low and one with a medium NPI relapsed. Another patient with low NPI showed in situ carcinoma at the other breast. One patient with low NPI had bone metastasis and another one with a high NPI had liver metastasis. Conclusion: According to our results it seems that NPI is not an independent prognostic factor but it must be taken in consideration with the histological and immunohistochemical profile of a breast carcinoma to predict its possible biological behavior.

PP2-29
ANDROGEN RECEPTOR (AR) IS FREQUENTLY EXPRESSED IN BREAST CARCINOMA AND CO-EXPRESSED WITH ER AND PGR. A NEW MOLECULAR MARKER?
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Background: ER and PgR are important prognostic and predictive factors involved in development, growth and differentiation in breast carcinoma; related with low-grade tumors and inversely correlated with Her2/neu. AR has been studied recently as a new molecular marker in breast carcinoma, expressed approximately in 35-70%. We analyzed the expression of AR correlated with another prognostic molecular factors in breast cancer patients. Methods: We studied a group of 35 patients with diagnostic of ductal carcinoma, all the cases had histopathological confirmation. We assessed the expression pattern of AR and ER by immunoblotting and IHQ in primary tumor samples; also we analyzed the expression of PgR and Her2/neu by IHQ. Chi-square and log rank tests were used to determine differences between proportions of each AR and ER isoforms and mortality and survival distributions respectively. Results: The median follow-up was 56 months (1-77mo); the median age of the patients was 48 years (30-81yr). In 46% of the cases (16/35) AR was expressed. ERα and ERβ were expressed in 34% (12/35) and 54% (19/35), respectively. Seven of 35 (20%) patients over-expressed Her2/neu. Eleven of 35 (31%) cases over-expressed AR/ERα (P=0.0068) and 17/35 were AR/PgR (49%) (P=0.0437). Four of 35 (11%) patients developed metastases, all of them were positive for AR. Twenty of 35 (57%) patients are still alive and 10 of them (50%) were AR positive. Conclusion: In this study we demonstrated the frequent expression of AR in breast carcinoma (46%) and the co-expression with ER and PgR. AR may be a predictive factor for adjuvant treatment in patients with metastases. Further studies are needed to demonstrated clinical significance in these patients.

PP2-30

SECRETORY BREAST CARCINOMA PRESENTED WITH METASTATIC AXILLARY LYMPH NODE

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Background: Secretory mammary carcinoma is a rare breast neoplasia originally described in children but sometimes also found in adults, presenting at the average age of nine with range of 3 to 87. It presents a more favourable outcome than the more common histological types of breast carcinoma; published literature in fact reports only a few cases with axillary lymph node metastases and only four cases with distant metastases. We report a rare case of secretory breast carcinoma with axillary lymph node metastases. Case : 62 year old woman presented with palpable axillary lymph node. The histopathological examination of the incisional biopsy revealed a carcinoma with CK7, GCDFP, ER positivity and CK20, TTF-1, thyroglobulin negativity. After that, a mass smaller than 1 cm was recognized radiologically on the lateral quadrant of right breast. The mass was excised, frozen section was performed; sclerosing adenosis and ductular proliferation areas were seen in microscopic examination at frozen section. Modified radical mastectomy was performed. Grossly, tumor was 0.9 cm in greatest diameter with irregular outline, had a hard and gritty cut face and white to yellow color and was submitted completely. Microscopically the lesion showed solid nests consisting of small, uniform, well differentiated cells, with granular cytoplasm. Mitoses was absent and atypia was mild. Immunohistochemically the neoplastic cells showed a strong positive staining for alpha-lactalbumin and milk fat protein. They were positive for ER and S-100. So the histological and immunohistochemical findings of the permanent sections of the mass were consistent with secretory carcinoma. There was one metastatic lymph node in the axilla. Conclusion: Secretory breast carcinoma is known as a juvenile carcinoma but has been found in patients ranging from 3 to 87 years of age both in men and women. It is not a aggressive tumor and presents an excellent prognosis. It may be difficult on hematoxylin –eosin slides and even more difficult at frozen section to decide how much of the tumor is invasive and how much is in situ. Treatment should be as conservative as possible.

PP2-31

MUTATION ANALYSIS OF EXONS 1 AND 2 OF CTNNB1 (BETA CATENIN) IN INVASIVE BREAST CARCINOMAS

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Introduction: beta-Catenin has a crucial role in cell–cell adhesion as well as a signaling role as a member of the Wnt pathway. Mutations in the beta-catenin gene have been found in numerous cancers; however, in breast and prostate cancer, mutations of beta-catenin have been found to be infrequent. The aim of this study was to identify new mutations in exons 1 and 2 of the beta catenin gene and correlate these with the clinicopathological parameters and overall survival of the patients in breast cancer. Materials and Methods: A total of 36 paraffin-embedded breast tissue specimens were available from patients with clearly invasive breast carcinomas, aged 25–84 years (mean age 56.43 years). DNA extraction from paraffin embedded tissues was performed using the Qiamp DNA Mini Kit (Qiagen) according to manufacturer's instructions. 10ul of the purified DNA were used as template for the PCR reaction using Platinum Pfx read-proof DNA polymerase (Invitrogen) and the following primers for exon 1:forward 5'ATTTTAAGCCTCTCGGTCT3',reverse 5'AAGCACCTCAGGGGAACA3', and for exon 2: forward 5'CAGGTATCCCAGTGACTTAGGAG3',reverse 5'GCAGAGCCCCAATTCAGTAAC3'. The amplified bands were gel extracted and sequenced using the same primers in an ABI PRISM 3100 Avant DNA sequencer (Applied Biosystems). Results: For exon 1 mutations were detected in 13 out of 36 samples (36%) and for exon 2 mutations were detected in 21 out of 36 samples (58,3%). The mutations comprise of point substitutions, missing bases, and a probable increase in the beta catenin gene copy number/cell. Specifically for exon 2 a certain point substitution at position 405 that corresponds to the 2nd intron of the gene (GenBank Accession Number X89579) was detected in 19 out of 21 mutated genes (90,47%) and seemed to correlate with lower cytosolic localization of beta-catenin in cancer cells ($p=0.009$, $\chi 2$ test). No correlation was observed with clinicopathological parameters and patients' survival. Discussion: This is the first report of mutations of beta-catenin gene in breast cancer. Previous work concerning mutations of beta-catenin and breast cancer was limited to the mutation detection in the 3rd exon of the gene. We have mapped the 405 point mutation in the second intron of beta-catenin gene but in order to make any correlation with clinicopathological parameters or any biological markers we need a larger line of patients.

PP2-32

PREVALENCE OF HUMAN PAPILLOMA VIRUS IN BREAST LESIONS IN MEXICAN PATIENTS

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Background: Breast cancer is the second most common female cancer in Mexico. Some classical risk factors are well recognized; nevertheless up to 80% of patients with this tumor do not have any recognizable risk factor. In the world, there is some evidence of an association between breast cancer and viruses, such as human papilloma virus and mouse mammary tumor virus (MMTV). At present time there is no report of detection of HPV sequences in mexican patients with breast cancer. Materials and Methods: From January 1999 to December 2003, 65 cases of breast cancer were selected from the pathology files. One representative paraffin block was selected, HPV DNA was analyzed by polymerase chain reaction (PCR) and sequenced for different types of HPV. Of all selected cases 51 specimens showed DNA integrity by b-globin amplification. These cases were matched by age and size with 51 cases of benign conditions (fibroadenoma, fibrocystic disease and phyllodes tumor) and HPV DNA was analyzed by polymerase chain reaction (PCR). Descriptive analysis of clinical and pathological variables was performed and comparisons between positive and negative cases were done. Results: Of all selected cases with breast cancer 51 specimens were evaluated, mean age was 53.3, cervicovaginal cytology was evaluated and only 1 patient (1.5%) had HPV no cases were diagnosed with cervical dysplasia. 92.3% of tumors were ductal carcinomas, mean tumor was 9.4cms, SBR 8 and 9 were 11(16.9%) and 10 (15.4%), respectively. 36 (70.5%) were negative to HVP and 15 (29.4%) were positive, when typing was performed, 10(66.6%) were positive for HPV 16, 3(20%) for HPV 18, and two cases (13.3%) were positive for HPV 16 and 18. When comparisons between HPV positive and negative were done in relation to all clinical and pathological variables the only one which was significant was tumor size, since only one tumor larger than 4cms was positive for HPV (p=0.008). In the group

of benign conditions all were negative to HPV-DNA Conclusions: A high prevalence of HPV in breast tumors in Mexican woman was found, similar to other reports in the literature, the most common type was HPV 16. Tumors larger than 4 cms rarely have HPV DNA, this feature is similar to large carcinomas of uterine cervix. Study of a larger series of cases need to be analyzed in order to establish the exact role of HPV virus in the pathogenesis of breast cancer.

PP2-33
IMMUNOHISTOCHEMICAL DEFINITION AND PROGNOSTIC SIGNIFICANCE OF SPORADIC BASAL-LIKE BREAST CARCINOMAS. A GRADE 3 COHORT OF PATIENTS.

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Introduction: Breast basal-like tumors are high grade carcinomas with a charactheristic morphologic appearence and agressive clinical behaviour. Although there are still not universal accepted immuohistochemical definition for these subgroup of tumors, Nielsen et al using a panel of four antibodies (ER, HER1, HER2 and cytokeratin 5/6) could identify them with a specificity of 100% and a sensitivity of 76%. Nevertheless, it is still unclear whether these tumors maintain their properties when compared to a similar grade matched subgroup of tumors. Aims: 80 grade 3 breast carcinomas were subclassify using expression profiles studies into different subgroups. First of all, we tested the tumors included into the basal-like subgroup for Nielsen et al immunohistochemical profile. Secondly, we tried to identify an immunohistochemical panel which higher sensitivity testing 30 immnuohistochemical markers using TMA. Moreover, we evaluated the prognostic significance of our findings. Results: Data still being evaluated.

PP2-34
ACINIC CELL CARCINOMA OF THE BREAST

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Background: Acinic Cell Carcinoma of the breast (ACC) is the rare counterpart of a similar tumor that occur in the salivary glands. It was described quite recently by Roncaroli et al. in 1996 and has a unique immunohistochemical profile. Eleven cases have been reported in the international bibliography so far. We present a very interesting new case. Methods: A 65-year old patient was presenting an asymmetric density during the repeating mammographic screening the past 5 years. The last mammography of the right breast showed an area of distorted architecture and a stellate radio-opaque change in its center. The FNA examination was positive for adenocarcinoma and the patient was treated by a modified radical mastectomy. Grossly, the tumor measured 7cm, with infiltrative margins, whitish to greyish-pink pallor and a consistency that varied from outer soft to inner hard. Results: Histologically, the core of the stellate lesion consisted of pleomorphic tumor cells embedded into an exuberant desmoplastic stroma (md: 1.5cm), while the surrounding projections consisted of a ductal type adenocarcinoma with nodular, solid, microglandular tumor nests or combination of these patterns. These nests of neoplastic cells were delimited by a network of thin connective tissue. The tumor cells were strikingly clear in most areas or had an abundant granular eosinophilic cytoplasm. Clear cells were also noted in numerous foci of lobular cancerization.The glandular structures were filled with eosinophilic PAS positive material. The nuclei were round and irregular with a single eosinophilic nucleolus. The 26 excised lymph nodes had no metastases. The immunohistochemical profile of the tumor showed intense staining with S-100, EGFR, CK7, EMA and PAS. Ki67 and p53 were positive (>10-49%, 80% respectively). ER, PR receptors, SMA and HER-2 neu were negative as well as the GCDFP-15 protein. Conclusion: In our case was a pure ACC coexisted with a small NOS and had all the morphological and immunohistochemical characteristcs which were identical to that of the acinic cell carcinoma of the salivary glands. Differential diagnosis involved microglandular adenosis, adenomyoepithelial carcinomas, secretory and glycogen-rich adenocarcinomas. On conclusion, the rarity of ACC, its excellent prognosis, the low rate of lymph node metastases and the good response to neoadjuvant chemotherapy, make its diagnosis important.

PP2-35
ANALYSIS OF ESTROGEN RECEPTOR POSITIVE (ER+), PROGESTERON RECEPTOR NEGATIVE (PR-) PHENOTYPE AND HER2 STATUS IN BREAST CANCER (BC) PATIENTS

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BACKGROUND: The most important morphological prognostic factors in BC are tumor size, axillary lymph node involvement and histological grade. The main non morphological prognostic factor is hormone receptors and HER2 status.HER2 overexpression usually correlates to a poorer prognosis and higher metastatic risk. ER and PR expression are the only predictive factors with proven usefulness in selecting patients who are likely to respond to adjuvant endocrine therapy. Various clinical studies indicate that patients with ER+/PR- BC have a different HER2 phenotype possibly resistant to tamoxifen therapy.
MATERIALS AND METHODS: From December 2005 to April 2007, two hundred and fifty nine BC evaluated using immunohistochemistry (IHC)for ER, PR and HER2 receptor. HER2 expression was interpreted as negative(0,1+) or positive(2+,3+). In addition, six HER2 2+ BC were also evaluated using chromogenic in situ hybridization (CISH). Independent variables such as age, tumor size, nodal status, grading and histological type were also included in the analysis.
RESULTS: Of the 259 cases, 137 (52,9%) BC were ER+/PR+/HER2- , 44 (17%) were ER-/PR-/HER2-, 28 (11%) were ER-/PR-/HER2+, 11 (4,2%) were ER+/PR+/HER2+, 3 (1,1%) were ER-/PR+/HER2- and 36 (13,9%) were ER+/PR-. Seven (19,4%) of the 36 ER+/PR- BC showed HER2 3+ membrane staining, 23 (63,9%)HER2 0 and 1+, and 6 (16,7%) showed HER2 2+ membrane staining. For HER2-2+ by IHC, two (2/6) were positive by CISH and both patients had poorly differentiated ductal BC. In the group ER+/PR-/HER2 3+, all tumors were ductal histological type, 4 (57,1%) were grade 2 and 3 (42,9%) were grade 3. Median age was 56 (range 44-67), median tumor size 36mm (range 23-54) and 5 (71,4%) patients had positive lymph nodes. In group ER+/PR-/HER2– BC, the majority of tumors were grade 1 (30,4%) and 2 (60,1%),and the commonest histological types were ductal (47,8%) and lobular (34,8%). Median age was 64 (range 49-76), median tumor size 34 mm (range 14-68) and 11 (47,8%) patients had positive lymph nodes. CONCLUSION: ER+/PR- status does not preclude a positive HER2 status. 19,4% of the ER+/PR- BC showed overexpression HER2. BC with ER+/PR-/HER2+ phenotype had higher histological grade and bigger stage of the disease than ER+/PR-/HER2- BC.

PP2-36
CHONDROLIPOMA OF THE MALE BREAST
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Background :Cartilage is the most often found component in malignant mesenchymal and epithelial tumors of the breast. The benign cartilage containing tumors of the breast are very rare. Chondrolipomas are benign lesions consisting of fat, cartilage and fibrous tissue. They are well circumsribed, firm nodules that clinically resemble fibroadenomas. The question of whether these lesions are neoplasms, hamartomas or choristomas is unsettled. Case presentation : We present a case of well circumscribed, painless breast mass in a 60 year old man. A few cases of chondrolipomas in female patients have been reported so far. To our knowledge, this is the first case in a man to be reported in English literature.

PP2-37
AN IMMUNOHISTOCHEMICAL EVALUATION OF CD44+/CD24-/LOW CELLS IN INVASIVE BREAST CANCER: CORRELATION WITH ANGIOGENETIC PHENOTYPE
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BACKGROUND: CD44+/CD24-/low breast cancer cells (cells with strong expression of CD44 adhesion molecule and no/very low expression of CD24 adhesion molecule) resemble normal stem cells with respect to their ability to self-renew, to proliferate and to differentiate into various cell types. Previous studies showed the ability of CD44+/CD24-/low cells to form new tumors in mouse models. In human breast cancer, the identification of these tumorigenic cells is crucial for the development of effective targeted therapies. The aim of our study was to identify CD44+/CD24-/low cells in invasive breast cancer and to correlate them along with classical clinicopathological parameters and angiogenetic factors. METHOD: Double-staining immunohistochemical technique was performed on paraffin-embedded tissue specimens from 138 invasive breast carcinomas to detect the CD44+/CD24-/low cells. CD44+/CD24-/low expression was assessed in combination with classical clinicopathological parameters (menopausal status, histological type, tumor size, stage, nuclear and histological grade, lymph node metastases), ER and PR receptor status, p53, Ki67, c-erbB-2, VEGF-A, VEGF-C, Flt-1 and Flt-4. The results were estimated by automated image analysis and statistically processed using chi-square test. Overall and disease-free survival distribution curves were assessed by Kaplan-Meier test and log-rank statistics. RESULTS: The phenotype CD44+/CD24-/low ranged from 0% to 70% in breast cancer specimens. The prevalence of CD44+/CD24-/low cells was ≤10% in the majority of cases (85.2%) and >10% in 14.8% of cases. There was no significant correlation between CD44+/CD24-/low prevalence and clinicopathological parameters, biological markers (ER, PR, p53, Ki67, c-erbB-2) as well as overall and disease-free patients' survival. CD44+/CD24-/low phenotype was associated with VEGF-C and Flt-1 ($p=0.028$ and $p=0.023$ respectively), while no relationship was found between CD44+/CD24-/low phenotype and VEGF-A or Flt-4 expression. CONCLUSION: CD44+/CD24-/low tumor cells are associated with Flt-1 and VEGF-C. Both of them are well known angiogenetic and lymphangiogenetic factors contributing to tumor metastasis. In a recent study (Abraham et al., 2005) breast cancer stem cells were found to be associated with distant metastases. However, this observation needs further investigation in order to clarify the role of these cells in the metastatic process.

PP2-38
HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILES OF BREAST CANCER IN POSTMENOPAUSAL WOMEN
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Background: Breast cancer in the postmenopausal women is a common disease that is ussualy undertreated compare to younger pacients. The proportion of estrogen receptor-positive tumors is increased in postmenopausal women. Although the observations support the perception that breast carcinoma tends to have less aggressive biologic features and a more favorable clinical course in the elderly, there are no significant differences in prognosis for patients younger than 35 years and older than 75 years, on the basis of tumor stage. Material and method: We retrospectively analyzed 120 cases of breast cancer in women between 50 and 85 years. All H&E sections from the cases were reviewed and specific changes were classified. Selected blocks were immunohistochemically evaluated with the following markers: estrogen receptor (ER), progesterone receptor (PR), HER2/neu, E-cadherin, and pan-cytokeratin. Results: There were 46.66% invasive ductal carcinomas, 18,33% invasive lobular carcinomas, 2.5% (3 cases) in situ ductal carcinomas, 1.66% (2 cases) lobular intraepithelial neoplasia (LIN), and 30.83% other types. From the last cathegory, the entities were represented, with decreasing frequency, by medullary carcinoma, mucinous carcinoma, Paget disease, inflammatory carcinoma. 15.45% presented pre-operative core biopsies and frozen sections were performed in 18.66% of cases. Intra-operative diagnoses corresponded to subsequently histopathological diagnoses. 26.66% of cases presented axillary lymph node metastases and 2.5% of cases presented ovarian metastases. Conclusions: Our data show that the incidence of breast carcinoma in postmenopausal women is increased compare to younger patients. Although invasive lobular carcinoma is relatively more common in elderly women, in our study the predominant histological type was invasive ductal carcinoma. The patients were generally diagnosed as T3 and T4. Because most of them had estrogen-receptor positive tumors, they could benefit to an adjuvant tamoxifen therapy. The proportion of estrogen and progesterone receptor-positive tumors did not increase significantly in postmenopausal women older than 65 years, fact which is in concordance with the existing data from the literature.

PP2-39
PERIPHERAL MULTIPLE RECURRENT PAPILLOMAS OF THE BREAST. A CASE REPORT
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Introduction: Peripheral multiple breast papillomas are benign lesions which are often clinically occult. They are defined as papillary projections within dilated lumina of terminal ductules lined by epithelial and myoepithelial (ME) cells. They are frequently associated with atypical ductal hyperplasia (ADH), and have a significant risk of carcinoma. Case history: A 52-year-

old female underwent a left-sided mastectomy for recurrent (four) multiple intraductal papillomas in spite of free surgical resection margin (with 20 years of follow up). All histologic sections were assessed for the presence of coexisting fibrocystic lesions, including florid ductal hyperplasia and ADH. The retrospective study of Mib1 prolifrative index and DNA ploidy on several samples revealed evolutive lesions, which could be predictive of a malignant degeneration. The immunohistochemical study shown the following results in ME cells: CK5/6+++, Actin++, P63+++, and CD10++, and epithelial cells CK7+++, CD10++, ER++, PR++, Bcl-2++, cyclin D1+++, c-erB-2-. The foci of ADH were faintly positive with ME markers cyclin D1 and p53. Conclusion: This observation illustrate that the immunophenotype of the papillary lesions of the breast is a useful tool in anticipating the invasive carcinoma and in differentiating between papillomas and invasive carcinomas.

PP2-40
GASTROINTESTINAL METASTASES OF BREAST CANCER
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Background: Gastrointestinal (GI) metastases of primary invasive breast cancer are rare, and they often mimic primary carcinomas of the GI tract. Breast cancer can metastasize to any part of the GI tract. Distinguishing metastatic disease from a second primary tumor can become a challenging issue from time to time, however, it has great significance for management of patients. Design: We identified 55 patients with metastatic breast cancer to the GI tract, treated at M.D. Anderson Cancer Center between 1986 and 2007, and reviewed the clinicopathologic features of these patients. In selected cases, immunohistochemistry for estrogen receptor was performed. Results: The median patient age at the time of GI metastasis was 57 years (range 31–81). Primary diagnoses included invasive ductal (46%), invasive lobular (40%), and mixed invasive ductal and lobular (7%) carcinomas. In 7%, the histologic type of primary breast cancer was unknown. In 64% of patients, GI tract was the first distant metastatic site, as part of systemic metastatic disease. The metastatic sites included stomach (44%), small intestine (31%), colon/rectum (38%), liver (11%) and the appendix (2%). Histologically, 56% of the metastatic sites had poorly differentiated carcinoma, and 31% had lobular carcinoma with focal signet ring cell morphology. Transmural involvement of the GI tract was identified in 16% of the cases. 78% of metastatic tumors were positive for estrogen receptor. Conclusion: Stomach is the most common site for GI metastases of primary invasive breast cancer, and lobular histology is the most common histologic type. Metastatic tumors commonly have features of poorly differentiated carcinoma, and most of the tumors are estrogen receptor positive. In patients with the diagnosis of breast cancer, estrogen receptor staining may be helpful to determine primary versus metastatic nature on GI biopsies.

PP2-41
ASSOCIATION BETWEEN HER2 STATUS AND HORMONE RECEPTORS IN INVASIVE BREAST CARCINOMA
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Background: Clinical experience indicates that efficacy of endocrine treatment invasive breast carcinoma (IBC) is impeded in tumors that express estrogen receptors (ER) at low levels, those that lack progesterone receptors (PR), and in HER2 positive disease. Data on quantitative associations between ER, PR and HER2 in IBC is scarce and our aim was to analyze these relationships. Methods: A total of 1457 consecutive primary IBC were analyzed. HER2 was assessed by IHC (HercepTest™) and FISH (PathVysion™). Strong protein expression (3+) and/or gene amplification indicated positive HER2 status (HER2+). HR were evaluated by IHC and tumors were categorized in three ways based on: 1. cut-off ≥10% as ER+/- and PR+/-, 2. semi-quantitatively evaluated HR expression, in 6 groups (1: 0%, 2: 1–9%, 3: 10–40%, 4: 41–60%, 5: 61–80% and 6: 81–100% positive cells) and 3. joint HR expression, in 4 groups: ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR-. Results: 14.6% of tumors were HER2+. Among ER- tumors there was no significant difference in proportion of HER2+ cases between groups 1 (39.1%) and 2 (37.0%); on the other hand, among ER+ cases there was a significant continuous decrease of HER2+ cases from group 3 (22.4%) to group 6 (8.0%; $rs = -0.137$, $p < 0.001$). In addition, in ER+/HER2+ tumors, the average level of HER2 gene amplification showed a steady decrease from group 3 to group 6 ($rs = -0.279$, $p = 0.03$). Among PR- tumors, the proportion of HER2+ cases was significantly higher in group 1 (31.9%) than in group 2 (14.8%); among PR+ tumors the decrease of HER2+ cases from group 3 (14.7%) to group 6 (6.4%) was also significant ($rs = -0.130$, $p < 0.001$). In PR+/HER2+ tumors, a decrease in the average level of HER2 gene amplification from group 3 to group 6 was also observed ($rs = -0.186$, $p = 0.05$). 9.2% of ER+/PR+ tumors were HER2+. This proportion was significantly higher in ER+/PR- tumors (16.2%) and was highest in ER-/PR- (38.5%) and ER-/PR+ tumors (41.2%); the difference between the last two groups was not significant. Conclusion: This report presents, for the first time, results on association between HR status of IBC evaluated semiquantitatively by IHC, and HER2 status evaluated both by FISH and IHC. In IBC, HER2 positivity is closely associated with absence and lower levels of ER and PR expression, which might explain the relative resistance of HER2+ tumors to hormonal therapy. In addition, level of gene amplification is inversely related to level of ER and PR expression.

PP2-42
MORPHOLOGIC CHARACTERIZATION OF THE ESTROGEN RECEPTOR-NEGATIVE AND THE ESTROGEN-RECEPTOR POSITIVE INVASIVE DUCTAL BREAST CARCINOMAS
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Background: Estrogen receptor (ER) status is a prognostic factor for patients with breast cancer, as well as a therapeutic one. ER-negative ductal breast carcinomas are usually poorly differentiated and carry a poor prognosis. The aim of this study was to assess the morphological characteristics and immunohistochemical profile of ER-negative and ER-positive tumors, in an attempt to understand the different nature of these lesions. Methods: In total, 120 cases of invasive ductal breast carcinomas, 31 (25.8%) ER-negative, and 89 (74.2%) ER-positive, as assessed immunohistochemically, were included in this study. The clinicopathologic characteristics analyzed were the size of the tumor, axillary lymph node status, tumor grade, lymphoid stroma, comedo-type necrosis, central tumor necrosis/fibrosis, p53, Ki-67, Her-2/neu, angiogenesis, and patient age. Results: ER-negative status was seen in 31 (25,8%) cases. ER-negativity was present in 12 of 63 T1 lesions (19%), in 11 of 44 T2 lesions (25%), in 3 of 7 T3 lesions (43%), and in 5 of 6 T4 lesions (83%), ($p<0.01$). ER- negative status was seen in 12 of 21 grade III tumors (57%), in 18 of 72 grade II tumors (25%), and in 1 of 27 grade I tumors (4%), ($p<0.01$). ER-negative tumors showed Her-2/neu protein overexpression in 14 (45%) cases, whereas ER-positive tumors showed Her-2/neu protein overexpression in 9 (10%) cases ($p<0.01$). ER-negative status was seen in 29 of 93 high Ki-67 tumors (31%), whereas ER-negativity was detected in 2 low Ki-67 cases (7%). ER-negative tumors showed p53 protein expression in 14 (52%) cases, whereas ER-positive tumors showed p53 protein expression in 13

(14%) cases. The presence of comedo-type tumor necrosis and lymphoid infiltration were associated with ER-negativity with statistical significance ($p<0.05$ and $p<0.01$, respectively). Conclusion: ER-negative status was significant associated with some poor prognostic tumor characteristics such as large tumor size, high histologic grade, Her-2/neu protein overexpression, high Ki-67 proliferative index, p53 protein expression, comedo-type tumor necrosis, and lymphoid infiltration. There was no association with patient age, lymphonodal status, central tumor necrosis/fibrosis or angiogenesis.

PP2-43

SECRETORY BREAST CARCINOMA IN A 48 YEAR OLD FEMALE

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Introduction Secretory breast carcinoma (SBC) is considered to be a subtype of infiltrating ductal carcinoma of the breast. It is a quite rare (<1% of infiltrating carcinomas) low grade breast carcinoma, composed of cells with abundant granular or clear vacuolated cytoplasm. Historically, this carcinoma has been named as "juvenile breast carcinoma" or "juvenile secretory carcinoma", because it usually develops in females under the age of 30. Only a few cases of secretory breast carcinoma have been reported in the medical literature. Case Report At the pathology department four segments of mammary parenchyma, measuring 2.3x2x1.5cm, 2x1.5x1.3cm, 1.8x1.2x0.7cm and 2x1.4x0.8cm respectively, were sent from a 48 year old Greek female. Dissecting the mammary parenchyma segments, four solid tumors were detected measuring 1.5cm, 0.4cm, 0.6cm and 0.6cm in diameter respectively. The diagnosis was secretory breast carcinoma. As far as immunohistology is concerned, a full set of tests was performed. Estrogen Receptor (ER) and Progesterone Receptor (PR) markers were highly (in a few, scattered cells) and moderately (in 20-25% of cells) expressed, respectively. Special staining for protein p53 (D07) showed moderate to high expression (10-15% of the cell population). Oncoprotein c-erbB-2 (CB11) staining was negative. MIB-1 index revealed that 5-10% of the cell population was actively dividing. Staining for protein B-Cell Lymphoma 2 (BCL-2) and Epidermal Growth Factor Receptor (EGFR) oncogene (31g7-Zymed) were negative and positive respectively. Conclusions Secretory breast carcinoma (SBC) is associated with a better prognosis compared to the other types of invasive ductal carcinomas (even when axillary metastases are present). Treatment involves simple mastectomy and axillary dissection. The prognosis seems to correlate with age. The younger population has a 100% survival rate at 5-years, whereas the adult population with SBC has a much poorer prognosis on par with infiltrating ductal carcinoma. Unfortunately, due to the fact that our case is recent, we cannot comment on its survival and its course. Size and extent of infiltration may be the most important determinants of metastatic potential in adults.

PP2-44

CARCINOSARCOMA OF THE BREAST - CASE REPORT

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Breast carcinosarcoma (BCS)is very rare mixed epithelial/mesenchymal metaplastic tumor. It appears in <0,1% of all invasive mammary carcinomas. Most of the patients with BCS are postmenopausal (70%) with average age at presentation of 55 years. In the present case is described a 56 years old woman with two well circumscribed palpable masses in upper outer quadrant of the right breast. They measured 1-2cm in diametar.On cut surface they were firm and white. Microscopic examination revealed an invasive well to moderate differentiated ductal carcinoma with malignant proliferation of spindle cells. Mitotic activity was 8-10mt/10hpf.On IHC analysis the carcinomatous component of the tumor showed intense reactivity for EMA and Polycytokeratin(MNF116). The sarcomatous component showed Vimetin, SMA and MNF116 strong positivity. Immunoreactivity for NSE and S100 protein was strong too.The immunoreactivity of the p53 gene product was positive in more then 60% of the tumor.The same IHC results were present in metastatic tumor tissue in axillary lymph nodes.IHC analysis of HER2/neu, ER+ and PR+ receptors was negative. The patient received adjuvant chemotherapy and radioactive therapy too. She developed diseminated metastatic disease after four years time period and died. In conclusion, BCS is very aggressive tumor especially because of infiltrative high proportion of metaplastic mesenchymal stromal element, probably of myoepithelial origine showing typical S100 and NSE immunoreactivity.

PP2-45

PRIMARY BILATERAL ANGIOSARCOMA OF THE BREAST: CASE REPORT

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Angiosarcoma of the breast is a rare and aggressively malignant tumor with high mortality rate and a very poor prognosis.This tumor tends to grow very quickly and is generally difficult to treat successfully. This is a case report of a 37-year old woman who had primary bilateral angiosarcoma of the breast. The patient presented with a lump in her left breast, which she had noticed three months earlier. The tumor was located at the outer upper quadrant. An incisional biopsy showed moderately differentiated angiosarcoma, which had already infiltrated the surrounding breast and fibrofatty tissue. Mastectomy was performed without axilary dissection. No further therapeutic procedure was undertaken. Two years later the patient noticed a lump in the upper inner quadrant of her right breast. The lump was sonographically and mammographically detectable. Tumorectomy was performed. Histopathologic analysis showed a well differentiated angiosarcoma. Immediate mastectomy was suggested to the patient, but she didn't accept it. A year later the patient noticed a lump under the postoperative scar in her right breast. Biopsy revealed a local recurrence of the tumor with a well differentiated angiosarcoma pattern. Radical mastectomy with large excision of the great pectoral muscle and dissection of the axillary lymph nodes was done. The pectoral muscle was partially infiltrated. Two years after the operation, the patient got pregnant and unfortunately there was a relapse of the illness. She decided to bring the pregnancy to term. Multiple bone and right ovary metastases were detected. Four months after the metastasis was found on the right ovary an ipsilateral adnexectomy was done. After the operation she got six lines of chemotherapy. Five months later craniotomy and cranioplasty followed due to local recurrence. The patient died two months later. Early diagnosis, surgical treatment and low histological grade play an important role in survival, whereas the benefit of adjuvant therapy remains to be demonstrated. The decision to treat should be preceded by a preoperative multidisciplinary assessment.

PP2-46

COMPARISON OF HER-2, EGFR AND CYCLIN D1 IN PRIMARY BREAST CANCER AND PAIRED METASTATIC LYMPH NODES: AN IMMUNOHISTOCHEMICAL AND CHROMOGENIC IN SITU HYBRIDIZATION STUDY

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The significant advance in the development of molecular-targeting drugs has made an evaluation of Her-2, EGFR and cyclin D1 an important clinical issue in breast cancer patients. This study compared the Her-2, EGFR and cyclin D1 statuses of primary tumors as well as their matching lymph node metastases using immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) in 73 breast cancer patients. Her-2, EGFR and cyclin D1 protein showed concordance between the primary lesion and the metastatic regional lymph nodes in 82%, 90% and 60%, respectively. CISH also revealed 92%, 93% and 85% concordance in the gene amplification status of the Her-2, EGFR and cyclin D1. There was a reasonable correlation between amplification of the Her-2, EGFR and cyclin D1 genes in axillary metastatic lymph nodes and the amplification of these genes in primary tumors. On the other hand, in terms of the expression of their protein, there was statistically significant agreement in only Her-2. We conclude that Her-2 status can be reliably assessed on primary tumor but a possible difference in EGFR and cyclin D1 status between the primary and the metastatic sites would be concerned for breast cancer patients considering molecular targeting therapy.

PP2-47

DNA PROMOTER HYPERMETHYLATION OF BLU GENE IN INVASIVE BREAST DUCTAL CARCINOMA IN TUNISIA

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Background: Many distinct regions of chromosome 3p shows frequent allelic losses in a wide range of tumor types comprising breast cancer, suggesting the presence of several tumor suppressor genes in these regions. The BLU candidate tumor suppressor gene located in a gene-rich critical deleted region in 3p21.3 was found to be inactivated by promoter CpG islands hypermethylation in several human tumors. Data about the frequency of hypermethylation of the promoter of BLU gene in breast cancer are largely lacking. The present study was undertaken to evaluate the prevalence and the clinicopathological significance of hypermethylation of BLU promoter in breast cancer. Methods: DNA extraction, bisulfite treatment, and methylation specific polymerase chain reaction (MS-PCR) was performed on 70 frozen tissues obtained from patients with invasive ductal carcinomas of the breast collected in the laboratory of pathology, Farhat-Hached Hospital, Sousse, Tunisia. Both specific methylated and unmethylated primers were used for PCR and the products were visualized with agarose gel electrophoresis. Results: Promoter hypermethylation of BLU was found in 28 (40%) out of 70 breast cancer samples. No correlation was found between hypermethylation of BLU promoter status and clinicopathological parameters including age, tumor size, lymph node metastasis, histological grade, and immunohistochemical status of estrogen and progesterone receptors. Conclusion: This study shows that hypermethylation of BLU promoter is frequent in breast cancer. Further analysis of a much larger number cases with clinical follow-up are needed to clarify the prognostic value of promoter methylation of this gene and to clarify its role in breast cancer.

PP2-48

IMMUNOHISTOCHEMICAL AND MORPHOLOGIC FINDINGS IN COLUMNAR CELL LESIONS COEXISTING WITH INVASIVE BREAST CARCINOMAS

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AIM: Columnar cell lesions (CCL) of the breast are classified as with atypia and without atypia. CCL with atypia (flat epithelial atypia) is considered as DIN (ductal intraepithelial neoplasia) 1a. CCL with or without atypia frequently coexist with invasive or in-situ carcinomas. In this study, CCL coexisting with invasive breast carcinomas are investigated. Expression of estrogen receptor (ER), progesterone receptor (PR) and p53 antibodies in CCL and coexisting invasive tumors, type of invasive tumor, histopathological grade and presence of atypia in CCL have been studied. MATERIALS AND METHODS: Thirty-nine mastectomy specimens containing CCL's coexisting with invasive carcinomas were retrospectively analysed for cellular characteristics and structural pattern of CCL neighboring the tumor. Histopathological type-grade of invasive tumor and existence of atypia in neighboring CCL have been identified. RESULTS: Histopathological types of carcinomas and coexisting CCL atypia existence are given as follows: 21 were with invasive ductal carcinoma, 7 of which were CCL with atypia and 14 without atypia, 7 were with invasive lobular carcinoma, 3 of which were CCL with atypia and 4 without atypia, 4 were with mixt carcinoma, 2 of which were CCL with atypia and 2 without atypia, 5 were with tubular carcinoma, 3 of which were CCL with atypia and 2 without atypia. Eight of the invasive carcinomas were ER (-). However, in 6 of them coexisting CCLs were ER(+) and without atypia. Coexisting CCLs in the other two invasive carcinomas were with atypia and one was ER (+) and the other was (-). PR was (-) in 4 invasive carcinomas. All of the coexisting CCLs were PR (+) and 3 were without atypia and 1 was with atypia. Two of the CCLs neighboring PR(+) invasive carcinomas were PR (-), one of them with atypia and one without atypia. p53 positivity rate in invasive carcinomas was 33% (13 cases). Among those, 6 had coexisting CCL with atypia (46%) and 7 had coexisting CCL without atypia (54%). P53 expression was present only in 2 (9%) of all 23 CCLs without atypia and 1 (6%) of all 16 CCLs with atypia. CONCLUSION: CCL with atypia was determined frequently in tubular carcinoma 60%, mixt carcinoma %50, invasive lobular carcinoma %43 and invasive ductal carcinoma %33. Correlation of histopathological grade and existence of atypia in CCL has not been observed. ER and PR expressions in CCL was not correlated with ER and PR expressions in coexisting carcinoma. Existance or non-existance of atypia in neighboring tumors was not correlated with the expression of p53 in invasive tumor.

PP2-49

HISTOPATHOLOGIC STAGING OF BREAST CANCER IN PATIENTS ATTENDING A MEDICAL UNIT IN CAMEROON

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Background: the histopathological study of tumor stages on surgical specimens is important for diagnosis purpose but can also be used as an element of evaluation of the effectiveness of breast cancer screening in the population.With this in mind, we carried out a retrospective study with the objective to analyse the histopathologic characteristics of cancers found in breast surgical specimen. Material and methods: Pathology reports on surgical specimen (nodulectomy, quadrantectomy, mastectomy, with or without axillary currage) issued between 1st April 2002 and 31st March 2007 were consulted: the tumor size, the grade (differentiation) using Scaff Bloom and Richardson Scale for carcinoma, the presence or absence of metastasis were analysed. We used the TNM system. Results: 1-The average age of our patients was 38 years with a range between 20 and 67 years. 2-

Tumor: 63 cases of breast cancer were diagnosed, with 60 cases of carcinoma, 2 cases of liposarcoma, 1 case of kaposi sarcoma and 1 case of lymphoma. 2a) Size: 8 out of 60 (13, 4 %) had carcinoma in situ; 13 out of 60 (21,6%) had a tumor of PT1 of whom 7 patients had PT1c. 27 out of 60 (45,0%) cases had PT2 carcinoma. Tumors of PT2 and above were found in 12 (20%) of the 60 cases. The two cases of liposarcoma were PT3. The Kaposi sarcoma came from a HIV positive Patient. 2b) Grade: 47 out o 52 invasive carcinoma (90, 3%) were ductal carcinoma, 4 (7,6 %) lobular carcinoma, and 1 (2,1%) medullary carcinoma. 10 (19,2%) cases were grade 1, 39 (75,0%) were grade 2 and 3 (5,8%) were grade 3. 3- Metastasis: 33 specimens were accompanied by axillary dissection: At least 3 lymph nodes invasions were present in each case. All of these cases were PT2 and above. Conclusion: Most of the patients received in our service for breast carcinoma were diagnosed in the late stage. The prognosis was thus poor. A lot still has to be done in the domain of screening in our milieu.

PP2-50

ESTROGEN RECEPTOR EXPRESSION AND Ki67 PROLIFERATIVE ACTIVITY IN NORMAL BREAST, COLUMNAR CELL LESOINS AND DCIS GRADE I: A PILOT STUDY

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Background: Columnar cell lesions (CCLs) of the breast present a spectrum of histologic alterations that have in common the presence of columnar epithelial cells lining variably enlarged terminal duct lobular units, ranging from those that show little or no cytologic or architectural atypia to those that show sufficient cytologic and architectural features to warrant a diagnosis of atypical ductal hyperplasia or DCIS.Estrogen has proliferative effects on breast epithelium and disregulation of proliferative activity is importat in carcinogenesis.In this study we evalaute the expression of ER and prolifertative activity in normal breast, CCLs and DCIS grade I. Material and Methods: Breast biopsies were re-evaluated for CCLs using the criteria proposed by Schnitt and Vincent-Salomon. For grading DCIS Van Nuys classification was used. ER expression and Ki67 proliferative activity were assessed immunohistochemically in 9 normal breast tissues from benign breast biopsises, 13 CCLs and 9 DCIS grade I. ER and Ki67 immunoreactivities were examined under x20 objective and positively stained nuclei were counted in 4 consequative fields. Results: Of 306 breast biopsies 13 (4.4%) were CCLs. Of 13 cases 7 (53.8%), and 6 (46.2%) were nonatypical and atypical CCLs, respectively. The median values and standart deviations for ER in normal breast, nonatypical CCLs, atypical CCLs and DCIS grade I were, 309±97.20, 1316.0±309.89, 1192.0±280.37 and, 1917.0±1561.45, respectively. The median values and standart deviations for Ki67 immunoreactivity in normal breast, nonatypical CCLs, atypical CCLs and DCIS grade I were, 60.0±44.02, 39.0±10.73, 140.50±127.35 and 205.0±163.75, respectively. There were statistically significant differences between normal breast tissue and all other groups and atypical CCLs- DCIS I for ER expression. There were significant differences between normal breast-atypical CCLs, normal breast-DCIS I, nonatypical-atypical CCLs for Ki67 reactivity. Conclusions: Increase in ER expression started from CCLs which were phenotypically normal. The proliferative activity of nonatypical CCLs was less than atypical CCLs and there was no difference between proliferative activities of atypical CCLs and DCIS I. Therefore nonatypical and atypical CCLs were different lesions and, atypical CCLs can be early stage of low grade DCIS.

PP2-51

EXPRESSION OF E-CADHERIN, HMWKER34βE12 AND CK 5/6 IN INVASIVE BREAST CARCINOMAS. CORRELATION WITH OTHER CLASSICAL PROGNOSTIC MARKERS

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Background: The invasive ductal and lobular carcinomas of the breast have usually distinct histological pattern. However, some cases are indistinguishable on the basis of morphology. Specific antibodies in immunohistochemical level, as shown in literature, can help in such cases. The aim of this study is to investigate the expression of E-Cadherin, HMWKer34βE12 and CK5/6 in invasive breast carcinomas. E-Cad demonstrates strong membrane staining of luminal cells in normal breast tissue and ductal carcinoma, but it is absent in lobular breast cancer. The HMWKer34βE12 constitutes a useful marker for lobular differentiation. The CK5/6 expresses in basal/myoepithelial cells of breast ducts and in a small number of high grade ductal carcinomas. Furthermore, we tried to correlate the invasive breast carcinomas with classical prognostic and predictive markers. Method: We investigated 100 cases of invasive breast carcinomas, of which 71 were ductal (IDC), 25 lobular (ILC) and 4 mixed types (IMC). Paraffin-embedded archival tissue was used with monoclonal antibodies against E-Cadherin, HMWKer34βE12, CK5/6, p53, HER-2, ER/PR. Results: Group IDC (n=71). 66 cases (93%) were E-Cadherin positive, of which six were moreover focal positive for HMWKer34βE12. The remaining 5 cases were E-Cadherin(-). 4 cases of them were HMWKer34βE12 and CK5/6 negative and the fifth case was CK5/6 positive. 6/71 cases (8,4%) were E-Cadherin, HMWKer34βE12 and CK5/6 positive, of which five were ER/PR/HER-2 negative. Group ILC (n=25). 24/25 were E-Cadherin and keratins negative. The remaining case was E-Cadherin(+) and focal HMWKer34βE12(+). Group IMC (n=4). 4/4 were E-Cadherin (+) only in the ductal component. All cases were negative for keratins. Conclusion: Evaluating of E-Cadherin expression alone in breast cancers is useful for distinguishing lobular from ductal carcinomas. E-Cadherin expression inversely correlates to tumour size and histological grade. Loss of E-Cadherin correlates with ER negativity. There was no association between E-Cadherin positivity, ER/PR status and p53 expression in our study. The majority of poorly differentiated invasive ductal carcinomas (84%) looses expression of HMWKer34βE12. The five CK 5/6 positive ductal carcinomas were ER/PR/HER-2 negative and probably represent basal-type carcinomas.-

PP2-52

SINGLE PROBE BRIGHTFIELD ISH IS A ROBUST AND REPRODUCIBLE TECHNIQUE FOR THE ASSESSMENT OF HER2 STATUS IN BREAST CANCER.

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Background: Brightfield ISH has recently begun receiving wider recognition as a useful tool in the assessment of HER2 status in

breast tumours. Whilst there have been numerous reports indicating the high level of concordance of these techniques with the widely accepted dual colour FISH assay, the lack of a single test incorporating the HER2 probe and a chromosome 17 probe has hampered the acceptance of brightfield ISH techniques. The aim of this study was to highlight the robustness and reproducibility of these assays in the assessment of breast cancer cases for eligibility for treatment with Herceptin. Design: Eight laboratories with expertise in brightfield ISH techniques were identified and invited to assess 30 breast cancer cases previously tested by dual colour FISH and IHC. Many of the cases were chosen because they had borderline scores and were difficult to interpret. The lab standard protocols and interpretation methods were used. Results were collated and assessed for concordance and a review of any discordant cases at a multi-headed microscope is planned. Results: Preliminary data available from two laboratories indicate that CISH was technically successful in all but one case where repeat analysis was needed. The agreement between two labs was total in all but 2 cases. One, with a FISH ratio of 2.69, was interpreted as low amplification by one centre and negative by the other. The second discordant case had a FISH ratio of 2.9 but was interpreted as negative by one centre and positive by the other. Conclusions: The success rate of CISH was high in this study indicating that it works well on routine formalin-fixed paraffin-embedded tissue. The laboratory agreement of CISH interpretation was excellent but revealed potential problems with low amplification and different cut-off values between CISH and FISH. This large inter-laboratory study will enable us to assess the problem of evaluating cases with very low level amplification and the effect of variability in threshold values.

PP2-53
COMPARISON OF FISH AND IMMUNOHISTOCHEMISTRY IN BREAST CANCER

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The role of HER-2 (HER -2/ neu or c-erB-2) has increased as a prognostic and predictive factor. The human epidermal growth factor receptor 2 oncoprotein is overexpressed in one third of invasive breast carcinomas. It is an independent prognostic marker of clinical outcome as predictor of benefit from chemotherapy regime and for metastatic disease for benefit from anti-HER -2 antibody therapy. Various laboratory methods have been used for this purpose. Results obtained in the routine practice by immunohistochemistry (IHC) and fluorescence In Situ hybridization (FISH) in determination of HER-2 status. We analyzed the concordance between immunohistochemical analysis and Fluorescence In Situ hybridization (FISH) for HER 2 status. We studied paraffin blocks of 632 mammary tumors between 21.12.2004 and 15.02.2007, in Okmeydani Training Hospital, Pathology Department. Before 2001 we applied alcalen phosphatase method, after 2001 we applied immunoperoxidase technique. We studied Dako, Biogenex, and NeoMarkers antibodies. Immunohistochemical results were interpreted as negative (0), or positive (1+, 2+,3+) as membraneous staining intensity. All this cases were interpreted by independent pathologists. None of 421 cases (% 66.61) revealed immune staining. 63 cases (% 9.96) were scored immunohistochemically (1+) , 70 cases (% 11.7)(2+), and 78 cases (%12.34) (3+). FISH test were performed by using Vysis PathVysion method. In 16 cases which were 2+ tumors, all of the patients were females, 35-83 years old, median 52,7, SD:11,4 . Only 1 case was invasive lobuler carcinoma and the others were invasive ductal carcinoma (Grade II-III) and one of these cases had suspect metastatic lung disease and one patient had multipl bone metastases, in one case there was local recurrens.In 7 cases both ER and PR receptors both were negative. In 4 cases were ER negative, PR weakly positive. In 5 cases both were ER and PR positive, and other cases were variable. Six of these test results were avaliable (positive), in 10 cases there was discrepancy between the FISH test and IHC. Our study revealed no correlation with ER and FISH method (p:0,36); PR and FISH method (p:0,89), IHC and FISH method (p:0,45) Best results will be obtained by immunohistochemistry of indeterminate results with complementary FISH testing .

PP2-54
PRECANCEROUS LESIONS IN BENIGN BREAST MASSES: A HISTOPATHOLOGICAL STUDY IN YAOUNDE-CAMEROON

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Background: Benign breast diseases raise a problem of their relationship with cancers of this gland. In Cameroon, breast cancers constitute one of the most common malignancies in women and are often diagnosed at a late stage. Risk factors are numerous, some of which are histopathological and are often described as precancerous, necessitating specific follow up of the patients. Material and methods: The purpose of this study was to highlight the relationship between some benign histopathological lesions of the breast that could be considered as risk factors for malignant evolution and the occurrence of the breast cancer in Yaounde-Cameroon. Slides bearing a record of benign lesion, associated or not to a malignant non invasive tumor, observed from 1st October 2002 and 28 February 2006 were re-examined. Results: of the 212 cases recruited in our study, we observed: -110 (51,9%) fibroadenoma amongst which 2 cases had atypical lobular hyperplasia, from two patients aged 45 and 55. -60 (28,3%) fibrocystic mastopathy among which 30 (50%) had no or slight ductular/lobular epithelial hyperplasia, 16 (26,7%) moderate or atypical hyperplasia and 14 (23,3%) carcinoma in situ. All cases of carcinoma in situ were associated with atypical or moderate epithelial hyperplasia. -38 (17,9%) fibrous mastopathy. -Other benign lesions were less frequent and included 4 (1,8%) lipoma, 1 (0,1%) benign phylloide tumor. -12 papillomas, 8 associated with fibrocystic mastopathy, 3 with fibrous mastopathy and 1 not associated with a significant lesion. 5 of the papillomas were multiple. Conclusion: breast masses considered as benign are present in our milieu. Some are known to have the risk of cancerous evolution, but this risk seems variable with the histopathological type of lesion, the predominant forms in our series being fibrocystic mastopathy with moderate or atypical (ductular/lobular) hyperplasia.

PP2-55
PRIMARY INVASIVE MICROPAPILLARY CARCINOMA OF THE MAMMARY GLAND IN CATS: A CLINICOPATHOLOGIC, DNA PLOIDY AND SURVIVAL ANALYSIS

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Background: Carcinomas account for 80-90% of the feline mammary gland tumours, and are known for their unfavourable prognosis due to a strong tendency for local recurrence and metastasis. The World Health Organization (WHO) classification of feline mammary lesions is a descriptive histological classification difficult to reproduce, because mammary carcinomas of queens present several histological patterns, a mixture of which is found in the same tumour. Histologic assessment of these tumours is not sufficiently discriminator in predicting prognosis, as it was demonstrated in various studies. However, there are some cases in which the histology justifies its individualisation by indicating a more or less aggressive behaviour. Micropapillary invasive pattern is a distinct histological type associated with poor prognosis. The World Health Organization (WHO) of feline mammary lesions does not recognize the micropapillary pattern, which is included in the spectrum of papillary tumours. Material and methods: In this study we report 16 carcinomas of the feline mammary gland displaying histological features that correspond to the invasive micropapillary carcinoma (IMC) of the women breast. The clinicopathological findings, overall survival, disease-free survival and nuclear DNA content of these cases were compared with 65 more common invasive mammary carcinoma of non-specified type (other FMCs). Results: IMC cases were associated with higher histological grade ($p<0.0001$), deeper muscle invasion ($p=0.004$), more frequent lymphovascular invasion and nodal metastases ($p=0.009$ and $p=0.001$, respectively) than other FMCs. The aneuploid pattern was more frequent in IMC lesions. IMCs were also associated with lower survival rates. Conclusion: All cases of feline IMCs were associated to pathological features of high biologic aggressiveness. In addition to histology, they also presented DNA content pattern, and survival rates that justify its individualization as an independent histotype of the mammary gland of queens.

PP2-56

NUCLEAR PLANIMETRY ON THE SAMPLES FROM CYTOLOGICALLY SIMILAR BREAST LESIONS

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BACKGROUND: Discrimination of low-grade invasive ductal carcinomas and tubular carcinomas from cellular fibroadenomas on the fine-needle aspiration materials may sometimes be problematic. In some cytological samples from such benign or low-grade malignant breast tumors, both the cells' nuclei and patterns of cell clusters show substantial overlapping morphologic characteristics. Therefore, most of the time, histopathological confirmation from small tissue samples is needed prior to any major surgical intervention. Nuclear morphometry on cytological materials is suggested as an ancillary method for the differential diagnosis in many lesions including breast tumors. METHODS: Twenty-five cytological samples obtained from patients with lesions, which were histopathologically confirmed as grade I invasive ductal carcinomas (n=10), tubular carcinomas (n=5) and fibroadenomas with focal proliferative features (n=10) were utilized in this study. Eight geometric features of about 2000 nuclei in total from these tumors, such as perimeter, nuclear area and circular form factor, were measured with the aid of a computer assisted image analysis system. Discriminant analysis was performed on this data set in order to test the correct classification based on the eight measured variables. Statistical analyses were carried out with two fundamentally different approaches: In the first one, the entire data from all measured nuclei were used for classification. In the second one, a subset of data representing the ten percent most deviated values of variables, was extracted from the entire dataset to simulate the selective examination performed during classical morphologic evaluation. RESULTS: When the entire data was used in discriminant analysis, the overall performance in correct classification rate was found approximately 50%, which was considered as an unacceptable value in routine diagnostic practice. In the data set constructed with our systematic and reproducible selection bias, the overall performance of correct classification rate of same discriminant model was improved dramatically up to the 97%. CONCLUSIONS: A pathologist always exploits the most abnormal findings encountered during screening to reach a final decision. Therefore, morphologic examination is actually based on selection. Using data obtained from all of the cells in morphometry, as always used in nearly all of the statistical methods, may cause a masking effect in diagnostically important features. Morphometric studies may seem to be useless when this effect is not taken into account. But with a systematic and reproducible selection of the values with a proper bias, morphometry may provide some discriminatory information in overlapping lesions.

PP2-57

PLANIMETRIC FEATURES OF CELL CLUSTERS IN FIBROADENOMAS: A PRELIMINARY STUDY

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BACKGROUND: In addition to the nuclear and cytoplasmic features, morphologic appearance of cell clusters usually provide considerable diagnostic clues in cytological diagnosis for many lesions. Most of the fibroadenomas are easily recognized from cytological specimens. However, aspirates from cellular fibroadenomas, sometimes cause diagnostic difficulties because of their resemblance to the low-grade malignant lesions. Morphometric assessment of cell cluster morphology may contribute to routine cytological examination by emphasizing its value. In this preliminary study, we investigated whether the cell cluster segmentation on cytological specimens is possible. METHOD: This study was carried out on 45 fine-needle aspirates reported as consistent with fibroadenoma and confirmed histopathologically. A blank-field image for shading correction and three digital photographs from the most representative areas on slides belonging to each case were taken under x25 magnification and saved as tiff images in 768x576 pixel resolution. Morphometric measurements were realized using a series of small macro programs prepared by one of the authors (YK) in ImageJ, an open source image processing and analysis software supported and maintained by NIH. Every recognizable cell clusters which are not touching to the boundaries of images measured automatically for eight different variables, such as area, perimeter and circularity. Resulting data set was composed of 1448 records. Two-step cluster analysis was performed on these data to assess the heterogeneity of cell clusters. RESULTS: Statistics showed that the cell clusters in fibroadenomas may be classified into two different groups. Major group (83%) contained predominantly the small cell clusters. The second – inconsiderable- (17%) group contained large cell aggregates. Our test results do not indicate any importance attributable to this categorization. CONCLUSION: This preliminary study showed that the morphometric cell cluster segmentation and analysis are possible in the fine needle aspirates. In an ongoing study, we planned to include benign and malignant lesions that have overlapping features with fibroadenomas. We hypothesized that the cell cluster shape may contribute to the cytological assessment in breast lesions as a stand alone variable. However, this hypothesis needs to be tested in similar studies.

PP2-58
PEUTZ-JEGHERS SYNDROME WITH BILATERAL BREAST AND OVARIAN CANCER (A CASE REPORT)

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Abstract Peutz-Jeghers syndrome is a rare autosomal dominantly inherited condition, characterized by the presence of hamartomatous gastrointestinal polyps and mucocutaneous pigmentation. Patients with this syndrome can be associated with other neoplasms such as ovarian neoplasms known as sex-cord tumor with annular tubules that is associated in one third of the cases with this syndrome and other type of malignancies. We report a 42 year-old woman with a history of Peutz-Jeghers Syndrome (PJS) and bilateral breast cancer that presented with abnormal uterine bleeding. Total abdominal hysterectomy with bilateral salpino-oophorectomy (TAH & BSO) was done and an ovarian sex cord tumor with annular tubules was incidentally diagnosed. By reviewing of literatures and in agreement with pervious studies we suggest routine screening for malignancies in patients with peutz-jeghers syndrome.

PP2-312
EVALUATION OF BIOMARKERS IN LOBULAR CARCINOMA IN SITU

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Background: Classic lobular carcinoma in situ lesions (LCIS) are associated with low grade morphology, low proliferation rate, positive ER, PR and negative HER-2 expression. Variants of LCIS have been described which exhibit high grade nuclei, pleomorphism and/or necrosis. The biomarker profile of spectrum of LCIS lesions has not been well defined. In the present study, a panel of biomarkers was analyzed and correlated with histomorphological features. Design: We reviewed consecutive cases of LCIS in surgical specimens from 2004 to present and selected LCIS cases based on negative E-cadherin, positive high molecular weight cytokeratin staining, and involvement of at least 3 lobules in at least 1 slide. Morphological features including nuclear grade, presence of apocrine features or signet ring cells, discohesion, and degree of distention of lobular units were recorded. Nuclear grading was similar to that used for DCIS (per CAP recommendations). We grouped lesions with nuclear grade 1 in low grade category and grades 2 and 3 in high grade category. Immunostaining for ER, androgen receptor (AR), HER-2, Ki-67, and p53 was evaluated in the corresponding LCIS foci. Result: The median age of 53 patients was 57 years (range 35-82 years). LCIS was associated with invasive carcinoma in the same specimen in 72% (38/53) cases. Low nuclear grade was assigned to 23 cases and high nuclear grade to 30 cases. Nuclear grade was associated with presence of necrosis (p=0.045) and showed borderline significant association with apocrine features (p=0.06). It was not related to presence of invasion, signet ring cells, or degree of distension of lobular units. LCIS cells showed strong ER expression in 95% of cases. AR expression (74% cases) was significantly associated with high nuclear grade (p=0.026). Increased expression of p53 (>10% nuclei) and Ki-67 (more than 10% nuclei) was limited to high grade cases (4%). HER-2 was positive (3+) in a single case of high grade LCIS (2%). Conclusion: Although rare cases of high grade LCIS show an aggressive biomarker profile (decreased ER, increased Ki-67, p53, or overexpressed HER-2), unlike DCIS, the majority of LCIS lesions appear to show a benign biomarker profile unrelated to morphological features. The only exception is AR expression which is associated with high grade LCIS with apocrine morphology. Further molecular studies may be required to classify and predict the biological behavior of these lesions.

PP2-59
THE HISTOLOGY OF CARDIOESOPHAGEAL JUNCTIONAL MUCOSA IN PEDIATRIC PATIENT POPULATION

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Background. The origin and histology of cardiac mucosa remains controversial. The classical concept that the cardiac mucosa is of gastric origin has been challenged by those who consider that the cardiac mucosa is metaplastic esophageal mucosa. By the latter theory, cardiac region does not exist early in life and direct continuity between the esophageal squamous and oxyntic mucosa is normalcy. The morphology of the gastric cardia in children and the significance of inflammation in this region have been studied only in a few series. The aim of this study was to evaluate the morphologic features of the cardia in an unselected pediatric population and to determine the significance of inflammation in this region. Method. Fourty five consecutive patients (age range 1 – 17 years) in whom cardiac mucosa was present in biopsy samples from the gastroesophageal squamocolumnar junction were selected. Biopsies that straddled the squamocolumnar junction represented the distal esophagus and proximal stomach, i.e. cardia. In addition, each patient had biopsies of the duodenum and gastric antrum and body. Inflammation of the cardia, irrespective of its exact anatomic location, was defined as carditis and classified as active or chronic, based on the type of inflammatory cells present. Inflammation of the esophageal mucosa in biopsy specimens was also recorded. Patients with Barrett's esophagus were excluded. Results. None of the patients had pure oxyntic mucosa (OM) at the squamocolumnar junction, adjacent to squamous epithelium. All patients had cardiac mucinous mucosa (CM) (17/45), or oxyntocardiac mucosa (OCM) (28/45). CM was highly associated with inflammation (14/17; $p<0.05$), contrary to OCM (17/28; $p>0.05$). Overall, carditis was seen in 31/45 patients (69%) ($p<0.05$). Of these 31 patients, 9 (29%) had Helicobacter pylori (H. pylori) carditis, which was always associated with H. pylori gastritis, and 3 (10%) had non-H. pylori carditis and gastritis (in corrosive injury, ulcerative colitis and lymphocytic gastritis). The remaining 19/31 patients (61%) had non-H. pylori isolated carditis, without any concomitant gastritis. Esophagitis was highly associated with non-H. pylori isolated carditis (17/19; 89%) ($p<0.01$). There was no significant association between esophagitis and H. pylori carditis ($p<0.01$). Conclusion. The finding of uninflammed gastric cardiac mucosa supports the concept that the gastric cardia is a normal structure. Normal cardia consists of CM and OCM mucosa. The etiologies of carditis are H. pylori infection and gastroesophageal reflux disease.

PP2-60
FLOW CYTOMETRIC VARIABLES AS PROGNOSTIC FACTORS IN SURGICALLY RESECTED GASTRIC CARCINOMA: A 7-YEAR PROSPECTIVE STUDY

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Background. DNA ploidy and S-phase fraction (SPF) measured by DNA flow cytometry (FC) were shown to correlate with several clinicopathological variables in several types of tumours. Materials and Methods. DNA FC was performed on multiple frozen tumour samples obtained from 115 patients undergoing curative surgery for gastric cancer (GC), and the findings were prospectively correlated with traditional clinicopathological indicators of prognosis. Vital status at 7 years was assessed in all patients. The crude survival probability was estimated by the Kaplan-Meier method and differences between patient groups were compared by the log rank test. Cox regression models were used to evaluate the risk of dying. Results. Overall, 20 tumours (17.4%) were diploid, 46 (40.0%) monoclonal and 49 (42.6%) multiclonal. Excluding 4 patients who died within 1 month of surgery, high SPF (>9.6%) was detected in 55 patients (49.6%), and was found to be significantly associated with vascular invasion and multiclonality ($p=0.02$). An association of borderline statistical significance emerged with macroscopic type ($p=0.06$) and pN and pM status ($p=0.07$). Multivariate regression analysis did not show a significant effect of SPF ($p=0.11$) or DNA ploidy ($p=0.28$) on 7-year survival. Conclusions. Investigations carried out on fresh tissue, with multiple sampling of single lesions, allow cytometric data to be correctly acquired. Aneuploidy appears to be a prognostic factor of low penetrance, whereas SPF is a more promising parameter of tumour aggressiveness in patients with GC.

PP2-61
MESOTHELIN EXPRESSION IN PANCREATIC CANCER

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Background: Mesothelin is an emerging marker for cancer diagnosis and target based therapy. In addition to malignancies of mesothelial origin, mesothelin has been shown to have positive staining by immunohistochemistry in malignancies from the ovary, and more recently from the pancreas. The purpose of this study is to evaluate the expression of this monoclonal antibody in pancreatic carcinoma versus chronic pancreatitis at our institution. Methods: The authors reviewed 74 cases of pancreatic ductal carcinomas (both biopsies and resections) from the files of McMaster University Medical Centre. Representative blocks of tumour and adjacent non-neoplastic pancreas were selected for immunohistochemistry. Staining was performed using a well-characterized mesothelin monoclonal antibody (5B2, Nova Castra). Staining of greater than 25% of tumour cells was considered to be a strong positive, less than 1% was considered negative and the remaining cases were called focally positive. Intensity of staining was semi quantitatively graded on a scale of 1-3 (weak, moderate and strong). Result: Mesothelin staining was seen in 90% of pancreatic ductal carcinomas, of which 78% of cases showed staining in 25% or more of the tumour cells. The staining intensity was graded as 2 in the majority of the positive cases. 36 cases of chronic pancreatitis and 5 cases of normal pancreatic tissue were included. Cases of chronic pancreatitis showed only occasional focal positive staining and the normal pancreatic tissue was negative in all cases. Conclusion: We conclude that mesothelin is a useful marker for the diagnosis of pancreatic ductal carcinoma and may be helpful in discriminating cases from chronic pancreatitis.

PP2-62
CANCER PROGRESSION OF HYPERPLASTIC POLYPS AND SERRATED ADENOMAS OF THE COLORECTUM

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The serrated polyps of large intestine, including hyperplastic/metaplastic polyps (HPs) and serrated adenomas (SAs), are characterized by sawtooth-like infoldings of the crypt and surface epithelium. The current molecular studies proposed the serrated neoplasia pathway as a model of colorectal carcinogensis through the HPs and SAs. The aim of this study

was to clarify the clinicopathological and immunohistochemical characteristics of SA with carcinoma (CA) and HP with CA, and to provide morphological evidence that HP and SA are the precursor lesions of colorectal carcinomas arising along the serrated neoplasia pathway. A clinicopathological study of 14 SAs with CA and 11 HPs with CA of the large intestine was carried out. 64% of SA with CA and 80% of HP with CA were located in the caecum, ascending colon, and transverse colon and they were frequently observed in the right-sided colon rather than in the left-sided colon. The average largest diameter of SA with CA and HP with CA was approximately 16mm. As for the macroscopic features of SA with CA, there were many pedunculated and subpedunculated elevated lesions, and there were many sessile elevated lesions in HP with CA. Most of the CA components were intramucosal, well differentiated adenocarcinoma, and the proportion of CA component was mostly less than that of HP and SA in their respective lesions. Immunohistochemically, the cytokeratin 7 (CK7)-positive rate was as follows; 35.7% of SAs and 50% of CAs in the SA with CA group, and 27.3% of HPs and 36.4% of CAs in the HP with CA group. The CK7 expression was more frequent in the CA than in SA and HP. 50% of CAs in SA with CA group presented a CK7+/CK20+ pattern. Concerning the expression of the human apomucins, in the SA with CA group, the mixed mucinous phenotype combining both gastric foveolar and intestinal mucin (MUC5AC+, HGM+/MUC2+) was 78.6% of SA and 42.9% of CA, and all of the HP and 90.9% of CA were mixed phenotype in the HP with CA group. The expression of gastric mucin was increased in the SA with CA group, and most of the lesions in the HP with CA group expressed the gastric mucin. In conclusion, the CAs accompanied by SA and HP have immunohistochemical characteristics which are similar to those of SA and HP, and are different from those of common colorectal carcinomas. These findings indicate that SA and HP are the precursor lesions of some colorectal carcinomas.

PP2-63
CPP32, CD34 AND VEGF EXPRESSION IN COLORECTAL CRYPTAL HYPERPLASIA, ADENOMA AND MALIGNANT ADENOMA

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Background. We investigated immunohistochemically the expression of cpp32, CD34 and VEGF on tissue samples of 104 endoscopically removed colorectal polyps. 21 tumors were classified as carcinoma-in-adenoma, 19 as colorectal adenoma with high grade atypia, 39 as adenoma with low grade atypia and 25 as cryptal hyperplasia, respectively. The aims of our study were to describe cpp32, CD34 and VEGF expression in colorectal adenomas and in malignant adenomas and to investigate the correlation between these markers and dysplasia in adenomas. Method. For immunohistochemical detection of cpp32, CD34 and VEGF on formalin-fixed paraffin-embedded tissue sections mouse monoclonal antibodies were used. The percentage of positive tumor cells was estimated and scored semiquantitatively. Tumors showing more than 10% positive cells were considered positive. Results. 8% of carcinomas-in-adenomas compared to 21% high grade adenomas, 43% low grade adenomas and 87% cryptal hyperplasia, respectively, were cpp32 positive (p<0,000l). In contrast 63% of carcinomas and 74% high grade adenomas were VEGF positive, compared to 57% low grade adenomas and 21% cryptal hyperplasia (p<0,0001). CD34 expression could be demonstrated in 64% carcinomas-in-adenomas, 68% high grade adenomas, 70% low grade adenomas and 61% cryptal hyperplasia, respectively (p>0,05). Conclusion. The expression of cpp32 and VEGF clearly shows differences between cryptal hyperplasia, adenoma and malignant adenoma. Based on these markers subgroups of patients could be identified who might be high risk patients and possibly need a closer follow-up after polypectomy.

PP2-64
MATRIX METALLOPROTEIN EXPRESSION IN GASTROINTESTINAL STROMAL TUMORS

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AIM: The growth of a cancer is accompained by progressive infiltration, invasion, penetration and destruction of the surrounding tissues. Next to development of the metastasis, invasiveness is the most reliable feature that differentiates malignant from benign tumours. Many criteria are considered to determine the malignant potential of the gastrointestinal stromal tumors (GIST) but the unique criterium of malignancy is metastasis. A group of enzymes which are involved in the degradation of different components of extracellular matrix, matrix metalloproteinases (MMP), have important roles in tumor invasion and metastasis. We aim to determine the expression profiles of MMPs and investigate whether MMPs correlate with the metastasis and prognosis of GIST. METHODS: Using immunohistochemistry, we examined the expression of MMP-2, MMP-9, and MMP-14 in 18 gastrointestinal tumors. For each tumor, the immunohistochemical panel of CD-117, CD-34, alpha sm-actin, S-100, antibody were performed, Ki-67 proliferation indices were determined. RESULTS: The expression of MMP-2 was not detected in GIST. MMP-9 and MMP-14 expression were detected both in the metastatic or nonmetastatic GIST lesions. In all of the GISTs moderate and strong MMP-9 and MMP-14 expression were detected. When we compared according to the staining intensity, the strong expression of MMP-9 and MMP-14 were significantly correlated to mitotic and Ki-67 indices ($p<0.05$). And also in all of the metastatic cases , strong expression was detected, but there was not any relation with respect to the size, localization, cellularity, cell type, degree of atypia, presence of necrosis, lymphoid infiltration, with the margins of the tumor, mucosal infiltration, and the immunstaining properties of the antibodies at the panel. CONCLUSION: The prominent overexpressions of MMP-9 and MMP-14 were detected in GIST lesions that were having mitotic indices higher than 5/50BBA, this correlation showed that those played a key role in the tumor progression. Mitosis as a criterium to determine the risk of malignancy, had a main role. So we considered that the strong expression of MMP-9 and MMP-14 could be a feature of high risk of malignancy. The number of metastatic cases were not enough to determine the role of those MMPs on the prognosis of the tumors.

PP2-65
EVALUATION OF EGFR GENE COPY NUMBER IN SELECTED IMMUNOHISTOCHEMICALLY EGFR – POSITIVE PATIENTS WITH COLORECTAL CANCER

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Treatment of patients with recurrent or metastatic colorectal cancer with anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies demonstrate a clinical response in about 10% cases. At present the main clinical problem is the selection of patients for therapy with anti-EGFR monoclonal antibodies who will have benefit from this treatment. EGFR analysis by immunohistochemistry does not seem to be a useful tool, because no correlation between EGFR expression and clinical answer was found. The goal of our study was to investigate EGFR gene copy number in patients with colorectal cancer with EGFR positive status examined by immunohistochemistry as a possible method useful in selecting patients for anti-EGFR monoclonal antibodies treatment in the future. Patients and methods Our material

consisted of 104 patients with colorectal cancer. EGFR expression was determined on paraffin - embedded and formalin fixed histological specimens by immunohistochemistry, using system kit EGFR pharmDx (DakoCytomation). EGFR status was evaluated as positive if the percentage of membrane stained tumor cells was ≥ 1%. Sixty-five (63%) patients were evaluated as EGFR - positive and 39 (37%) as negative. In selected group of 30 patients with positive EGFR status we evaluated EGFR gene copy number using fluorescence in situ hybridization (FISH). Results Based on EGFR gene status patients were classified into two groups: EGFR FISH – positive cases, when a ratio of EGFR gene to chromosome 7 was ≥ 2, or high amplification (presence of tight EGFR gene clusters) was found and EGFR FISH – negative group when a ratio was < 2. To EGFR FISH – positive group 5 patients (16%) were qualified: in one case gene amplification was found and in 4 cases a ratio was ≥ 2 (range from 2 to 3,69). Twenty-five (84%) patients were found to be EGFR FISH-negative (ratio < 2). Conclusion In our material 16% patients with immunohistochemically EGFR – positive status have high EGFR gene copy number. It needs further investigation, whether the correlation between increased EGFR gene copy number in colorectal cancer and the clinical response for therapy with anti-EGFR monoclonal antibodies exists. These group of patients is clinically closely follow - up.

PP2-66
COLONIC ADENOCARCINOMA REVEALING SMALL INTESTINAL MULTIPLE CARCINOID TUMORS

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Background: Neuroendocrine tumors of the small intestine are also called carcinoids. Most are non-functionning and they are often discovered accidentually. This tumor can be multiple and can be associated with others tumors, particularly gastro-intestinal malignancies. Method: Among a case of a 68-year-old man in which we discovered a multiple neuroendocrine tumors in the small intestine associated with colonic adenocarcinoma, we discuss the epidemiology, the clinicopathologic features, the pathological diagnosis problems and the surgical approach of this association with review of the literature. Results: A 68-year-old male patient was admitted for exploration of an abdominal pain with subocclusive syndrome. Colonoscopy showed a budding tumor 60 cm far from the anal margin. Biopsy concluded to an adenocarcinoma. Per-operative exploration revealed an expended small intestine with multiple polyps. Total colectomy with resection of the last portion of the ileum was achieved. After histopathological study, the diagnosis of a colonic adenocarcinoma associated with multiple neuroendocrine tumors was established. Conclusion: Through this observation, we emphasize the concept of multiple neuroendocrine tumors and the possibility of synchronous malignancies.

PP2-67
PRIMARY MELANOMA OF THE SMALL BOWEL: A CASE REPORT AND REVIEW OF THE LITERATURE

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Background: Malignant melanoma is the most common tumor metastating to the gastrointestinal (GI) tract, especially to small intestine. Although small bowel involvement by malignant melanoma is most frequently metastatic, a primary origin at this site has been reported in rare cases. Case presentation: From January 1997 to March 2007 we diagnosed 14 patients as primary malignant melanoma originating from GI tract. Localizations of tumors are anorectal region (12 patients) and small bowel (2 patient). Endoscopic biopsies of 2 and wide excisions of 12 were available in the archieve of our department. Amongst them we report a case (76-year-old woman) of primary melanoma of the jejenum. The patient was admitted to the emergency department with abdominal pain for 15 days. She had complains of abdominal distention and melena for 3 months. Laboratory investigation revealed mild anemia. Abdominal ultrasound was interpreted as acute appendicitis by radiologists. Mild anemia, melena, abdominal distention and critical age were the clues for probability of malignancy. Exploratory laparotomy revealed multiple masses and partial obstruction of the jejenum. Histopathological examination of the resected small bowel specimen was confirming the diagnosis of melanoma. A thorough postoperative investigation did not reveal a primary lesion of the skin, anus, oculus, or any other location. Thus, we diagnosed the tumor as a primary intestinal melanoma. Conclusion: Malignant melanoma is the most common metastatic tumor of the gastrointestinal tract and can present with fairly common constitutional symptoms. Though the possibility of primary malignant melanoma in the small intestine do exist, the incidence is extremely low.

PP2-68
IMPORTANCE OF ANGIOGENESIS IN COLORECTAL ADENOCARCINOMAS AS A PROGNOSTIC PARAMETER

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Background: Angiogenesis is an important step in tumor biology and prognosis. Research in angiogenesis and relation to pathogenesis is rewieved in a large group of tumor such as malignant melanoma, lung, ovary, prostate and brain carcinoma. Several growth factors have been identified that regulate angiogenesis in colorectal cancer; the most important of these are vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF) and of several angiogenic factors. The aim of this study is to evaluate the angiogenic factors in colorectal adenocarcinoma as a prognostic parameter and to search correlation with the basic prognostic findings. Method: In this study, H-E slides of 39 cases of colorectal adenocarcinoma were (5 of stage 2, 17 of stage 3, 17 of stage 4) reexamined and vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF) were evaluated with immuno-histochemical technique. Staining intensity of both parameters was correlated with microvessel density (MVD showed by using CD31 antibody) and with the tumor stage, histological grade, lymph node metastasis and vascular invasion. Results: Significant correlations were found; between the pathological tumor stage and MVD ($p<0.05$), histological grade and MVD ($p<0.05$) and also VEGF staining and MVD ($p<0.05$). Conclusion: Our results support that VEGF and MVD may be used as an important prognostic parameters in the colorectal adenocarcinoma. But in a larger series with different tumor stages and the follow up studies are needed to define the role of the tumor associated angiogenesis in the biologic behaviour of colorectal adenocarcinomas.

PP2-69
THE IMPORTANCE OF THE MARKERS IMMUNOHISTOCHEMICAL: APOPTOTIC GENES, PROLIFERATION FACTORS AND ENDOTHELIAL ANTIGENS IN BARRETT ESOPHAGUS AND ITS COMPLICATIONS

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Introduction. The most serious complication of the chronic reflux esophagitis, or Barrett esophagus (BE), with glandular metaplasia is secondary esophagial adenocarcinoma. Purpose: Checking the link between the progress of Barrett's metaplasia to adenocarcinoma and the application of tumor markers involved in increased proliferation aspects with presence of apoptotic genes (p-53, bcl-2) proliferation factors (PCNA, Ki-67) and endothelial antigenes (CD 34) Material and methods. The study included 52 cases, mainly males, aged between 23 and 65, who were subjected to endoscopic and histopathologic investigation. Biopsies were fixed in 10% formol and parafin included.. Than ue used HE and histochemical staining for neutral and acid mucopolysaccharides and IHC staining: (indirect tristadial Avidine-Biotine-Peroxidase method). Results: The 52 cases, diagnosed by endoscopic and histologic methods, classified as follows: 20 cases of Barrett esophagus with specialised epithelium, 6 cases of non-determined metaplasia, 8 cases of initial intraepithelium neoplasic lesions, 5 cases of advanced intraepithelium neoplasic lesions and 13 cases of moderate and undifferentiated carcinoma (one of them abounding in Russell bodies). Immunohistochemical, Mutated tumor marker p-53 evidentiation was expressed in 61% cases (high grade of displasia of Barrett's esophagus and adenocarcionoma). The second antiapoptotic marker bcl-2 was inconstantly positive, especially in the lymphocytes of lamina propria. Monoclonal PCNA and 85% of Ki-67 antibodies were positive especially in the undifferentiated carcinoma. Endothelial antigenes (CD 34) were positive especially in the areas with the highest vascularization of well differentiated carcinoma. Follow-up studies are necessary to evaluate the prognostic value of BE complications. Conclusion: To diagnose the distal esophagus precancerous and cancerous conditions, assess the prognosis and risk of progression and to determine effective treatment, a wider panel of tissue tumour markers could help.

PP2-70
MYCOPHENOLATE MOFETIL RELATED COLITIS

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Background: Enterocolitis causing afebrile diarrhea is the most common adverse effect in mycophenolate mofetil (MMF) treated renal transplant patients. However, the histological lesions are still obscure and the pathogenesis has not yet been clarified. The aim of this study is to describe the histological features of the MMF-colitis and to define diagnostic criteria. Histological findings of possible pathogenetic significance are also discussed. Materials and Methods: The examined material consists of colonic biopsies which were obtained from seventeen renal transplant recipients having persistent afebrile diarrhea during treatment with an MMF-containing immunosuppressive regimen. Cases with colonic lesions attributed to other causative agents (CMV, cryptosporidium, bacteria) were excluded. Results: Three cases showed no significant changes. The rest fourteen cases displayed drug induced colitis which was mainly localized in the cecum and ascending colon and exhibited the following histological features: oedema (10), moderate cellular infiltrate (12), increased number of eosinophils (14), small number of neutrophils (9), crypt distortion (10), mucosal atrophy (5), crypts with flattened epithelium and cell apoptosis (5), crypt abscesses (7), erosions and ulcerations (4) and lymphoid aggregates (2). Crypt abscesses were usually few and often contained neutrophils, eosinophils and apoptotic bodies admixed with mucinous material. Conclusions: MMF-related colitis is often associated with a distinct histological pattern resembling chronic IBD. Crypt cell apoptosis in the absence of cryptitis speaks in favor of direct drug toxicity while mucosal architectural disarray and atrophy provide some indications of impaired epithelial regeneration.

PP2-71
MMP-7 EXPRESSION IN COLORECTAL CANCER AND RELATION TO E-CADHERIN AND BETA-CATENIN EXPRESSION

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BACKGROUND:It is widely known that colorectal cancer formation is a complex, multistep process involving the accumulation of genetic lesions in genes that regulate the pathways of cell proliferation, adhesion, differentiation, and death required for normal development. E-cadherin/β-catenin complex has a critical role in cell–cell adhesion. β -catenin is a critical component of the highly conserved Wnt signaling pathway that regulates cell proliferation and differentiation When the Wnt pathway is activated, β -catenin ubiquitination and degradation are inhibited and β -catenin enters the nucleus where it complexes with transcription regulator proteins activating target genes and the proteolytic enzymes, such as matrix metalloproteinase-7 (MMP-7, matrilysin). MMP-7 is thought to be directly involved in the processes of growth, invasion, and metastasis of colorectal cancer. METHOD: We were analyzed the expression pattern of E-cadherin, β -catenin and MMP7 in a group of 42 patients with colorectal cancer, 25 of them with lymph node metastases or distant metastases, on immunohistochemical staining using the labeled streptavidin-biotin method. RESULT:MMP-7 staining was significantly higher for colorectal cancer (61.9 %) than for colonic mucosa (19 %) ($p < 0.001$). Cytoplasmic or membranous expression of β -catenin and E-cadherin were observed in 76.2 % and 75.7 % of tumor, respectively. Nuclear expression of β -catenin was detected only in 26.2% of tumors. β -catenin expression scores were closely associated with E-cadherin expression scores. CONCLUSION: There was no association expression of matrilysin or b-catenin and lymph node metastases or distant metastases. The results of this study indicate that expression of MMP-7 may play a role in carcinogenesis of colorectal cancers and correlated with β-catenin and E-cadherin expression.

PP2-72
ASSESSMENT OF Ki-67 IMMUNOHISTOCHEMISTRY IN GASTROINTESTINAL STROMAL TUMOURS (GIST)

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Background The prognostic significance of Ki-67 proliferation marker in GIST is debatable. Only a few reports have shown that

Ki-67 index should be used as predictor for the malignant potential of GIST. Therefore, we examined 33 cases of GIST immunohistochemically and focused on the differences between benign and malignant groups. Methods The paraffin embedded tissues were immunostained for c-kit, CD34, and Ki-67 antigen by employing labeled streptavidin-biotin (LSAB) method. For Ki-67 index, the results were classified into two groups: stained cells < 10% and > 10% in 50 randomly selected high power fields. Statistical evaluation was carried out with the software SigmaStat for Windows v 3.0 (SPSS Inc., Chicago, IL, USA). Results Of the 33 cases, 17 classified as benign and 16 as malignant. Immunoreaction for c-kit was demonstrated and strongly expressed in all GIST. The almost half the tumours were stained positively for CD34. The total cases stained for Ki-67 >10% were 19/33 (57.58%). The Ki-67 index gretaer than 10% in benign GIST were significantly lower than in malignant GIST (7/17, 41.18 % vs 12/15, 80 %, P<0.05). Conclusion The expression of Ki-67 proliferation marker clearly show differences between benign and malignant GIST and it is useful as a most important prognostic parametar.

PP2-73

EXPRESSION OF AMACR AND THYMIDYLATE SYNTHASE IN RECTAL CANCERS AND CLINICAL OUTCOME

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Background: AMACR or P504S (α-methylacyl coenzyme A racemase) is a mitochondrial and perioxisomal enzyme involved in β-oxidation of dietary branched-chain fatty acids. It is overexpressed in various malignant neoplasms, such as prostate and colon cancer. Thymidylate synthase (TS), in turn, is involved in 5-fluorouracil (5-FU) metabolism and has been used as an important biomarker in predicting tumor response to 5-FU-based therapies. The aim of this study was to analyse AMACR and thymidylate synthase expression in rectal cancers and to find correlations with histopathological features as well as clinical outcome. Method: Study cases included surgically resected primary rectal carcinomas from 36 neoadjuvantly treated patients (25 males and 11 females; median age 61) and 20 patients without preoperative treatment (10 males and 10 females; median age 64.5). Immunohistochemical studies were carried out using anti-AMACR (Polyclonal, 1:100, Dako) and anti-TS (Monoclonal 1: 25, Dako) antibodies. The staining was evaluated using three different scoring systems: intensity, pattern and intensity + pattern. Results were compared with histopathological features such as grade, morphological pattern, pT, pN, tumor response after treatment as well as clinical features such as preoperative CEA level and disease-free survival. Results: 91 % of cases showed positive expression of AMACR. Diffuse pattern and strong staining intensity in case of AMACR expression was correlated with decreased disease-free survival (p=0.027). Positive expression of TS was observed in 71 % of cases. There was a significant correlation between preoperative CEA level and staining intensity as well as stainig pattern of TS expression in patients without lymph node metastases (p=0.020). However, there was no significant correlation of grade, morphological pattern, pT, pN and tumor response after treatment or type of treatment with AMACR expression or TS expression. Conclusions: Additional studies are required to confirm the prognostic significance of AMACR expression in rectal cancers.

PP2-74

MORPHOGENESIS AND PECULIARITIES OF ACUTE MUCOSAL PEPTIC LESION REPARATION IN GERD

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The topicality of GERD problem is due to its high incidence. Purpose: to investigate morphogenesis and peculiarities of peptic erosive-ulcerous lesion reparation in GERD. 600 samples taken from esophageal mucosa (EM) of 206 patients were studied. 58 patients underwent reexaminations in the course of treatment. In addition to standard histological methods, staining with combined method of alcian blue+PAS-reaction was used. Peptic erosions and ulcers occurred in 52,9% of examined cases, in 68,5% and 31,5% respectively. The most severe lesions were localized in gastro-esophageal junction or in the area of stratified squamous epithelium (SSE). Acute peptic lesions characterized by necrosis of collagen and muscular fibers and vascular walls. There are 2 possible variants of peptic lesion healing. In typical cases 4 stages can be noted: of leukocyte infiltration and necrotic mass rejection, of granulating, of epithelization (either by SSE or by columnar epithelium (CE) and of tissue remodeling after scarring. The second way of healing is that of encapsulation and organization of necrotic masses and their transformation into hyalinotic sites. In these cases leukocyte and macrophage infiltration and angiogenesis are poorly marked, typical granular tissue being not formed. Re-epithelization becomes impaired; active SSE and CE cellular proliferation and hyperplasia of mucous glands are noted at defect margins. This background promotes precancerous epithelial changes. If columnar cell metaplasia (CM) of EM was having been formed, peptic lesions localize superficially. Mucosal lesions result in active formation of different mucous glands; its secrete becomes amphophilic. In the course of GERD treatment 3 variants of its dynamics are possible – partial SSE restoration; keeping CE of a gastric type invariable; transformation of a mixed type CE into gastric epithelium. When not treated, cell containing intestinal mucus, including goblet cells predominate in mucosa. Conclusion: acute peptic erosive and ulcerous lesions of EM in GERD have definite stages of development and present an obligatory stage in “esophagus Barrett” forming. There are 2 types of their healing; CM development of EM, either of a gastric or of an intestinal type, being possible at any of its stage. CM should be regarded as a body compensatory adaptive reaction under the effect of gastro-duodenal content.

PP2-75

RELATIONSHIP BETWEEN INVOLVED LYMPH NODE, METASTATIC NEST DIAMETERS AND PROGNOSIS OF COLORECTAL CANCER

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Introduction: Nowadays colorectal cancer is the third cause of mortality from cancer. For this reason, there have been expanded studies about the effect of various factors, such as depth of tumoral invasion, size of involved lymph nodes, number of these nodes, their place and the extent of metastatic nest, on prognosis of patients with colorectal cancer. We decided to study on relationship between metastatic nest diameter to involved lymph node diameter ratio and prognosis of patients with colorectal cancer. Materials and methods: This historical cohort study was done on Alzahra hospital patients with colorectal cancer who have had metastasis to lymph nodes, it has been at least 1 year since diagnosis of their disease and they have been treated with the same chemotherapy protocol in less than 2 month from surgery. In order to collect data, pathologic samples of 63 patients were prepared and size of lymph nodes with metastasis were registered, by teacher-student microscope, then borders of metastatic nest was marked by a marker and microscope magnification (40). Also, size of metastatic nest was registered with the ruler, and finally, metastatic nest to involved lymph node ratio was calculated. Then, data were entered to SPSS soft were, and Kaplan Meier and Acturial methods in results' analysis and cox regression, Log rank test in comparing the survival appurtenances were used. Results: Based on the results of this

study, about the effect of metastatic nest on prognosis of patients, no meaning full relationship was observed between size of metastatic nest and prognosis (p=0.07). In the case of involved lymph node's size, no meaningful relation with prognosis was seen (p=0.1). Also, no meaningful relationship was seen between metastatic nest to lymph node ratio and prognosis of patients (p=0.2). Conclusion: Despite the fact that our study did not show a clear relation between size of involved lymph node and metastatic nest to involved lymph node ratio and prognosis of patients with colorectal cancer, concerning discrepancies in results of studies by different researchers, more studies are likely necessary. Metaanalytic researches that will possibly be based on all the existed studies, will lead us to a definite conclusion. Key words: Colorectal cancer, Metastatic nest, Prognosis, lymph node.

PP2-76
CLINICOPATHOLOGIC AND IMMUNOPHENOTYPIC FINDINGS IN 4 CASES OF ENTEROPATHY-TYPE T-CELL LYMPHOMA

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Background: Enteropathy-type T-cell lymphoma (ETCL) is a rare tumour often occurring in a background of coeliac disease (CD). ETCL is often disseminated at diagnosis, and extraintestinal presentations are not uncommon in the liver/spleen, skin, nasal sinus, and brain. We evaluated ETCL from 4 patients presenting with a history of adult CD. Design: Patients were identified from our hospital lymphoma database. All four cases were studied with respect to clinical (staging, treatment, and outcome), histopathological, and immunophenotypic features. We performed immunohistochemistry using a broad panel of antibodies for CD20, CD3, CD4, CD8, CD30, CD45Ro, CD5, CD23, CD7, CD43, CD15, CD56, perforin, granzyme B, ALK, cytokeratins, and EMA, in formalin-fixed, paraffin embeded tumors and adjacent intestinal mucosa. Results: There were 3 men and 1 women with a mean age of 51.5 yrs (range: 49-54). All patients had a documented clinical history of adult-onset CD. The presenting symptoms were weight loss, small-bowel perforation or B symptoms. One patient had extraintestinal presentation of the lymphoma, in the maxillary sinus. In all four cases, there were clinical and histopathologic evidence of CD and failed to respond to gluten-free diet. Histologic examination of the tumor revealed aggregates of neoplastic pleomorphic large cells, with vesicular nuclei, prominent nucleoli, and abundant cytoplasm. Intramucosal spread of an epitheliotropic T-cell population was observed in the vicinity or even at distant segments of the small bowel. All cases were CD30+, and all showed evidence of T-cell lineage with cytotoxic potential by expression of cytoplasmic CD3, CD43, or CD45RO, or perforin; and all tumors were CD8 negative. Granzyme B was found in two of four cases, mostly in a smaller number of tumour cells. All cases were ALK negative, corroborated by the absence of the t(2:5). Two cases showed cavitation of mesenteric lymph nodes. After diagnosis of ETCL, the patients were treated with chemotherapy. Of the total 4 patients, 3 have died, from progressive disease (dissemination into the CNS, spleen, and colon) or from complications of the disease and/or its treatment. Conclusions: The enteropathy-associated T-cell lymphoma is an aggressive and fatal complication of CD. Loss of response to a gluten-free diet is a complication of CD that may progress to ETCL. The phenotype (cytoplasmic CD3+, CD8-, CD30+), and the intraepithelial accumulation of lymphoma cells observed in the adjacent surviving mucosa are clues to the diagnosis.

PP2-77
PRECANCEROUS ESOPHAGEAL PROCESSES IN GASTROESOPHAGEAL REFLUX DISEASE (GERD)

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Two types of malignant tumors – squamous cell carcinoma (SCC) and adenocarcinoma (AC) occur in esophagus. Obligate precancerous processes include high-grade dysplasia of stratified squamous epithelium (SSE) and cylindrical epithelium (CE). Facultative precancerous process is GERD, which is traditionally associated with adenocarcinoma; real epithelial dysplasia incidence and its character in GERD have remained unknown. Purpose: to investigate metaplastic and dysplastic SSE and CE changes in GERD by biopsies from esophageal mucosa. 600 samples taken from 204 patients were studied. In addition to standard methods, the preparations were stained with alcian blue in combination with PAS-reaction, methylene blue and selectively, with monoclonal antibodies to mutant P53, HPV, VEB viruses. Chronic esophagitis of one or another grade was revealed in all the patients. Superficial catarrhal esophagitis and severe erosive – ulcerous lesions were observed in 25,9% and 52,9%, respectively. SSE columnar cell metaplasia (CM) was revealed in 38,7% of cases, 42,7% of them being of a gastric type, 40% - of a mixed type and 17,3% - of an intestinal type. In CM of a gastric type PAS+ mucus was revealed in the cytoplasm of surface and glandular epithelial cells. In incomplete intestinal metaplasia (second type), both gastric and intestinal mucus was revealed in the cells. The least common forms were those in which intestinal goblet cells predominated. Mainly mild dysplastic epithelium changes in esophageal mucosa were revealed in 14 patients (6,8%), changes in SSE and CE making up 64,3% and 35,7%, respectively. Discorrelation of the layers due to basal cells proliferation and nuclear hyperchromasia were noted in SSE. Signs of HPV, HSV and VEB infection were demonstrated in 50%. In CE dysplasia there was increase of glands in number, piling of cells on each other, cellular atypia and nuclear hyperchromasia. 4,9% of the patients had cancer: SC (70%), low-differentiated (10%), glandular (10%), glandular-squamous (10%). Low- and high-grade changes up to Ca in situ typical for SSE dysplasia were noted in areas adjacent to the tumor in all cases of SCC. Besides, in 3 cases of SCC these changes were noted against the background of CM with numerous goblet cells containing intestinal mucus. Conclusion: in esophageal mucosa SSE cellular dysplasia occurred almost twice as often as cylindrical one in case of GERD. In 30% of cases SCC developed against the background of intestinal metaplasia of surface epithelium. Thus, GERD is significant for developing not only AC, but SCC, as well.

PP2-78
A CLINICOPATHOLOGICAL ANALYSIS OF GASTRIC LYMPHOMAS

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Background: The stomach is the most common site of primary extranodal Non-Hodgkin's Lymphomas (NHL). The aim of this study was to evaluate retrospectively 29 gastric lymphoma cases in accordance to the criteria of the new WHO classification for neoplastic diseases of the hematopoietic and lymphoid tissues. Material-Method:To obtain information, we examined charts of all 29 cases of primary gastric NHL between January 2000 and December 2006. Our cases affected all age groups in both genders and for each group, the rate was higher among men than women, with an overall gender ratio of 1,23. The age of patients ranged from 30 to 69, with a median of 54,07 for males and 61,8 for females. The mean tumor size was 9.4 cm. In 10 of the 21 patients, lymph node involvement has been observed (%49). 13 cases were extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT)-type(MALT lymphomas) (%44), 10 were diffuse large B-cell lymphomas(%30) and 1 was follicular lymphoma(%6), the rest were diagnosed as NHL NOS(Not otherwise specified)(%20). Conclusion:. Clinical and histopathological characteristics of our cases were characterized

by a higher incidence of stomach location(50%), MALT type (mucosa associated lymphoid tissue)(40%) and B-cell type(90%) The subtype range of our cases was almost similar to those of the cases in Western countries with a few differences.

PP2-79
THE ROLE OF THE DIFFERENTIAL EXPRESSION OF FASCIN IN CHRONIC PANCREATITIS AND PANCREATIC ADENOCARCINOMA

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Chronic pancreatitis is a condition that in some cases may simulate adenocarcinoma. The purpose of this study was to evaluate the usefulness of fascin, a monomeric actin filament bundling protein, which is known to be expressed in ductal pancreatic carcinoma, as a potential differentiating marker between the former and chronic pancreatitis when routine immunohistochemistry is inconclusive. Materials and methods: 48 specimens obtained from patients diagnosed with chronic pancreatitis and 44 specimens from patients with ductal pancreatic adenocarcinoma were studied. Whole tissue sections were immunohistochemically stained and expression of fascin was evaluated. Results: Fascin was positive in 33 out of 44 specimens with pancreatic adenocarcinoma studied. On the contrary, no positivity was observed in all of the 48 chronic pancreatitis specimens studied. In addition, fascin expression in cases of ductal pancreatic adenocarcinoma was of variable intensity related to the differentiation of the tumors with intense expression observed especially in less differentiated tumors. Discussion and Conclusions: The up-regulation of fascin in pancreatic adenocarcinoma related to the progression of the neoplasm, as well as its expression in tumors of the large bowel, the lung and skin have been well documented in previous studies. The fact that none of the chronic pancreatitis cases expressed fascin, leads to the suggestion that it could possibly be used in every day practice, along other tumor markers, especially in cases with limited available tissue samples. Therefore, the differential expression of fascin could be of diagnostic utility in cases when these two entities are hardly distinguished.

PP2-80
THE SIGNIFICANCE OF FASCIN IN THE DIFFERENTIAL DIAGNOSIS OF AMPULLARY VS PANCREATIC ADENOCARCINOMA

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Purpose: Adenocarcinomas of the ampulla of Vater are rare neoplasms, the distinction of which from pancreatic adenocarcinoma is not an easy task in every day practice. The purpose of this study is to clarify the mode of expression of human homolog of the sea urchin fascin, an actin-binding cytoskeletal protein implicated in cell motility, in neoplasms encountered in the ampullary area in comparison to its expression in ductal pancreatic neoplasms. Materials and methods: We examined 6 specimens from patients diagnosed with adenocarcinoma of the ampulla of Vater, one of which showed features of a villous adenocarcinoma and 45 specimens from patients diagnosed with pancreatic adenocarcinoma. Immunohistochemical staining for fascin was performed using the ABC method in formalin fixed, paraffin-embedded sections. Results and Conclusions: Only one specimen of ampullary adenocarcinoma expressed intense positivity of fascin, whereas two specimens showed weak focal positivity of tumor cells. The remaining three specimens, including the one with villous adenocarcinoma features, were negative. 34/45 pancreatic neoplasms expressed fascin with variable staining intensities of tumor cells. These findings suggest a possible synergic utility of fascin in diagnosing neoplasms originating from the ampulla of Vater, especially those with features of pancreatobilliary-type tumors.

PP2-81
SYNCHRONOUS OCCURENCE OF GASTROINTESTINAL STROMAL TUMORS AND OTHER PRIMARY GASTROINTESTINAL CARCINOMAS

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Gastrointestinal (GI) stromal tumor (GIST) is the most common mesenchymal neoplasm of the GI tract wall. This tumor aries in the muscularis mucosa and muscularis propria layers anywhere from the esophagus to the rectum, and its most common anatomic sites of origin are the stomach (60-70%), small intestine (20-30%), colon and rectum (5%), and esophagus (<5%). Syncronous accurence of epithelial and GIST in the GI tract is uncommon. We present a series of eight patients, from two institutions, with GIST and a second primary neoplasm occuring synchronously. The syncronous GISTs were located in the stomach (5 cases), small intestine (3 cases) and were incidentally found during the operation. The coexistent tumors were pancreatic duct adenocarcinoma, gastric adenocarcinoma (5 cases), and small intestinal adenocarcinoma (2 cases). The syncronous occurence of GISTs and other gastrointestinal malignancies is more common than it has been considered. Various hypotheses have been proposed regarding this simultaneous development, and it is not known whether or not such an association is a simple incidental coexistence or whether the two lesions are connected by causal relationship.

PP2-82
THE EFFECTS OF ERDOSTEINE, N-ACETYLCYSTEINE ON INTESTINAL TISSUE PATHOLOGICAL DAMAGE AND APOPTOSIS INDUCED BY SEPSIS

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This study investigated the frequency of apoptosis in rat small intestinale enterocyt after intraperitoneal endotoxin (LPS) injection, the effects of LPS on inflammatory markers [myeloperoxidase (MPO), tumor necrosis factor alpha (TNF-α), and vascular endothelial growth factor (VEGF)] in small intestinale damage and the protective effects of two known antioxidant agents, erdosteine and N-acetylcysteine (NAC). Male Wistar rats were divided into six groups, each composed of nine rats: two control groups, two LPS-treated groups, one erdosteine-treated group (150 mg/kg), and one NAC-treated group. LPS was intraperitoneally injected at a dosage of 20 mg/kg. Following LPS injection, the antioxidants were administered orally. The rats were killed 24 h after LPS administration. Intestinale tissue samples were stained with hematoxylin–eosin (H&E) for histopathological assessments. Apoptosis level in enterocyt was

determined by using TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick endlabelling) method. Staining of cytoplasmic TNF-α and VEGF in enterocyt, and epithelial MPO activity were evaluated by immunohistochemistry. Posttreatment with erdosteine and NAC significantly reduced the increases in the local production of TNF-α and VEGF, and epithelial MPO activity. The effects of NAC on apoptosis, the increases in the local production of TNF-α and VEGF, were weaker than the effects of erdosteine. This finding suggests that the effects of erdosteine at the administered dose on apoptosis regulation are stronger than that of NAC.

PP2-83
GASTROINTESTINAL STROMAL TUMORS – EPIDEMIOLOGY, MORPHOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF 53 CASES FROM TWO CENTERS IN BULGARIA

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For a period of five years (2002-2006) 53 cases of stromal gastrointestinal tumors (GIST) were diagnosed in pinch biopsies and surgical materials. The analysis of cases including immunohistochemical typization by CD 117, S100 protein, CD 34, smooth-muscle actin and vimentin. Only CD 117 positive tumors were recognized as GISTs. The tumors were distributed according to the patient age and gender, tumor location (esophagus, stomach, small and large intestine, or extragastric). GIST tumors were more common in women, from the middle age-group intervals (60-74 and 45-49 years of age). For all tumors the grade of malignancy was evaluated, according to the size, invasive growth, and the presence of necroses and mitotic activity. Immunohistochemical typization allows differentiation of the typical (true type) GISTs, from the other types, which show definite differentiation (leiomyomatous, myoblastic or neurogenic).

PP2-84
THE EFFECTS OF ERDOSTEINE, N-ACETYLCYSTEINE ON LIVER TISSUE PATHOLOGICAL DAMAGE AND APOPTOSIS INDUCED BY SEPSIS

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This study investigated the frequency of apoptosis and expression of VEGF in rat hepatocyt after intraperitoneal endotoxin (LPS) injection, the effects of LPS and vascular endothelial growth factor (VEGF)] in liver damage and the protective effects of three known antioxidant agents [N-acetylcysteine (NAC), erdosteine] on liver tissue. Male Wistar rats were divided into six groups, each composed of nine rats: two control groups, two LPS-treated groups, one erdosteine-treated group (150 mg/kg), and one NAC-treated group. LPS was intraperitoneally injected at a dosage of 20 mg/kg. Following LPS injection, the antioxidants were administered orally. The rats were killed 24 h after LPS administration. Liver tissue samples were stained with hematoxylin–eosin (H&E) for histopathological assessments. Staining of cytoplasmic VEGF in epithelial cells and in Apoptosis level in epithelial cells was determined by using TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick endlabelling) method. Posttreatment with erdosteine and NAC significantly reduced the rate of LPS-induced epithelial cell apoptosis and in the local production of VEGF. The effects of NAC on apoptosis, the increases in the local production of VEGF, were weaker than the effects of erdosteine. This finding suggests that the effects of erdosteine at the administered dose on apoptosis regulation are stronger than that of NAC.

PP2-85
PROGNOSTIC IMPLICATIONS OF IMMUNOEXPRESSION OF CELL CYCLE REGULATORY PROTEINS IN GASTROINTESTINAL STROMAL TUMOURS

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract with wide spectrum of biological behaviour. The evaluation of malignancy of GISTs is still difficult and risk grading for possible adverse outcome based on tumour size and mitotic counts proposed by Fletcher in 2002 have been widely accepted. The aim of this study was to elucidate the prognostic values of alterations of cell-cycle related proteins and correlate them to risk grades and mutation status of GIST. We reexamined clinically, histologically and immunohistochemically 124 cases of paraffin-embedded GISTs noted in the Tumor Registry of Clinical Centre of Serbia from the total of 418 patients operated for abdominal mesenchymal mostly visceral tumours between 1992 and 2004. There were 25 cases of low-risk grade, 24 cases of intermediate-risk grade and 69 cases of high-risk grade. In addition, mutation status in 91 cases were examined showing 4 subgroups, according to Kit mutations on exon 9 in 7 cases (7,69%) exon 11 in 59 cases (64,84%), PDGFrA mutations on exon 12 in 3 cases (3,30%), exon 18 in 7 cases (7,69%) and wildtype mutations in 15 cases (16,48%). Immunohistochemistry was performed for several cell cycle related proteins including Ki-67, p16ink4a, p21WAF1/CIP1, p27KIP1, p53 and bcl-2. The positive immunophenotypes were as follows: p53 40.68%, p21 32.2%, bcl-2 57.63%, Ki-67 47.46% (cut off values were 5% for p53 and Ki-67, and 10% for others). However, loss of expression of p16 and p27 was found in 85,6% and 83,05% of cases respectively (cut off values 50%). Altered expression of cell cycle related proteins in our series were statistically significant only for positive p53 and Ki-67 as well as negative p16 expression. PDGFrA exon 18 point mutations and exon 12 size mutations are more likely to be associated with high risk GIST. Therefore, they might be potentially useful for predicting aggresive behaviour of GIST.

PP2-86
REGULATION OF NICOTINE-INDUCED APOPTOSIS OF LIVER AND SMALL INTESTINAL CELLS BY POSTTREATMENT OF ERDOSTEINE AND N-ACEYLCYSTEINE

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Nicotine is a major toxic component of cigarette smoke that induces a wide range of biological effects in human cells. The aim of this study was to determine the effect of nicotine in small intestinal and liver tissue and to determine the protective effects of three known antioxidant agents (N-acetylcysteine (NAC) and erdosteine). Sixty three Wistar rats were divided into seven groups, each composed of nine rats: two negative control groups, two positive control groups, one erdosteine-treated group (500 mg/kg), one NAC-treated group (500 mg/kg), and one vitamin E-

treated group (500 mg/kg). Nicotine was injected intraperitoneally at a dosage of 0.6 mg/kg for 21 days. Following nicotine injection, the antioxidants were administered orally, treatment was continued until the rats were killed. In small intestinal and liver tissue samples were stained with hematoxylin–eosin (H&E) for histopathological assessments. The apoptosis level in the hepatocyte and cyrpt epithelium was determined by using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) method. Cytoplasmic TNF-α in the hepatocyte and crypt epithelial cells and MPO activity were evaluated immunohistochemically. Posttreatment with erdosteine and NAC significantly reduced the rate of nicotine-induced epithelial cell apoptosis. Posttreatment with erdosteine and NAC significantly reduced the increases in the local production of TNF-α and VEGF, and epithelial MPO activity. The effects of NAC on apoptosis, the increases in the local production of TNF-α and VEGF, were weaker than the effects of erdosteine. This finding suggests that the effects of erdosteine at the administered dose on apoptosis regulation are stronger than that of NAC.

PP2-87

CAR EXPRESSION IN GASTROINTESTINAL AND PANCREATIC ADENOCARCINOMA

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Background: Coxsackievirus adenovirus receptor (CAR) functions as the primary receptor for both coxsackie B viruses and adenoviruses, playing a crucial role for their entry into host cells. CAR expression on target tumor cells is correlated with their sensitivity to adenoviral infection. Tumor cells lacking or expressing low CAR levels are resistant to adenovirus infection and to efficient oncolysis by recombinant adenoviruses. The aim of our study was to examine CAR expression on clinical material of gastric, colon and pancreatic adenocarcinoma. Methods: CAR expression was detected immunohistochemically on paraffin-embedded tissue sections of 44 gastric, 64 colon, and 53 pancreatic adenocarcinoma cases. CAR positivity and overexpression (expression in more than the mean percentage value) were correlated with patients' gender, age, TNM stage; tumors histopathological grade of differentiation and proliferative capacity (Ki-67 immunohistochemical expression); and with patients' survival. Results: CAR positivity was noted in 26 out of 44 (59%) gastric, 53 out of 64 (82.8%) colon and 44 out of 55 (80%) pancreatic adenocarcinoma cases, examined. CAR overexpression was noted in 14 out of 26 (53,8%) gastric, in 23 out of 53 (43,4%) colon and 22 out of 44 (50%) pancreatic adenocarcinoma CAR positive cases. CAR positivity and overexpression were not statistically significantly correlated with patients' gender, age, TNM stage, tumors histopathological grade, proliferative capacity and patients' survival. Additionally, in gastric carcinoma CAR positivity and overexpression were not statistically significantly correlated with histopathological type (diffuse vs intestinal). Conclusion: Our data reveal, immunohistochemical expression of CAR protein in clinical material obtained from patients with gastric, colon and pancreatic adenocarcinoma. CAR expression was not correlated with clinicopathological variables of prognostic significance and the patients' final outcome. However, CAR expression is considered of possible clinical importance in future cancer gene therapy, as this receptor appears to modulate the proliferation and characteristics of tumor cells.

PP2-88

THE COMPARISON OF NUCLEAR MORPHOMETRIC ANALYSIS WITH CLASSICAL PROGNOSTIC FACTORS IN COLORECTAL CARCINOMAS

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Colorectal carcinomas are one of the most commonly seen malign tumor in the world. Despite innovations in diagnosis and treatment, it has been continuing to become serious problem as regards mortality and morbidity. In order to recognize the prognosis in colorectal carcinomas, a lot of clinic and pathologic parameters. As most of these parameters are subjective, there need more objective and useful prognostic factors in determination of suitable treatment options by assessing the whole biologic behavior before. In this study, operation materials belonging to 83 colorectal carcinoma cases were examined retrospectively. In the study, by mean of morphometric method, nuclear area, nuclear surrounding, minimum and maximum diameter in tumor cells were carried out. We couldn't find relationship between morphometric data and age, sex and tumor localization. Nevertheless, there was statistical relationship between the result of morphometric measurements and histological type, grade, invasion depth of the tumor, lymph node metastasis, vascular invasion, inflammatory reaction, stage and life durations. It was found out that nuclear area became related to stage, life duration and histological type. The maximum nuclear diameter was related to histological grade. In conclusion; colorectal cancers, the values of nuclear morphometric measurement obtained by means of image analysis method are worth of being investigated as the indicator of prognosis.

PP2-89

GASTROINTESTINAL LYMPHOMAS: CLINICOPATHOLOGIC AND IMMUNOPHENOTYPIC FEATURES

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Gastrointestinal lymphomas (GIL) comprise a group of distinctive clinicopathological entities of B- or T-cell type. Most low-grade B-cell gastrointestinal lymphomas are of mucosa-associated lymphoid tissue (MALT) type and most MALT lymphomas arise in the stomach to arise as a reaction to infection of Helicobacter pylori. Low-grade MALT lymphomas, which usually have a very favorable clinical course, may undergo high-grade transformation but high-grade diffuse large B-cell lymphomas may also arise de novo. In this study, clinicopathologic and immunophenotypical features of GIL which were diagnosed in last 5 years, at our department were reviewed. Method: During the period from 2001 to 2006, 36 gastrointestinal resection specimens were diagnosed as GIL in our department. Their hematoxylin-eosin stained slides were reexamined by standard light microscope and an immunhistochemical panel was used including Ki67, CD20, CD3 or CD5 for all and bcl-2, bcl-6 and others for some cases. The features were statistically analyzed by multiple comparison tests and the results were discussed. Results: Of the 36 patients age range was from 17 to 81 with a median age of 54.5 years. 20 cases were female, while 16 were male. The most common location of tumors was stomach (22 cases), 5 cases located in the small bowel, 4 were in the colon, 2 of them were both in the small bowel and colon together. 3 cases took place in salivary gland. All of the cases were B-cell type lymphomas, as all expressed B-cell phenotype immunohistochemically. 22 cases were diffuse large B-cell type (DLBCL) where 8 cases were MALT lymphomas. 4 DLBCL cases had MALT components. 12

cases which all were DLBCL had high proliferation rate with Ki67 score over than 50%. However, there were no statistical differences between the clinicopathologic features of MALT type and DLBCL and proliferation rates of DLBCL cases with or without MALT components. Conclusion: Primary GIL represents the most common location of extranodal lymphomas and gastric lymphomas are the majority of them. Gastric MALT lymphoma occurs predominantly in individuals over 50 years. Some DLBCL cases of the stomach in which a MALT lymphoma component cannot be detected are transformed MALT lymphomas that have been completely overgrown by DLBCL, but others are true primary DLBCL. However, there is no difference in the clinical behavior between transformed MALT lymphoma and primary DLBCL.

PP2-90

C-FLIP EXPRESSION IN COLORECTAL CARCINOMAS: ASSOCIATION WITH FAS/ FASL EXPRESSION AND PROGNOSTIC IMPLICATIONS.

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Backround: Disruption of apoptotic cell death has been implicated in tumour aggressiveness in colon carcinogenesis. The Fas-Fas ligand (FasL) system is involved in the execution of apoptosis induced by the immune system. c-FLIP protein constitutes an inhibitor of Fas and other (TRAIL) death receptor-mediated apoptosis. Published information regarding the significance of c-FLIP overexpression in human tumours has only recently begun to accumulate. However, there is as yet no study addressing the issue of concurrent Fas/FasL expression and c-FLIP overexpression in colorectal carcinoma. Therefore, in this study, we investigated the simultaneous expression of Fas, FasL and c-FLIP in relation to standard clinicopathological parameters and patients' outcome in this tumour. Methods: Fas, FasL and c-FLIP protein expression levels were quantified immunohistochemically in paraffin-embedded tissues from 90 patients with colorectal carcinomas, by using the following rabbit polyclonal antibodies, diluted 1:200: 1) Fas 2) FasL, and 3) c-FLIPS/L. The labelling index (LI) was defined as the percentage of neoplastic cells with clear cytoplasmic and/or cell surface immunoreactivity out of the total number of neoplastic cells counted. Basic statistical analysis was performed in order to determine the associations of LIs with several clinicopathological parameters as well as their prognostic significance. Results: Positive immunostaining was detected for Fas, FasL and c-FLIP in 71%, 35.5% and 68.8% of cases respectively. Concurrent expression of Fas/FasL was seen in 28 samples (31%) of which 24 (85.7%) also displayed c-FLIP positivity (p=0.04). Univariate logistic regression analysis showed that Fas, FasL and c-FLIP LIs were unrelated to grade, stage or tumour location (P>0.10). When analysis was performed on a categorical basis, c-FLIP over-expression (>10%) seemed to marginally prevail in higher stage tumours (III/IV vs I/II p=0.09). A positive correlation also emerged between c- FLIP and FasL expression (R=0.3115, P=0.0028). Additionally, FasL and c-FLIP labelling indexes adversely affected survival in both univariate (p=0.001 and p=0.0024 respectively) and multivariate analysis (HR=3.491, p=0.005 and HR=2.960, p=0.036 respectively). Conclusions: The frequent expression and co-expression of Fas, FasL and c-FLIP in colorectal carcinomas implicates c-FLIP as an inhibitor of the Fas-FasL-induced death pathway in these tumours. Moreover, c-FLIP conveys independent prognostic information in the presence of classical prognosticators.

PP2-91

HISTOPATHOLOGIC CHANGES OF GUT MUCOSA DURING ANESTHESIA AND ABDOMINAL SURGICAL PROCEDURES

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A perfusion and integrity of small bowel mucosa were examined during anesthesia and surgery on open abdomen in 60 patients in various length treatments. Patients (44 man, 16 woman) age range was 21 to 80 years (mean age 58 years). During the surgery, two samples of the intestinal mucosa were removed: T0 at the beginning of the procedure, and TN at the end (N = treatment duration in hours – 2 to 7 hours). All the biopsies were examined and classified according to the observed mucosal injury into 4 grades (Knichwitz: Crit Care Med 1998;26(9):1550-7): grade 0 – no injury, grade 1 – isolated hemorrhage in lamina propria, grade 2 – lifting of the villus epithelium and isolated hemorrhage, grade 3 – damaged villi and hemorrhage, grade 4 - damaged villi and ulcerations with necrosis. The changes of intestinal mucosa were found in 31 patients: in 19 patients grade 1 changes were recorded, in 10 patients grade 2, and in 2 patients grade 3. Grade 4 lesions were not recorded. During the anesthesia and surgery on open abdomen, the length of the procedure influences the grade of the intestinal mucosa injury. Aggravation of the patohistological changes in intestinal mucosa correlates with high lactate blood level, proving that the cause of these changes is connected with tissue hypoxia.

PP2-92

PSAMMOMATOUS AND DIFFUSE CALCIFICATION IN GASTRIC CARCINOMA: REPORT OF TWO CASES WITH LITERATURE REVIEW

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Background: Psammoma bodies are round, concentrically laminated calcospherits ranging from 5 m in diameter. They may indicate certain types of tumors includingμto 100 papillary carcinoma of thyroid, meningioma and papillary serous tumors of the ovary, and to a lesser extent contribute leiomyomas and angiomas of gastrointestinal tract. Distrophic calcification is uncommon in gastric cancer and displays either diffuse or psammomatous pattern. Diffuse type calcification is generically seen within the pools of mucin in advanced mucinous adenocarcinoma. Conversely, psammomatous calcification is associated with non-mucin producing carcinomas and detected not only within the carcinomatous glandular lumina but also in the stroma. Case: Total gastrectomy specimens of a 54 year-old female and 74 year-old male with ordinary moderately differentiated intestinal type adenocarcinomas revealed psammomatous and diffuse calcification, respectively. Conclusion: Although diffuse type calcification is well-documentated, psammomatous type is exceptionally rare and only 6 such cases have been reported in the literature; the current case represents the seventh one.

PP2-93
CK7 AND CK20 IMMUNOREACTIVITY IN PRIMARY CARCINOMAS OF THE COLON
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The expressions of cytokeratin (CK) 7 and 20 have been studied in various primary and and metastatic carcinomas, and their determination may help distinguish the site of origin of origin of metastatic carcinomas. Colorectal carcinomas consistently express CK20, whereas CK7 is found in many ductal and glandular epithelia, including the lung, breast, ovary, and endometium and their carcinoma cells. CK7 expression in colorectal carcinoma has been reported to be rare, and when present, a metastatic origin needs to be excluded. We investigated the expression of CK7 and CK20 in 107 cases of colon carcinomas. Parafin sections of 78 left side colorectal carcinomas, and 29 rigtht side colon carcinomas were immunostained for CK7 and CK20 with standart avidin-biotin complex method. Cytoplasmic CK20 immunoreactivity was detected in 65 of 107 (61%) while cytoplasmic CK7 immunoreactivity was detected 17 of 107(16%) colorectal carcinomas. In term of the combined expression patterns, CK20+/CK7- was the most common pattern, accounting for 51% of colon carcinomas. The CK20-/CK7+ pattern was seen in 7 cases (7%). The CK20+/CK7+ pattern was seen in 10 cases (9%). No association between CK7 expression and anatomic location of carcinomas, tumor size, and grade was found. There was a correlation between CK 7 expression and lymph node metastasis (p[LTEQ]0.02). No association was established among CK20 expression, and grade, and stage. CK20 positivity was more common in left side colon carcinoma (55/65, 85%). There was an association between CK20 expression and anatomical location of tumors which was statistically highly significant (p=0.001). Because of the CK7 expression in colon adenocarcinomas, when CK7 is applied to distinguish primary colorectal versus metastatic origin, its reactivity should be interpreted with caution and should not be used as the sole evidence for excluding a rectal primary, particularly in tumor involving the rectal or pelvic region.

PP2-94
A CASE OF GASTRIC LARGE CELL NEUROENDOCRINE CARCINOMA WITH PANETH CELL DIFFERENTIATION
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A distinct morphological variant of a high-grade gastric neuroendocrine carcinoma of nonsmall cell type (large cell neuroendocrine carcinoma) of the stomach with Paneth cell differentiation was reported. The tumor was a Borrmann's Type III carcinoma measuring 9.0 x 5.5 cm at the body along the lesser curvature. Histologically it was composed of Paneth cells in addition to poorly differentiated adenocarcinoma cells.. The Paneth cell differentiation was characterized histologically by cytoplasmic distinct coarse eosinophilic granules stained red with periodic acid-Schiff and Masson trichrome reagents and reddish brown with phosphotungstic acid hematoxylin, and immunohistochemically by lysozyme and alpha-1 antitripsyn positivity. Immunohistochemically the tumor with poorly differentiated adenocarcinoma morphology were strong and diffusely positive for synaptophysin and chromogranin A. This case is unusual, since Paneth cells occur only rarely in adenocarcinomas of the stomach. The incidence and significance of the Paneth cell and endocrine differentiation in gastric carcinomas were discussed with the review of the literature.

PP2-95
THE PROGNOSTIC IMPORTANCE OF EGFR EXPRESSION AND GENE AMPLIFICATION IN COLORECTAL ADENOCARCINOMAS
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BACKGROUND: Epidermal Growth Factor Receptor (EGFR) is a transmembrane protein located at 7th chromosome and which includes tyrosine kinase activity. EGFR and its ligand types provide the growth and regeneration of intestinal mucosa. Accordingly, it can be considered to be related with generation and improvement of gastrointestinal system tumours. In Gastric and Colorectal Carcinomas, by the combination of immunohistochemistry and Fluorescent in Situ Hybridization (FISH) methods, overexpression of EGFR has been shown. The main aim of this research is to show the relationship of EGFR protein expression and gene amplification with each other and with other morphological prognostic parameters at colorectal carcinomas. METHOD: Sixty colorectal adenocarcinoma cases diagnosed at Dr. Lutfi Kirdar Kartal Educational and Research Hospital were investigated. Gene amplification with EGFR overexpression was assessed by using immunohistochemistry and FISH methods. Morphological parameters were age of the patient, gender of the patient, histological grade, local invasion, vein invasion, perineural invasion, lymphoid metastases, stage and the lymphocytic response to the tumour. RESULTS: At immunohistochemical examination, no staining was observed with EGFR at 19 cases (31.6%). There was (+) staining at 13 cases (21.6%), (++) staining at 24 cases (40%) and (+++) staining at 4 cases (6.6%). Nine (75%) of the poor differentiated cases showed staining with EGFR while 3 (25%) of them had no staining. Five (40.2%) of the well differentiated cases did not stain with EGFR while 7 of them (50.8%) showed staining. With FISH method, 8 (13.3%) cases had gene amplification and 52 (86.7%) had none. At the cases showing gene amplification, EGFR expression was seen immunohistochemically. CONCLUSION: As the tumour differentiation becomes poorer and its stage becomes higher, EGFR expression will increase. However, a statistically significant relation between EGFR expression and prognostic parameters is not observed. Besides, at the 8 cases showing gene expression, although EGFR expression was seen immunohistochemically, no statistical significance was found between the parameters. Therefore, mechanisms other than gene amplification may be responsible for EGFR expression. These results may suggest that at colorectal adenocarcinomas, for the evaluation of the response to anti-EGFR monoclonal antibody therapy, molecular diagnostic values as FISH may not be appropriate.

PP2-96
THE DIFFERENCE IN CYCLIN D1, HSP 105, EGFR, RB, AND MSH2 GENE EXPRESSIONS IN CHRONIC GASTRITIS WITH AND WITHOUT HELICOBACTER PYLORI INFECTION
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Background: The main goal of the present study is to show differences in the expression of various genes in patients with chronic gastritis with and without Helicobacter pylori infection. Method: Sixty three samples between 2005 and 2006 were available for the study, including 20 samples with normal gastric fundic mucosa, 22 with moderate to severe chronic gastritis in

fundic mucosa with Helicobacter pylori infection, and 21 with moderate to severe chronic gastritis in fundic mucosa without Helicobacter pylori infection. No samples with chronic gastritis had intestinal metaplasia or dysplasia. Expression of cyclin D1, HSP 105, EGFR, RB, and MSH2 were detected by means of immunohistochemical method. Results and Conclusion: Expression of HSP 105, cyclin D1, and EGFR in gastric mucosal crypts were higher in patients with chronic gastritis without Helicobacter pylori infection than in those with Helicobacter pylori infection. In contrast, RB was higher in patients with chronic gastritis with helicobacter pylori infection. No reaction was detected for MSH2 in any of the samples. Similar results were observed in lymphoid infiltrate of gastric mucosa, excluding cyclin D1. The gene expressions in both crypt cells and lymphoid infiltrate of gastric mucosa with Helicobacter pylori infection were different from chronic gastritis without Helicobacter pylori infection. This result may have been due to the products of Helicobacter pylori. In addition to the bacterial products, some chemical mediators of inflammation in chronic gastritis with Helicobacter pylori infection may have an effect on immunohistochemical reactivity of some genes. Consequently, the immunohistochemical reactivity may be decreased or increased for different genes. These variations are possibly because of alteration in antigenic structure of gene products or changing of gene expressions. In conclusion, these different expression profiles for some genes may explain the difference in behavior of chronic gastritis with Helicobacter pylori infection from chronic gastritis without Helicobacter pylori infection.

PP2-97
MACROSCOPIC AND HISTOPATHOLOGIC EVALUATIONS OF SMALL INTESTINE AND COLON RESECTION SPECIMENS DIAGNOSED AS CROHN DISEASE

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PURPOSE: The aim of this study is to evaluate the macroscopic and histopathologic findings on the resection specimens of colon and small intestine on people who are operated with different reasons and diagnosed with Crohn Disease, to determine the frequency of findings resulted in Crohn Disease diagnosis, to establish the accuracy of histopathologic diagnosis on these cases which has pre-operative endoscopic biopsies and compare them with mucosal lesions in specimens.MATERIALS AND METHODS: The colon and small intestine resection specimens of 28 cases diagnosed as Crohn Disease which are hospitalized in Haydarpaşa Numune Education and Research Hospital Pathology Laboratory between July 2003-February 2007 are used as study groups. Among these cases, 11 had pre-operative endoscopic biopsies. Macroscopic findings evaluated from their pathologic reports and frequencies were determined. Cases' preparations prepared with Hematoxylen-Eosine were reevaluated and frequency of histopathologic properties stated after comparing them to microscopic findings on pathology reports. The accuracy of pre-operative endoscopic biopsy diagnosis was determined. Mucosal lesions of endoscopic biopsies and resection pieces were compared.RESULTS: Cases include 19 male and 9 female patients. Ages are between 17-81. 14 patients' pre-operative diagnosis was Crohn Disease. The other 14 patients were operated because of 2 acute appendicitis, 6 masses, 2 intestinal obstructions, 1 relapse Crohn Disease or mass, 1 terminal ileitis, 1 enterocutanous fistulae, 1 mesentery ischemia. From pathological reports 15 cases were directly diagnosed as Crohn Disease and 13 were said to be related with Crohn Disease after the histopathologic descriptions were done. In the macroscopic evaluations of resection specimens; % 39.2 gato formations, % 23 membrane appearances on serosal surface, %7.14 antimesenteric lipid appearance, %17.8 thickening on intestine wall and firm consistency and %17.8 construction of lumen were found. On mucosa in addition to normal appearance pili disorder as focal centers, hyperemia and edema was %17.8, %67.8 ulcers, % 35.7 disappearances of pilis and %10.7 cobble stone appearances were established. Microscopic findings include; %92.8 focality (structural anomaly of crypt and villus and lymphoplasmasiter cell infiltration in lamina propria), %57.1 focal mucosal ulcer, %7.1 common mucosal ulcer, %46.4 fissure, %17.8 basal plasmasitosis, %35.7 antral metaplasia. Transmural lesions were determined as; %46.4 submucosal edema and fibrosis, %35.7 lymphoid aggregate, %39.2 neuronal hypertrophy, %57.1 serosal fibrosis and %17.8 granuloma. During our work, membrane appearance on serosa (peritonitis), gato formation, thickening of intestine wall and construction of lumen were the most frequently seen properties of macroscopic findings. Commonest histopathologic findings were focality, ulcer, fissure, submucosal edema and fibrosis, neuronal hypertrophy, transmural lymphoid aggregate and serosal fibrosis. 11 cases were evaluated with pre-operative endoscopic biopsy. 2 of these diagnoses were related with Crohn Disease mucosal lesions and 9 were nonspecific for Crohn Disease. These 9 cases were diagnosed as following; 2 focal active chronic colitis, 1 inflammatory pseudopolip, 1 acute and chronic nonspecific inflammation, 1 suspect lymphoma, 2 normal colon mucosa and inadequate small intestine mucosa.

PP2-98
MALIGNANT HISTIOCYTOFIBROMA OF THE PANCREAS / A CASE REPORT WITH REVIEW OF THE LITERATURE

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INTRODUCTION: The cancer of the pancreas remains bad forecast for the common forms of adenocarcinoma; it is not less in the unusual and rarer types like the sarcoma; since, in the large majority of the cases, the assessment of extension made at the moment of the diagnosis shows that the tumour was impossible to remove, either because of a vascular invasion, or because of the presence of nodes and/or hepatic metastasis. The clinical signs are not specific and are late, generally translating the invasion of the bodies of vicinity; the two most frequent symptoms are the abdominal icterus and pains. Material and Method: It is about the patient A.Z 57 years old who presented abdominal pain for 3 months and an important and generalized icterus . The scanner reveals a process of 3,5 cm in the head of the pancreas with proximal nodes. A cephalic duodenopancreatectomy was performed. The morphological study, showed an intracystic heterogeneous lesion of 4 cm in largest axis and altered by necrosis and haemorrhage corresponded to malignant histicytofibroma with infiltrated nodes. EVOLUTION: The patient had presented a syndrome of cytolysis and died after 6 days. CONCLUSION: This sarcoma is rare and of unusual localization whose fatal exit will always remain of dark forecast . No chemotherapy currently clearly was the proof of its effectiveness and the treatment remains palliatives for the most invalidating symptoms like the icterus and the pains.

PP2-99
MGMT EXPRESSION IN COLORECTAL CANCER: RELATION TO CLINICOPATHOLOGIC AND MOLECULAR FEATURES

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Background: The O6-methylguanine-DNA-methyltransferase (MGMT) is a DNA repair protein that removes mutagenic and cytotoxic adducts from O6-guanine in DNA. MGMT inactivation by promoter methylation is thought to play a major role in the development of a subgroup of colorectal tumors. Aim of this

study was to evaluate whether colorectal adenocarcinomas (CRCs) with MGMT inactivation display distinctive clinical, pathologic and molecular features. Methods: MGMT protein expression was evaluated by immunohistochemistry in a series of 196 surgically resected CRCs using the anti-MGMT monoclonal antibody MT3.1. Tumor DNA mismatch repair (MMR) status was determined by immunohistochemical evaluation of MLH1, MSH2 and MSH6 expression and microsatellite genotyping. In addition, MGMT and MLH1 promoter methylation were assessed in 57 tumors using methylation-specific PCR. Results: Loss of MGMT expression was detected in 44 (22.4%) of the 196 tumors examined. In most cases (40/44) a complete loss of MGMT immunoreactivity was observed. MGMT expression was closely related to MGMT methylation status. MGMT promoter methylation was observed in 11 of the 13 MGMT-negative (84.6%) and in 6 of the 44 MGMT-positive (13.6%) carcinomas examined (P< 0.001). MGMT-negative carcinomas occurred more frequently in the proximal colon (P= 0.02) and among patients older than 70 years (P= 0.01). No significant relationship was found with other clinical and pathologic features. MGMT expression was found to be related to the MMR status of the tumors. Infact, loss of MGMT reactivity was observed in 13 of 40 (32.5%) MMR-defective tumors and in 31 of 156 (19.9%) MMR-proficient carcinomas. Specifically, loss of MGMT expression occurred in 33 of 168 (19.6%) MLH1-positive carcinomas and in 11/28 (39.3%) MLH1-negative carcinomas (P= 0.02). To more properly define the relationship between MMR status and MGMT expression, we examined further 63 MSI-H colorectal adenocarcinomas and evaluated MLH1 promoter methylation in a subset of cases. Lack of MGMT expression was detected in 34/82 (41.5%) MSI-H MLH1-negative and in 1/21(4.7%) MSI-H MSH2 or MSH6-negative carcinomas (P= 0.01). A significant relationship between MGMT and MLH1 promoter methylation was observed in the 57 cases examined by methylation-specific PCR (P< 0.01). Conclusion: Our results indicate that MGMT-negative CRCs are characterized by certain clinical and molecular features. The role of MGMT expression in cancer recurrence and in disease-free and overall survival after curative surgery and chemotherapy needs to be carefully evaluated.

PP2-100

DIFFUSE NODULAR LYMPHOID HYPERPLASIA: CASE REPORT

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Introduction: Diffuse nodular lymphoid hyperplasia is a very rare lesion of the gastrointestinal tract which is especially encountered in patients with primary immune deficiency and is defined as the presence of multiple lymhoid nodules in the small and large bowels. The pathognesis of diffuse nodular lymphoid hyperplasia is unclear. It is two times more common in women. Diffuse nodular lymphoid hyperplasia is a risk factor for all gastrointestinal tumors but for gastrointestinal lymphoma in the first line. Case presentation: The endoscopic examination of a 42 years-old male patient with diarrhea revealed grape-bunch like nodular appearance in the stomach and duodenum and biopsies from the related regions were sent to our department. The histologic sections of the upper gastrointestinal endoscopic biopsies displayed superficial erosion, intestinal metaplasia and active-chronic gastritic changes of the epithelium. Additionally, the mucosa and the submucosa contained localized lymphoid follicles with prominent germinal centers. The following lower gastrointestinal endoscopy of the patient showed similar findings in the terminal ileum and colon. All biopsy samples were immunhistochemically stained with CD-3, CD-20, CD-43, CD-5, CD-10, Cyclin-D1 and bcl-2 for the differential diagnosis of lymphoma and no findings suggestive of lymphoma were observed. We found our case worth presentation because diffuse nodular lymhoid hyperplasia is a rare lesion which may mimic lymhoma both in endoscopic and histopathologic examination and it should be considered in the differential diagnosis.

PP2-101

NEOADJUVANT THERAPY IN RECTAL CARCINOMA: STUDY OF 28 CASES

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INTRODUCTION: Local advanced the rectal carcinoma has a private prognostic. However, since the introduction of the neoadjuvant radiochemotherapy, the evolution of these neoplasias has suffered deep modifications. However, until the moment, no histopathologic features has been told as predictive factor to the reply to the therapeutical neoadjuvant, even so some molecular alterations have been considered as potential markers. An increase in the supervened one of the patients with retal cancer is one of the biggest challenges of the oncologic surgery, a time that only half of the patients one has supervened average one of 5 years. Thus, different therapeutical protocols have been considered, such with association of the neoadjuvant radiotherapy and chemotherapy. This therapeutical boarding induces a tumoral regression of the 60% order, causing an important reduction the recurrence frequency local and an improvement in the taxes of overlife. The objective of the present study is to evaluate the impact of the radiochemotherapy preoperative in the patients with local advanced rectal carcinoma, through the study of clinical and anátomo-pathological characteristics. MATERIAL AND METHODS: To estimate the effects of the radio and chemotherapy preoperative in the rectal carcinoma, the authors had studied 28 surgical specimens of patients with advanced rectal carcinoma (Stage T3 and T4) diagnosed through biopsy. All the patients had been submitted the neoadjuvant radiochemotherapy. All the surgical specimens had been examined by an only pathologist and all the area of the injury is submitted the histopathologic examination. Using a standardized boarding, the following morphologic parameters had been evaluated: Index of tumoral regression; degree of cytological atipia; intensity of the inflammatory reaction and tumoral necrosis. The tumoral regression was graduated in agreement criteria praised for Mandard et al, that it used a scale of 1-5 established one in the absence of residual tumoral cells and in the extension of fibrosis. Other conventional parameters had been also analyzed, among these the age, the sex, the histologic type (WHO), the surgical depth of tumoral invasion, linfonodal status and still the clinical and pathological stage. RESULTS/CONCLUSION: Between the 28 patients who had been studied, it had complete tumoral regression in 10 (35.7%) and minim or no tumoral regression in 18 (64.3%).

PP2-102

INCREASED EXPRESSION OF NUCLEAR FACTOR-KAPPA B/P50 CORRELATES WITH DISEASE PROGRESSION AND PROGNOSIS IN COLORECTAL ADENOCARCINOMA

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Background: The nuclear transcription factor nuclear factor-kappa B (NF-kB) has been found to play a role in cancer development and progression through its influence on apoptosis. The aim of this study was to determine whether NF-kB is constitutively activated in colorectal cancer tissues and to determine the role of NF-kB in colorectal cancer development and tumor progression. Method: Immunohistochemical staining was performed using the p65 and p50 subunits of NF-kB in normal and neoplastic colorectal tissues. To identify the correlations between clinicopathological parameters and the survival, expression of both the NF-kB/p65 and NF-kB/p50 was assessed in 210 consecutive patients with colorectal carcinomas who had undergone colectomy or polypectomy due to intramucosal carcinoma arising from pre-existing adenomas. Results: The immunoreactivity of both the NF-kB/p65 and NF-kB/p50 was significantly increased in the transition from normal mucosa and adenoma with low dysplasia to adenocarcinoma ($p<0.05$). Among the 210 cases of adenocarcinoma, NF-kB/p50 immunoreactivity was significantly correlated with the angiolymphatic invasion ($p< 0.01$), lymph node metastasis ($p<0.05$), liver metastasis ($p<0.05$), distant metastasis ($p<0.05$), and increasing stages of the Dukes classification ($p<0.05$). On the other hand, well-differentiated adenocarcinoma is showed significantly higher expression of NF-kB/p65 than moderate and poorly differentiated adenocarcinomas ($p<0.05$). Importantly, the patients with expression of NF-kB/p50 (but not of NF-kB/p65) had significantly worse prognosis (Log-rank test, $p=0.001$). On multivariate analysis, Dukes stage ($p=0.001$) and NF-kB/p50 expression ($p=0.003$) were independent prognostic indicators. Conclusion: These results suggest that, both the NF-kB/p50 and NF-kB/p65 play an important role in colorectal tumorigenesis. Overexpression of NF-kB/50 is showed aggressive clinical biology and poor outcome; thereby suppression of NF-kB may be a useful therapeutic strategy for a subset of patients with colorectal carcinoma.

PP2-103
MICROVESSEL DENSITY AND IMMUNOHISTOCHEMICAL EXPRESSION OF ANGIOGENIC PROTEINS VEGF, VEGFR1 AND VEGFR2 IN GASTRIC ADENOCARCINOMA

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Background. Recently, many studies have demonstrated that angiogenesis is related to tumor growth and progression. Vascular endothelial growth factor (VEGF) is one of the most important angiogenic factors and acts through its specific receptors VEGFR1 and VEGFR2. In the present study, we analyzed the immunohistochemical expression of VEGF, VEGFR1 and VEGFR2 proteins as well as the microvessel density (MVD) in relation to the proliferation profile (expression of Ki67 and cyclin A proteins), p53 protein expression and clinicopathological parameters in a series of gastric adenocarcinomas. Materials and Method. Tissue specimens from 69 cases of surgically resected gastric adenocarcinomas were studied immunohistochemically for the expression of VEGF, VEGFR1 and VEGFR2 proteins. MVD was estimated after immunostaining for CD34 (MVD-CD34) and CD105 (MVD-CD105) and evaluated as the mean (±SD) vessel count/high-power microscopic field (HPF). Spearman's correlation coefficient test was used for the statistical analysis. Results. Expression of VEGF, VEGFR1 and VEGFR2 proteins was found in 49/69 (71.4%), 56/68 (82.4%) and 46/68 (67.6%) cases, respectively. The MVD-CD34 and the MVD-CD105 were 59.96 (±23.5) and 20.94 (±16.82), respectively. VEGF expression was positively correlated with VEGFR1 and VEGFR2 protein expression ($p=0.027$ and $p=0.011$, respectively). Moreover, a similar correlation between the expression of VEGF and Ki67, cyclin A and p53 proteins was found ($p=0.038$, $p=0.01$ and $p=0.001$, respectively). Conclusions. The results of this study suggest that in gastric adenocarcinomas a) the expression of the angiogenic proteins VEGF, VEGFR1and VEGFR2 is a common event, b) VEGF may act as an autocrine growth factor and c) VEGF protein expression may be regulated by p53.

PP2-104
CALBINDIN – D28K AND PARVALBUMIN IMMUNOREACTIVITY IN HUMAN GASTROINTESTINAL CARCINOMAS

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Calbindin D28k (Ca-D28k) and parvalbumin (PV) are calcium-binding proteins which maintain cellular calcium homeostasis. They express mainly in central and peripheral nervous system. Ca-D28k is thought to play a role in inhibiting apoptosis. The aim of this study was to investigate the expression of CA-D28k and PV in tumour tissue of gastrointestinal adenocarcinomas and neuroendocrine carcinomas using light and electron microscope immunohistochemical techniques. Material and methods. 17 adenocarcinomas and 15 neuroendocrine carcinomas with different differentiation were immunostained with the both antibodies using peroxidase - antiperoxidase technique and immunogold technique. Results. Light microscopically intracellular localization of Ca-D28k and PV was found in 11.7% /2 cases/ and 17.6 7% /3 cases/, respectively in adenocarcinomas and 66.6% /10 cases/ and 86.6% /13 cases/, respectively in neuroendocrine carcinomas. Immunoreactivity varied from weak to moderate. The both proteins were found in a few cells in gastric and intestinal mucosa next to the carcinomas. In well-differentiated neuroendocrine carcinomas Ca-D28k and PV were found mainly in the nuclei of tumor cells and perinuclear zones while in poorly differentiated neuroendocrine carcinomas the both peptides were localized mainly in tumor cell cytoplasm. In adenocarcinomas the expression was mainly in tumor cytoplasm. Ultrastructurally Ca-D28k and PV were found in the nucleus and next to nucleus in tumour cells in well-differentiated neuroendocrine carcinomas. In poorly differentiated neuroendocrine carcinomas and in the adenocarcinomas the both proteins were localized mainly in the cell tumour cytoplasm. Conclusion. Our results reveal that Ca-D28k and parvalbumin can be used in the diagnostic tumour immunohistochemistry. The presence of the both proteins in the nuclei and the perinuclear zone in well-differenciated neuroendocrine carcinomas suggest a participation in the regulation of tumour cell apoptosis.

PP2-105
A CASE OF COLLISION TUMOR OF THE RECTUM; COMPOSED OF A CONVENTIONAL ADENOCARCINOMA AND LARGE CELL NEUROENDOCRINE CARCINOMA

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Collision glandular-neuroendocrine tumors of the gastrointestinal tract are extremely rare. The term "collision" applies when glandular and neuroendocrine components are closely juxtaposed but the individual cell types are not mixed. The aim of our article

was to describe a collision tumor involving the rectum. Our patient was a 75-year-old man who presented with rectal bleeding. Two necrotic hypervascular masses were detected in the liver and the wall of the rectum by computerized tomography. Histological examination showed of a tumor composed of an adenocarcinoma of conventional type and a component consisting of large round to polygonal cells exhibiting clear-cut neuroendocrine differantiation morphologically and immunohistochemically. These two components were closely juxtaposed but the individual cell types were not mixed. Adenocarcinoma was located superficially and continued with dysplastic surface epithelium. The neuroendocrine component was deeply infiltrating. Metastatic tumor was found in 6 of regional lymph nodes. Although the metastatic lymph nodes contained both the conventional adenocarcinoma and a large cell neuroendocrine carcinoma, a needle biopsy from the liver masses were consisted only of the metastatic conventional colonic adenocarcinoma. To our knowledge, this is the first report of collision tumor occuring in the rectum.

PP2-106
MALIGNANT PERITONEAL DECIDUOID MESOTHELIOMA: A CASE REPORT

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Malignant Deciduoid Mesothelioma is a rare morphological variant of epithelioid mesothelioma. Frequently it arises from the peritoneum of young women. We present a case of malignant deciduoid mesothelioma in a 17 years old man. He presented with peritoneal mass and abdominal pain and his clinic was very aggressive. His physical examination revealed ascites. He underwent laparoscopic exploration and granuloma-like masses of the peritoneum, omentum and mesothelial surface were seen. Microscopically, there was diffuse deciduoid cellular proliferation. The immunohistochemical staining showed positivity for calretinin and the case was evaluated as malign peritoneal deciduoid mesothelioma. He progressed so quickly that he died four months after initial diagnosis. This observation confirms the poor prognosis of this rare entity.

PP2-107
UNUSUAL TUMOUR OF THE STOMACH

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Introduction: Gastro-intestinal stromal tumors (GIST) are rare accounting for 2.2% of malignant gastric tumors. Histologically, they vary from cellular spindle cell tumors to epithelioïd ones. Pleomorphic variant is rare. We report an unusual case of malignant GIST particular by marked and diffuse cellular pleomorphism. Observation: A 65-year-old woman had a 6 week-history of vague abdominal discomfort. The endoscopy and the computed tomography demonstrated a hudge epigastric mass that was 15 cm in diameter. The patient underwent a gastrectomy. On macroscopic exam, the tumor was intra-mural, protruding into the gastric lumen; on sectoning, the tumor was well circonscribed with massive necrosis. On histopathologic exam, the tumor was composed of slightly cohesive epithelioid cells with clear cytoplasm and pleomorphic nuclei mixed with numerous giant cells; the mitotic activity was high. The tumor showed a strong positivity for c-kit, CD34, vimentine and a slight positivity for protein S100. It was negative for smooth muscle actin (SMA). The diagnosis of pleomorphic variety malignant stromal tumor of the stomach was made. The patient have not received any adjuvant therapy and she was alive without evidence of disease 3 months later. Conclusion: The pleomorphic variety of GIST is very rare, the marked and diffuse pleomorphism is unusual and should alert to the possibility of other differential diagnosis. Among GIST, the pleomorphic variety suggests a worse biologic behavior.

PP2-108
SYNCHRONOUS GASTRIC ADENOCARCINOMA AND SQUAMOUS CELL LUNG CARCINOMA WITH LIVER AND NODE METASTASES

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Background: Gastric adenocarcinoma and squamous cell lung carcinoma are quite common entities. The purpose of this study is to present an unusual case of synchronous gastric adenocarcinoma and squamous cell lung carcinoma of the left lower lobe. The gastric adenocarcinoma showed metastasis to the paraesophageal and gastric lymph nodes, to one peribronchial lymph node and lymph nodes of the carina, as well as liver metastasis. The lung carcinoma showed metastasis to peribronchial lymph nodes. Method: The patient was male, 63 years old. Endoscopic examination revealed a gastric adenocarcinoma while CT scanning revealed tumors in the liver and the lower lobe of the left lung, as well as enlargement of the peribronchial lymph nodes and lymph nodes of the carina. Results: The patient underwent gastrectomy, lobectomy and limited hepatectomy including the tumor. Also subcarinal lymph nodes were excised. Microscopic evaluation revealed a moderately differentiated gastric adenocarcinoma of intestinal type according to Lauren classification, which infiltrated the whole gastric wall, with metastases to the paraesophageal and gastric lymph nodes. The hepatic nodule was a metastatic nodule of the adenocarcinoma. The lung tumor was a moderately differentiated squamous cell carcinoma with metastases to the peribronchial lymph nodes. Curiously, one of them showed metastasis of the gastric adenocarcinoma. Moreover, the subcarinal lymph nodes had metastases of the adenocarcinoma. Conclusion: Synchronous appearence of two carcinomas is unusual. The fact of lymph node metastasis to a peribronchial lymph node and to the subcarinal lymph nodes from the gastric adenocarcinoma while the others showed metastases from the lung carcinoma is quite curious. Eitherway, the prognosis is poor.

PP2-109
EFFECTS OF NIMODIPINE ADMINISTRATION ON SMALL BOWEL MUCOSA FOLLOWING LAPAROTOMY AND CONSEQUENT 48-HOUR STARVATION IN A RAT MODEL

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Background The combination of starvation and surgical trauma induces disturbances to the intestinal mucosal structure and function, as well as changes in mucosal barrier function in the rat small bowel. The aim of the present study was to evaluate the effects of nimodipine administration, on intestinal mucosal structural changes and enterocyte apoptosis, following laparotomy and subsequent postsurgical starvation (PSS) in the rat. Method Thirty Wistar rats were divided into two experimental groups: A: Control group (n=15), where the animal models underwent laparotomy and consequent 48-hours PSS and B: Nimodipine group (n=15), where the rats underwent

laparotomy, followed by intraperitoneal nimodipine administration and consequent 48-hour (h) PSS. Small bowel mucosal structural changes and enterocyte epithelial apoptosis were determined 48 h following laparotomy. Results Nimodipine rats (group B) demonstrated a significant decrease in small bowel villous height in jejunum (p=0.016) and ileum (p=0.002). Similarly, crypt depth decreased in jejunum (p<0.001) and ileum (p<0.001). Nimodipine group exhibited significantly higher apoptotic index in ileum compared to control rats (p=0.006). Conclusion Nimodipine did not protect the intestinal mucosa from damage caused by surgery and consequent PSS and had obvious damaging effects on intestinal mucosa with derangements to its structure and subsequent mucosal atrophy.

PP2-110
TUMOR BUDDING IN COLORECTAL CARCINOMAS

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Background: In colorectal carcinomas, tumor budding has been defined as the presence of isolated single cells or small cell clusters in the stroma at the invasive tumor margin. There are reports which determine tumor budding along the invasive margin is associated with tumor behavior. In this study the relation of tumor budding at the invasive margin with pathologic parameters is investigated. Materials and Methods: Retrospectively H&E slides of 73 of colorectal carcinoma specimens were evaluated for tumor budding. Of 73 case, 37 (%50.6) were well differentieted, 28 (%38.3) were moderately differentieted and 8 (%10.9) were poorly differentieted. There was lymph node involvement in 41 (%56.1) cases and no lymph node involvement in 32 (%43.8) cases. Vascular invasion was present in 27 (%36.9) cases and no vascular invasion was detected in 46 (%63) case. The intensity of tumor budding at the invasive margin was evaluated by 2 observers. After the specimens were assessed in general, the highest density of tumor budding area was counted within a microscopic field of x200. Tumor budding density of cases were separated into 2 groups as < 10 (low grade) and ≥ 10 (high grade) .The relation of these groups with histologic grade, vascular invasion and lymph node involvement were investigated. Results: Of the 73 colorectal carcinomas, 33 (%45.2) cases had low and 40 (%54.8) cases had high grade tumor budding density, respectively. Of 33 low grade group, 21 (%63.6) were well differentieted, 10 (%30.3) were moderately differentieted and 2 (%6.1) were poorly differentieted. Of 40 high grade case, 16 (%40) were well differentieted, 18 (%45) were moderately differentieted and 6 (%15) were poorly differentieted. Of the 33 low grade cases, there was no lymph node involvement in 23 (%69.6) cases, but there was lymph node involvement in 10 (%30.4) cases. Of the 40 high grade cases, there was no lymph node involvement in 9 (%22.5) cases but there was lymph node involvement in 31 (%77.5) cases. In the low grade group, no vascular invasion was detected in 27 (%81.8) cases and vascular invasion was present in 6 (%18.2) cases; in the high grade group no vascular invasion was detected in 19 (%47.5) cases and vascular invasion was present in 21 (%52.5) cases. Histologic grade (p= 0.042), lymph node involvement (p= 0.0001) and vascular invasion (p= 0.0034) were significantly associated with high grade tumor budding density. Conclusion: High grade tumor budding density is associated with aggressive phenotypical features in colorectal carcinoma.

PP2-111
PRIMARY GASTROINTESTINAL STROMAL TUMORS IN THE OMENTUM AND MESENTERY: REPORT OF THREE CASES

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Introduction: Gastrointestinal Stromal Tumors (GISTs) are the most common tumors of mesenchymal origin in the gut. Less than 1% of GISTs occur in the mesentery, omentum or retroperitoneum. Methods: We report three cases of GIST- two mesenteric with malignant potential and one of the omentum and their diagnosing with imaging and immunohistochemical methods. Results: The patients had been complaining of pain and oppression in the epigastric and abdominal area. Abdominal Ultrasound showed tumor formation with heterogeneous tissue. CT scan of the abdomen showed large round masses in the upper abdomen. The patients underwent an operation with biopsy. The macroscopical and histologic findings of the tumors were described. The mesenteric and omental tumors showed histologic features similar to GISTs with elongated spindle cells and moderate to high cellularity. Immunohistochemically, all tumors were positive for both KIT and CD34. Conclusion: The use of contemporary diagnostic technologies – ultrasound, CT and immunohistochemical methods are important factors for the diagnosis and staging of GISTs.

PP2-112
HISTOLOGICAL TUMOR REGRESSION PATTERNS OF PREOPERATIVE NEOADJUVAN CHEMO-RADIOTHERAPY APPLIED COLORECTAL CARCINOMA AND THE IMPORTANCE OF THE IDENTIFICATION OF ASSOCIATED RADIATION INDUCED BOWEL CHANGES FOR LIFE QUALITY

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Background. Several grading and staging systems have been proposed for the histological quantification of the tumor regression of colorectal cancer after chemo-radiotherapy. Radiation induced colitis and vasculopathy are also well established entities. We had observed different regressional changes during the histopathological investigation of these tumors and presented our group composed of 10 cases in Turkish National Pathology Symp. in 2006. The aim of this ongoing study is the assessment of the effects of different histological tumor regression patterns on the tumor regression biology, prognosis and the life quality of the patients. Method. Between 97-06, 18 patients who received preoperative neo-adjuvant chemo-radiotherapy followed by surgery for colorectal carcinoma were enrolled in the study. Two of them had mucinous adenocarcinoma. Histological regression patterns(HRP) have been described and subclassified as follows: Type-I-massive necrosis; Type-II-massive fibrosis (successor to tumor); Type-III-cystic degeneration with central necrosis of the tumor parenchyma with fibroplasia; Type-IV-massive microcalcification of tumor parenchyma with fibroplasia. In addition to common histological prognostic parameters of colorectal carcinoma, xantogranulomatous reaction and the changes of adjacent bowel wall and vessels were recorded. Results. The distribution of HRP in non-mucinous group were Type-I in 3 cases, Type-II in 5 cases, Type-III in 3 cases and Type-IV in 4 cases. There was not any regression in 1 case in this subgroup due to early developing generalized bowel infarction and died soon after. One of the patients had mucinous adenocarcinoma fully resistant to therapy, and after the local

recurrence she died in the 5th year. In another case with mucinous carcinoma, persistence of the acellular mucin pools were observed. Overall, complete regression was seen in 5 patients. The features of the chronic ischemic colitis and occlusive radiation vasculopathy were observed in all patients. Conclusion. For the evaluation of the co-efficiency of preoperative neo-adjuvant chemo-radiotherapy, there is a necessity for a worldwide consensus including grading, staging of the tumor regression, and histological regression pattern. However, the whole effort should not focus on the tumor. It is the responsibility of the pathologist to define and report the intensity and spread of the mesenteric ischemic properties of the resected specimen. Another point of study should be the standardization of the long term effects of ischemic parameters on the quality of life.

PP2-113

CYCLOOXYGENASE EXPRESSION IN THE COLON ADENOCARCINOMAS AND ITS RELATIONSHIP WITH OTHER PROGNOSTIC PARAMETERS

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Background: There are two isoforms of cyclooxygenase (cox) which takes place in the synthesis of thromboxanes and prostoglandines from arachidonic acid. Inducible form is cyclooxygenase-2 (cox-2) and its role in the pathophysiologic processes is well known. In this study immunohistochemical expression of cox-2 in the colon adenocarcinomas and its relationship with other prognostic parameters are investigated. Method: Paraffin embedded blocks of 59 cases of colon adenocarcinomas which diagnosed at the Dr.Lutfi Kirdar Kartal Education and Research Hospital, Pathology Department, were investigated for expression of cox-2 by immunohistochemistry. According to the extent of expression, cases are graded as score 0 for no expression and score 1, score 2, score 3 score 4 for the 1-9%, 10-29%, 30-60%, 61% and above, respectively. For the intensity of the staining, cases were graded as score 0 for no staining and score 1, score 2, score 3 for the weak, moderate and strong cytoplasmic staining, respectively. Extent and intensity of the immunostaining compared with the prognostic parameters as age, sex, tumor grade, stage, vascular invasion, perineural invasion, inflammatory response and lymph node metastasis. Results: According to histologic differentiation, our cases were 15.6% grade I, 59.3% grade II, 27.1% grade III. Intensity and extent of the cox-2 expression was significantly higher in the vascular invasion positive cases (p: 0.0218, p: 0.035, respectively) and lymphocytic response was weak (p:0.035). We also found reverse relationship with the lymphocytic response and the histologic grade (p: 0.069). Conclusion: In this study we found statistically significant relationship with cox-2 expression and vascular invasion which is an important prognostic parameter.

PP2-114

ADENOMYOMA OF THE GASTROINTESTINAL TRACT. REPORT OF THREE CASES AND REVIEW OF THE LITERATURE

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Adenomyoma of the gastrointestinal tract is a rare benign tumor-like condition characterized histologically by a mixture of glandular structures lined by tall columnar epithelium with intervening bundles of smooth muscle. The pathogenesis of adenomyoma is considered to be either a form of myoepithelial hamartoma or a pancreatic heterotopia. We describe three cases of adenomyoma. One was the jejunal adenomyoma that presented with intussusception occuring in a 48-year-old man. The other was gastric antral adenomyoma found incidentally in a 62-year-old man, during cholecistectomy. It had intra-abdominal localisation, origining from the antral subserous region. The third was the papilla of Vater adenoma, discovered during the autopsy if a 54-year-old woman. Clinical diagnosis was dysfunction of the papilla Vateri, associated with severe obstructive jaundice induced by its carcer. Formaldehyde fixed, paraffin sections were stained with H&E,Van Gieson, PAS and HID-AN pH=2,5 methods. Immunohistochemical LSAB2 immunohistochemical method was used for verification of epithelial, smooth muscles tissues and, nuclear proliferative activity (DacoCytomation). On histological examination, the lesions consisted of hyperplastic glandular lobules of the pylorus, exocrine pancreas and jejunum, covered by single-layer epithelium of cuboidal and columnar cell type. The cells showed no atypia; some ducts shoved cystic changes. The hyperplastic glandular lobules were surrounded by hyperplastic mesenchymal tissue, composed of bundles of smooth muscle. Immunohistochemically, intense expression of panCytokeratin (epithelial marker), as well as desmin (smooth muscle marker) was found. Ki-67 activity (proliferative activity marker) was negative. In conclusion, adenomyoma of the gastrointestinal tube is a rare benign lesion. The differential diagnosis from adenocarcinoma, especially in a frosen section, might be difficult. The main features are the absence of cytological abnormalities, the absence of desmoplastic stroma and the presence of smooth muscle bundles encircling the cysts.

PP2-115

FIBROMUSCULAR DYSPLASIA OF THE MESENTERIC VASCULATURE RESULTING CROHN LIKE AND ULCERATIVE COLITIS LIKE CHRONIC ISCHEAMIC BOWEL DISEASE IN YOUNG ADULTS

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Fibromuscular dysplasia (FMD) is described as a no inflammatory segmental disease of the arteries of unknown origin. FMD may affect arteries of all sizes and is commonest in middle-aged women, but it can occur in both sex and in all ages, including children. FMD of the mesenteric vessels is too rare and we couldn't find involvement of veins together with arteries in the literature. We report four cases with mesenteric FMD that cause to chronic ischemic bowel disease mimicking Crohn's disease in three cases and ulcerative colitis in the fourth case. Case 1. 38 years old female followed up as Crohn's disease since 1996, in addition to esophageal leiomyom. Case 2. 30 years old female followed up as Crohn's disease since 1994. Case 3. 36 years old male followed up as Crohn's disease since 1999. Case 4. 40 years old male followed up as Ulcerative Colitis since 2001, in addition to classical seminoma in the testis. Pathologic investigation of surgical resection specimens of all patients revealed that FMD involved extramural intestinal arteries and veins. The whole histologic spectrum (intimal, medial,

periarterial and also petal-like periadventitial patterns) of FMD was seen in the several vessel samples in all patients. Unexpectedly, venous involvement was equivalent or same degree with arterial involvement. Periadventitial hyperplastic smooth muscles, in bundle form, surrounding the artery/vein couple was recorded in these cases as an undescribed feature of FMD. The appearance of the whole thickness of the walls of the affected vessels and periadventitial petal like smooth muscles hyperplasia were very well organized and hamartomatous in nature. In first three cases the affected area was small bowel, in the fourth case it was large bowel. Affected small bowel expressed Crohn's like morphological changes radiologically, macroscopically and microscopically. Similarly, the affected large bowel expressed ulcerative colitis like changes. In conclusion, mesenteric involvement by FMD may cause chronic ischemia, not only acute ischemia. Chronic ischemic bowel disease may mimic idiopathic inflammatory bowel disease also in young adults, and not only elderly. FMD may involve veins. Further investigation is necessary for congenital etiology of the FMD characterized by whole thickness and periadventitial involvement of the couple of artery and vein.

PP2-116

ALTERED EXPRESSION OF MONOCARBOXYLATE TRANSPORTERS 1, 2 AND 4 IN COLORECTAL CARCINOMAS

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Background: In order to prevent apoptosis by cellular acidosis, tumour cells increase proton efflux through pH regulators, such as proton pumps, sodium-proton exchangers, bicarbonate transporters and monocarboxylate transporters (MCT). Since in ColoRectal Adenocarcinomas (CRC), data concerning expression of MCTs are scarce and controversial, present study was aimed at the assessment of the role of MCTs in CRC, by studying the immunohistochemical expression of the MCT isoforms 1, 2 and 4 in a well characterized series of CRC and searching for its correlations with clinico-pathological parameters Casuistic and Methods: Formalin-fixed, paraffin-embedded samples from 126 CRC were analyzed for MCT1, MCT2 and MCT4 amplified by avidin-biotin-peroxidase system. Consolidating a semi-quantitative assessment, cases were further subdividing as positive or negative for each MCT, yielding further comparison with 15 clinico-pathological variables. Results: Immunoexpression of all MCTs in tumour cells was significantly superior to the findings in adjacent normal epithelium (81.7 % vs 19.8% for MCT1,$p < 0.001$), 93.7% vs 45.8% for MCT2,$p < 0.001$, 95.2% vs 41.6% for MCT4, $p < 0.001$). Remarkably, there was a significant loss of plasma membrane staining in the transition to malignancy (118/126 cases, 93.7% vs 38/83 , 45.8% ($p < 0.001$). Such difference was not finding in plasma membrane immunoexpressions of MCT1 nor of MCT4. As regards clinico-pathological variables, MCT1 expression in plasma membranes pattern was found in a lower percentage of positive cases in adenotubulo-papilar carcinomas ($p = 0.023$). On the other hand, plasma membrane expression of MCT1 was directly related to the presence of vascular invasion ($p = 0.001$). Regarding histological differentiation, both MCT2 ($p < 0.001$) and MCT4 ($p < 0.001$) were significantly more frequently expressed in low grade tumors , whereas just a trend was found for MCT1 ($p=0.095$). Conclusions: The significantly higher expression of MCT 1, 2 and 4 in CRC as compared to corresponding non-tmoral areas found herein may serve as preliminary evidence for a pottentially important role of these MCTs in colorectal carcinoma . Further studies should be performed to assess whether this aberrant expression might justify the use of these proteins as targets in colorectal carcinoma drug therapy.

PP2-117

EXPRESSION OF bcl-2, P53, MDM-2 PROTEINS AND APOPTOSIS IN COLORECTAL ADENOMAS AND CARCINOMAS

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Background: Bcl-2, p53 and mdm-2 are genes implicated in the regulation of apoptosis. The aim of this study was to investigate the expression of bcl-2, p53, mdm-2 proteins and apoptosis in colorectal adenomas and carcinomas and to correlate them with clinicopathological parameters and patient's survival. Materials and Methods: Formalin-fixed paraffin- embedded tissue from 121 cases of colon adenomas and 46 colorectal carcinomas was stained by immunohistochemistry for bcl-2, p53 and mdm-2 protein. Apoptosis was measured by TUNEL method. The number of the positive stained cells was counted by using image analysis technique. Results: Bcl-2 cytoplasmic staining was found in 114/121 (96%) adenomas and in 18/46 (39%) colorectal carcinomas. P53 nuclear staining was found in 97/121 (80%) adenomas and in 19/46 (41%) colorectal carcinomas. Mdm-2 nuclear staining was found in 78/121 (78%) adenomas and in 25/46 (57%) colorectal carcinomas. Apoptotic index was higher in adenomas ($p=0.012$) in relation to carcinomas ($p=0.006$). P53 and bcl-2 protein expression was higher in tubulovillous-villous adenomas ($p=0.03$ and $p=0.02$, respectively) and in adenomas with size> 1 cm ($p=0.006$ and $p=0.03$, respectively). A statistically significant relationship between bcl-2 expression and the degree of dysplasia was observed ($p=0.009$). In carcinomas an inverse relationship between p53 and bcl-2 was found ($p=0.035$). In Cox regression analysis, Dukes' stage was the most significant independent prognostic indicator of a worse survival ($p=0.019$), whereas when stage was eliminated, bcl-2 expression was also a potential predictor for poor prognosis ($p=0.02$). Conclusions: Bcl-2 and mdm-2 expression seems to occur in the early stage of colorectal carcinogenesis. In large adenomas bcl-2 overexpression, in adittion to increased p53 expression, reduces apoptosis and give them a potential malignant phenotype. In carcinomas, apart from Dukes' stage, the bcl-2 expression seems to be an independent prognostic factor associated with an unfavourable outcome.

PP2-118

INTESTINAL BEHCET'S DISEASE: A CASE REPORT

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Behçet's disease (BD) is a multisystemic inflammatory disorder characterized by recurrent aphthous ulcers, genital ulcers, ocular and skin lesions. Hulusi Behçet, a Turkish Dermatologist first defined it in 1937 as a triad. Since its initial description involvement of many other organs has been reported. Gastrointestinal tract disease has been reported to occur in 3–60% of patients with BD. It is most frequent in patients from Japan (50-60%), followed by those from the UK and is least common in Turkish patients (2.8%). Any part of the intestine may be affected, but the ileocaecum is the most predominant site. We report on a 33-year-old male patient who was admitted to the hospital because of fever, abdominal pain, nausea, vomiting following a haemorrhoid operation. He has been diagnosed as BD 12 years ago, and received "colchicine" for 7 months. The treatment was quitted for its side effects as diarrhea, the patient remained untreated for 11 years. Although mucocutaneous lesions recurred meanwhile, ocular controls revealed any progression. He has been suffering from hematochezia related with hemorrhoids for 6 years. Following the haemorrhoid operation, massive hematochezia was developed. Due to previous history of BD he has taken puls steroid therapy that resulted in healing of symptoms. Abdominal ultrasound detected oedema in the walls of right colon and ileum. He underwent a colonoscopy which showed multiple ulcers in the right colon, with areas of closed perforation. A right hemicolectomy with distal ileal resection was performed. The resected specimen showed fibrinous exudate, congestion and serosal adhesions as well as closed perforations and mucosal ulcers. Histologic examination revealed superficial or penetrating ulcers with perforation, leading to peritonitis. There was a nonspecific inflammatory infiltrate in the neighbourhood of ulcers and focal areas of ischemic necrosis. The most striking feature was "Lymphocytic vasculit" observed in small to medium sized venules. The small and medium sized arteria and venules of serosa also showed thrombus, thrombosis and recanalization. Neutrophylic phylebitis was observed in small vessels. But no granulom formation or fibrinoid necrosis was present. The underlying pathology was consistent with vasculitis that affects both small and large vessels. With the aid of the clinical history, the case was considered to be an intestinal reflection of Behçet's disease and responded well to high dose steroid therapy and cyclophosphamid. The case was presented for its demonstrative histopathologic findings.

PP2-119

SOLID PSEUDOPAPILLARY TUMOR OF PANCREAS: IS IT REALLY RARE?

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Background: Solid pseudopapillary tumor is an uncommon neoplasia of pancreas presenting mostly at young age in women. These tumors show acinar, ductal and endocrine cell differentiation mostly with predominance of exocrine features. They are usually found incidentally at physical examination or they sometimes cause abdominal discomfort. The neoplasms present as large, round encapsulated and well demarcated masses. The cut surface is lobulated with necrotic and hemorrhagic areas. The microscopic examination revaeals solid monomorphic pattern with pseuopapillary component located more centrally which is the most distinctive feature of this tumor. The neoplastic cells occasionally contain diastase-resistant PAS positive globules. The most consistent positive immunohistochemical markers for this tumor are alpha-1- antitrypsin, alpha-1-antichemotrypsin, progesterone receptors (PR) and CD10. The citeria for malignancy are perineural invasion, angioinvasion or deep invasion to sorrounding tissue. Method and Results: In our study, we reviewed 13 cases of solid pseudopapillary tumor which accounted for about 14% of all exocrine tumors, diagnosed during 15-year period (1992-2007) and analyzed for clinicopathological features, expression of several markers including alpha-1- antitrypsin, neuron specific enolase (NSE), vimentin, PR, chromogranin and synaptopyhsin. The malignancy citeria were reviewed for each case. The mean age was 34,4 (range: 19-73) and all the patients were women except one. Two cases that had been diagnosed as cystic papillary neoplasia in the past had the same histology and the immunohistochemical properties as solid pseudopapillary tumor.. Positive immunohistochemical staining for endocrine markers was seen in some of the cases. Conclusion: Solid pseudopapillary tumor which are included in less common neoplasms of pancreas are probaby being diagnosed more frequently as the number and sensitivity of diagnostic imaging studies increase. Number of the solid papillary tumors diagnosed at our center during the last 4 years was 10 which constitutes the majority (the other 3 cases diagnosed within 11 years). So the pathologist should be alert when dealing with a large cystic tumor composed of a solid monomorphic morphology and pseudopapillary areas and should make a detailed workup including immunohistochemical panel for the diagnosis and malignancy potential.

PP2-120

CORRELATION BETWEEN TELOMERASE ACTIVITY, TUMOR BUDDING AND SEVERAL IMMUNOHISTOCHEMICAL MARKERS WITH THE STAGE INFLUENCING PARAMETERS IN COLORECTAL ADENOCARCINOMAS

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Background: Although may be out of date, evaluation of telomerase activity in human cancer and it's importance in tumor progression is still intriguing. Hoping to get some data about the correlation of telomerase activity to other, more widely evaluated parameters in colorectal adenocarcinoma (CA), including macro- and microscopic features influencing the disease stage, we performed the following study. Methods: We analyzed 52 specimens of resected CA with different AJCC stages, sent from the Clinic for Digestive Surgery. All of them were formaline fixed, and tissue samples for standard pathohistologic analysis were embedded in paraffin. Sections were stained with H.E and immunohistochemicaly (ICH) for p53, Bcl-2 and Ki-67 (DAKO Cytomation EnVision+ HRP system). Besides the analysis of standard macro- and microscopic parameters for staging, we analysed the tumor budding (TB) according to Moradomi's criteria. ICH stainings were evaluated semiquantitatively. From 22 specimens accepted at the Institute of Pathology as unfixed, samples from tumor tissue and control samples from the resection margins were frosen for telomerase activity analysis (RTA) using ''TeloTAGGG PCR-ELISA'' kit from Roche. Results: RTA was significantly increased (2,5 fold) in tumor samples compared to controls ($p<0,05$). Significantly lower RTA was detected in CA with 2+ Bcl-2 expression (1,8 fold increase, compared to controls), compared to Bcl-2 negative CA (2,4 fold higher,

compared to controls) (p<0,05). We found similar difference in RTA between tumors that haven't pnetrated the bowel wall, compared to those that have (p<0,05). RTA showed positive correlation to the extramural tumor spread (SpearmanR 0,517; p<0,05) and negative correlation to Bcl-2 expression (SpearmanR -0,60;p<0,01) and Ki-67 (SpearmanR -0,48;p<0,05). Our data showed significant differences between CA without distant metastases and CA that have metastasized concerning following parameters: TB, number of positive lymphnodes and extramural venous invasion (p<0,01). TB showed strong positive correlation to the following parameters: extramural spread, infiltrative growth pattern, number of involved lymph nodes, extramural venous invasion, and distant metastases (p<0,05), having the strongest impact on the number of lymph nodes affected (b=0,69;p=0,015). Conclusion: RTA is significantly increased in CA with extramural spread and Bcl-2 negative ICH staining. However, TB is cheaper and simplier to evaluate and is associated to more significant parameters such as lymph node and distant metastases.

PP2-121
GASTRIC CANCER-APPROPRIATE RESECTION WITH LYMPH NODE DISSECTION

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BACKGROUND: The extend of lympadenectomy and gastric resection remain controversial issues in management of early gastric cancer (EGC). The latest study was performed to appropriate gastric resection with lymph node dissection for early gastric cancer. AIM: We analyze the extension of gastric resection and lymphadenectomy in EGC and advanced gastric cancer. METHODS: 104 patients; 50 patients with early gastric cancer and 54 with advanced gastric cancer were operated in Oncology Center-Institute in Warsaw, from 1999 to 2005. 93 patients underwent total gastrectomy; 44 patients with early gastric cancer and 49 with advanced gastric cancer. 10 patients underwent subtotal gastric resection (5 cases with early gastric cancer and 5 cases with advanced gastric cancer). Endoscopic mucosal resection was performed in 1 cases with early gastric cancer. Lymphadenctomy was performed in 103 cases. RESULTS: In cases with EGC mucosal invasion was observed in 21 patients and submucosal invasion in 29 patients. From 2 to 48 lymph nodes were obtained in patients with early EGC and from 6 to 55 in patients with advanced gastric cancer). Lymph node metastases were found in 5 cases with EGC (4 cases with submucosal invasion) and 36 cases with advanced gastric cancer. CONCLUSION: Our study confirms that the depth of invasion and lymph node involvement is an extremely important prognostic factor. For the reason, D2 gastrectomy is the standard treatment even in EGC.

PP2-122
SERRATED POLYPS OF THE LARGE INTESTINE: CLINICOPATHOLOGICAL FEATURES OF SESSILE SERRATED ADENOMAS

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AIM: Serrated polyps of the large intestine, including traditional hyperplastic polyps (HP), traditional serrated adenomas TSA), and more recently described sessile serrated adenomas (SSA), have gained increased recognition in recent years because of growing evidence that one of these lesions, SSA, might be the precursor lesion for some cases of microsatellite unstable colorectal carcinoma. However, for many practicing pathologist, numerous diagnostic challenges exist to diagnose SSA. METHODS: A total of 236 colonic polyps from over a three-year period (2005-2007) were retrieved from the archives of pathology department. Endoscopic polypectomy biopsies diagnosed as tubular adenoma with low grade dysplasia (TA) (n=42), HP(n=54), TSA (n=18), SSA(n=5), abberrant crypt foci (ACF)(n=8) and normal mucosa (NM) (n=15)were retrospectively examined. "Architectural dysplasia" and "cytologic dysplasia" were evaluated. Among architectural findings; branching and dilatation of the base of the crypts, inverted T- and L- shaped crypts, the presence of mature cells with goblet cell or gastric foveoler phenotype at the base of the crypts replacing the proliferative zone, serration at the base of the crypts, foci of pseudostratification of the surface epithelium, eosinophilic change of the surface epithelium, subtle nuclear alterations were noted. All polyps were re-classified according to Snover et al. as HP, SSA, TSA, mixed serrated polyp (SSA+TA) and sessile serrated polyp (SSA+HP). RESULTS: Seven cases were re-classified as SSA (1 TA, 2 HP, 2 ACF, 3 NM), 5 cases as mixed serrated polyp (4 TA) and 3 cases as sessile serrated polyp (3 HP). The age range was 32-75 years (average 54 years). Twelve patients were females and 8 were males. The sites of SSAs were as follows: 6 in ascending colon, 5 in hepatic flexura, 4 in transverse colon, 5 in rectosigmoid colon. The diameter of the SSA ranged from 5 to 12 mm. Irregular shapes of the crypts with branching and dilatations in the basal parts and mature cells in the basal crypts were the two easily applied morphologic criteria in the diagnosis of SSA. In 2 patients with SSA there was synchronous adenocarcinoma of the colon. CONCLUSIONS: There is histologic overlap among the features of HP, TSA, SSA, and ACF which creates diagnostic difficulty. Accurate diagnosis requires the examination of the base of the crypts in well-oriented sections. Approximately 20% of SSA cases have areas of tubular adenoma which shows that the progression to a more aggressive form is not uncommon.

PP2-123
APOPTOSIS AND CELL PROLIFERATION IN GASTROINTESTINAL STROMAL TUMORS (GISTS)

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Background: Although GIST has been extensively investigated in an attempt to identify factors predictive to aggressive behavior, there are few molecular investigations directed towards determining importance of apoptosis in these tumors. In addition, relatively little is known about the mechanisms by which tumor cells growth and proliferate. In this study, the authors present the results of relation of apoptosis and cell proliferation in GIST, in correlation with tumor site and disease grade. Method: Eighty two cases (40 male and 42 female patients, age range 31-73 years) with surgically resected GIST were admitted into the study. The site of the lesion was the gaster in 35, small intestine in 28, colon/rectum in 7 and extraintestinal site in 12 cases. GISTs were reclassified into low-, intermediate-, and high risk group regarding their estimated potential for aggressive clinical behavior as proposed by NIH. Apoptosis was assessed using the TUNEL detection system (TdT-FragEL kit, Oncogene, San Diego, CA). Immunohistochemistry for Ki67 was performed using avidin-biotin-peroxidase complex technique (LSAB+/HRP kit; Dako, Denmark). Apoptotic index (AI) was calculated from the number of positive-staining cells in at least 1,000 tumor cells.

Ki67 labeling index (Ki67 LI) was determined by the quotient of Ki67+ cells and the total number of cells, and at least 300 cells were counted in 3 representative tumor areas. Results: Our analysis revealed that apoptotic index is associated with histopathological grade of tumors, and was significantly higher in intermediate- compared with low risk group (6.9±2.1 v.s. 4.97±0.74; $p<0.05$). Also, in intermediate and high risk groups was found that apoptosis is in correlation with proliferation ($p<0.01$). Refer to tumor localization, significant increase in apoptotic activity was found in extraintestinal tumors compared to lesions in colon/rectum (7.23±0.78 v.s. 6.64±1.82; $p<0.01$), while both proliferation (11.7±3.05 v.s. 6.92±1.39; $p<0.01$) and apoptosis (7.02±1.36 v.s. 5.67±0.69; $p<0.05$) was significantly increased in small intestine lesions compared to gaster. Contrary, in low risk group of tumors apoptotic index was found significantly increased in gaster compared to small intestine lesions (5.84±0.84 v.s. 3.41±0.84; $p<0.01$). Conclusion: Our results suggest that not only cell cycle regulatory proteins, but those who regulate apoptosis might reflect tumor grade and patients prognosis of gastrointestinal stromal tumor. Also, it seems that site of the lesions may be a contributing factor to the pathogenesis of GISTs.

PP2-124

SOLITARY LIPOMATOUS POLYP OR LIPOMA OF GASTROINTESTINAL TRACT: 3 CASE REPORTS

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Backgrounds: Lipomas are most common, benign, mesenchymal neoplasm consisting of mature adipous tissue in their stroma. Most of the lipomas are located superficially or deeply in the skin, although, they also can be seen in gastrointestinal tract and are usally insidental findings in the submucosa or serosa of the small and large intestine. The solitary lipomas of colon have been reported to have a tendency to occur in the vicinity of ileocaecal valve or the ascending colon. Lipomatous poliposis is a rare "non-inherited gastrointestinal polyposis syndrome" associated with diffuse lipomatous polips of colon. Here we reported 2 cases of solitary colonic lipomatous polips at distal colon and a case of duodenal lipoma in order to emphasize the confusion in the terminology of lipoma and lipomatous polip. Case 1: Sixty nine year's old, female complained of hematochesia for 3 weeks. She had the history of hypertension and hyperlipidemia. The physical examination, blood tests were within normal range. Colonoscopic examination revealed pancolitis associated with patch hemorrhagic areas and 1 cm sesile polyp in the rectum. The stroma of polyp was composed of mature fat tissue and fibrocollagen which were surrounded by normal rectal epithelium. Case 2: Seventy six year's old, female patient with hypertension and hyperlipidemia was investigated for constipation of one year duration which was recently accompanied by hematochesia. Her father and sister had the history of larynx and colon cancers, repectively. Her physical examination, blood tests were within normal range. There was a large tumor and 2 cm sesile polyp in the rectum. Pathologic examination revealed normal rectal epithelium lining the stroma of mature adipocytes and blood vessels at different sizes. Case 3: Fifty nine year's old female had been previously operated for breast cancer. On routine check up, all the laboratory tests were normal except for elevated erithrocyte sedimentation rate. A 3 cm sesile polyp had been detected at second portion of duodenum and polypectomy was applied to it. There was a thin fibrous capsule beneath duodenal epithelium and surrounding mature adipose tissue. Conclusion: We though that solitary lipomatous polips are much more rare than lipomas in the gastrointestinal tract. The differentiation of submucosal gastrointestinal lipomas from lipomatous polips can be based on the presence of a prominant capsule in a unpedinculated polyp. Although most of the colonic polyps are adenomatous, the differential diagnosis from lipoma or lipomatous polyps is necessary.

PP2-125

EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN GASTRO-INTESTINAL STROMAL TUMORS

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Epidermal Growth Factor receptor (EGFR)is a member of the ErbB family of receptors existing on the cell surface and activated by binding of its specific ligands, wich include epidermal growth factor and transformaing growth factor. Activation of EGFR is associated with cell growth and transformation. It is overexpressed in many types of cancers, especially colorectal cancer and seems to reflect more aggressive histological and clinical behaviors. Material and method: The aim of this study was to evaluate EGFR immunohistochemical reactivity in 20 gastrointestinal stromal tumours (GISTs) of the stomach (n = 12), small intestine (n = 7) and colon (n=1) using standard immunostaining techniques in paraffin-embedded sections. Result: 9 cases of GISTs (45%) expressed EGFR. EGFR(-)tumors(11 cases) measured 11±5.8 cm of size, were extragastric in 5 cases (45.5%), with high risk of malignancy in 7 cases. Mitotic index was 2.6±2.3 mitosis /50 HPF in GISTs EGFR (+) and 17.2±15.7 /50 HPF in GISTs EGFR (-) with significant difference ($p=0.012$). Proliferative index was estimated by MIB-1 expression and was evaluated to 1 ± 1.3 in GISTs EGFR (+) and 8.3±9.6 in GISTs EGFR (-) ($p=0.051$). Conclusion: These preliminary results suggest that loss expression of EGFR in GIST is correlating with aggressive biological tumors.

PP2-126

TRANSMURAL BACTERIAL AND FUNGAL INVASION AND MYCOTIC THROMBOSIS OF THE MESENTERIC VESSELS IN PATIENTS WITH ACUTE MESENTERIC INFARCTION. A BRIEF REPORT OF A PRELIMINARY STUDY

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Background: The translocation of commensal bacteria in patients with ischemic bowel, and development of septic and toxic shocks are well known incidents in practice. During our routine pathological examination, we have identified candida strains and some pleomorphic filamentous microorganisms invading necrotic bowel wall. The aim of this study is to determine the prevalence of the invasive fungi infestation of the infarctoid bowel and to specify the invasive filamentous microorganisms that cause mycotic thrombosis. Method: Seventy five consecutive patients in whom acute intestinal ischemia was diagnosed between 2005 and 2006 were retrospectively reviewed. Tissue Gram, PAS, Gridley, and Masson thrichrome stains were applied on the paraffin section of tissue samples that contain microorganisms. Results: There were 41 men and 34 women. Ages ranged from 24 to 87 years (mean 55,5 years). Only small bowel resection, only large bowel resection, both small and large bowel resections were applied in 55, 8, and 12 patients respectively. 4 patients had superior mesenteric vein thrombosis, 7 patients had superior

mesenteric artery occlusion, 5 patients had external herniation, 3 patients had internal herniation, 7 patients had bride ileus, 5 patients had previous abdominal radiation therapy, and 12 patients had previous abdominal surgery. The others had unknown etiology. Pathological examination revealed transmural infarction in 28, mural infarction in 12, mucosal infarction in 19 cases. Subacute ischemic enteritis with acute exacerbation was observed in 16 cases. In patients with transmural infarction, cocci and rods were invading the necrotic wall and the subserosal mesenteric fat tissue. Colonization of the candida strains were observed on the luminal aspect of the necrotic wall, under the denudated necrotic membrane. Candida colonization was also seen on the serosal surface in some patients with transmural infarction. In addition to these findings, some pleomorphic filamentous microorganisms were invading the whole thickness of necrotic and semiviable bowel wall, and subserous space. The lumen of the extramural arteries and veins were obliterated with these microorganisms that were PAS (-), and Gridley (-). Conclusion: Acute mesenteric infarction is still a disease with a consistently high mortality rate. If surgery is performed in an early stage, the survival rate increases regardless of age. One of the most important prognostic factors is the time interval between the onset of symptoms and surgery. We suspected that candidiasis and invasive filamentous microorganisms were probably seen in the patients that delayed diagnosis. The presence and identification of these microorganisms in the surgical specimens may be important in the post-operative management of the patients, especially in prophylactic therapy of the infections.

PP2-127

THE RELATIONSHIP OF K-ras MUTATION AND MUCINOUS DIFFERENTIATION OF COLORECTAL CARCINOMAS

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BACKGROUND: Accumulation of molecular alterations, including K-ras mutations are believed to be critical events in carcinogenesis. Mucinous adenocarcinomas of colon need to be more investigated for their different behaviour.We aimed that at clarifying the role of k-ras mutation related to mucinous differantiation in Colorectal Carcinomas (CRC). DESIGN: This retrospective study was composed of 33 sporadic colorectal cancer cases which had undergone radical surgery and diagnosed in Cerrahpasa Medical College. The cases were subdivided according to presence of extracellular mucinous secretions. K-ras mutations in codon 12 were examined in tumor tissues using PCR-RFLP analysis. RESULTS: Twenty one (66%) of the cases had either focally or no extracellular mucin secretion (M-). Other 12 cases showed extracellular mucin (M+) in different percent ranging 10-90% of all tumors. K-ras mutation was found in 15 (45,3%) of all cases and 8 (66,6%) of M(+) cases. Mucin secretion showed statistically significant correlation with k-ras mutation and depth of invasion ($p<0.05$. There was not found significant correlation between mucinous diferentiation and sex of the patients, age and tumor grade. CONCLUSIONS: Our data indicate that the presence of mucinous secretion in colorectal carcinomas is associated with presence of k-ras mutation. This finding is important to understand for distinct behaviour pattern of CRC with mucinous differentiation.

PP2-128

MOLECULAR ANALYSIS OF SERRATED POLYPS AND SYNCHRONOUS CANCERS THAT ARISE IN THE SETTING OF HYPERPLASTIC POLYPOSIS SYNDROME

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Background: Hyperplastic polyposis syndrome (HPS) is an uncommon condition, characterized by the presence of multiple, large hyperplastic polyps (HP), the majority of which is mainly located in the right colon. Though, the true risk is not known, HPS is associated with an increased risk for colorectal cancer (CRC) through alternative morphologic and molecular pathways. Molecular analysis of the carcinomas and representative premalignant lesions was performed in order to understand the morphologic and genetic changes that lead to the development of carcinoma in this syndrome. Method: Two cases of HPS comprising two male patients of 51 (case 1) and 75 (case 2) years old underwent rectosigmoidectomy and total colectomy, respectively, for CRC. Case 1 had 20 in the descending colon while case 2 showed 25 hyperplastic polyps (HP) and/or sessile serrated adenomas (SSA) in transvers and rectosigmoid colon. DNA extraction was performed from carcinomas, three representative polyps, and from normal mucosa in each case. Mutation analysis was performed using PCR and sequencing exon 2 and exon 3 of KRAS gene. Microsatellite instability status was determined using primers for BAT25, BAT26, D5S346, Mfd15 and D2S123. MSI-high (MSI-H) cases showed mutation in two of five markers whereas in MSI-low (MSI-L) phenotype only one of five markers was unstable and in microsatellite stable (MSS) cases none was mutated. Immunohistochemical detection of mismatch repair gene proteins was performed using antibodies to hMLH1, hMSH2, hMSH6 and PMS2. Tumour cells without nuclear staining, in the presence of positively stained stromal cells were considered to have an abnormal pattern of expression. Results: Both tumours were moderately differentiated adenocarcinomas. KRAS mutation was found on exon2, codon12 (glycine to aspartat) in the tumor sample of case 2. There was no evidence of microsatellite instability at any of the five loci examined, in either of the carcinomas and both tumours were positively stained with mismatch repair gene proteins. HPs or SSAs did not show KRAS mutations or MSI in the representative samples. Conclusion: The molecular analysis of HPS showed no evidence of MSI in either the carcinomas or any of the precursor lesions examined, including HPs and SSAs. The neoplastic progression in HPS seems to follow the classical genetic pathway rather than the “serrated pathway” of colorectal carcinoma which occurred synchronously with mutation of KRAS in one of the tumours studied.

PP2-129

THE CORRELATION BETWEEN SERUM VEGF LEVELS AND TUMOR-ASSOCIATED MACROPHAGES IN COLORECTAL CANCER

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Background: The angiogenesis process is stimulated by angiogenic factors released by tumoral cells, but other cells as

such tumor-associated macrophages (TAM) can also contribute to increase angiogenic proccess in colorectal cancer (CRC) producing the proangiogenic molecule vascular endothelial growth factor (VEGF). Aims: To determine in primary CRC patients the contribution of VEGF expression of TAMs and tumor cells in circulating VEGF levels, and their relation to p53 expression. Methods: Immunohistochemistry was used to identify TAMs, p53 protein, and to evaluate the VEGF expression by TAMs and tumor cells in 110 primary CRC patients. Serum VEGF levels were determined before and after surgery using an enzyme immune assay (ELISA). Results: VEGF expression in tumor cells was significantly greater in stage III carcinomas than in stage II and also correlated with lymph node metastases. There was a greater expression de VEGF in tumours with p53 positive immunohistochemical stain than in p53 negative. Macrophage index was not related to tumor VEGF secretion. We observed no significant association between serum VEGF values and VEGF tumor expression, node status, histological grade or p53. However, patients had high number of TAM expressing VEGF showed higher presurgery serum VEGF levels than those patients with median or low number of VEGF positive TAM (378 vs 246 or 251 pg/ml). Surgery produced an increased of serum VEGF levels at 6-8 hour after tumor removal (354 vs 285 pg/ml). Conclusions: Our data suggest that in primary CRC, presurgery circulating VEGF levels are mainly related to VEGF secreted by TAM instead of VEGF secreted by tumor cells. Abbreviations: microvessel density (MVD), vascular endothelial growth factor (VEGF), tumor-associated macrophages (TAM), enzyme-linked immunosorbent assay (ELISA).

PP2-130
SERRATED ADENOMAS AND SESSILE SERRATED POLYPS

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Background: Serrated adenoma (SA) is characterized by having combined architectural features of hyperplastic polyps and classical adenomas.A recently recognized lesion is a variant of polyps characterized by relatively large size, atypical histology and proximal location in the colorectum. Because this lesion lacks the traditional cytology of colorectal adenoma and in order to avoid confusion with SA, it is referred to in this review as sessile serrated polyp (SSP). In this study, the incidence and clinicopathologic features of SAs and SSPs previously diagnosed were investigated. Design: Between 2002 and April 2007, archives of Cerrahpaşa Medical Faculty, Department of Pathology were reviewed in both resection materials and endoscopic biopsies, all the cases diagnosed as SAs, adenomas with SA components and SSPs were collected. Their slides were reexamined and clinicopathologic features were discussed. Results: 173 polyps were reexamined in 165 cases. 84 cases were diagnosed as SAs, 58 cases as adenomas with serrated components (Mixed polyps) and 4 cases were SSPs. 26 of them were in resection materials whereas 139 cases were in endoscopic biopsies. Female/male ratio was nearly 1/2 and the mean age was 62,58. The most common location of SAs was rectosigmoid, however 2 cases of SA were seen in stomach, 2 cases in ampulla Vateri and small bowel, 3 cases in appendix, 9 cases in transvers colon, 14 cases in descending colon,25 cases in caecum and ascending colon,76 cases in rectosigmoid and 42 cases at specifically unknown location. SSPs were located mostly in caecum and ascending colon (3 cases). The diameter of SAs and SSPs ranged between 1,5cm. and 0,2 cm., 1,3 cm. and 0,1cm., respectively. In 7 of SAs and 9 mixed polyps development of carcinoma was observed. 9 SAs and 2 mixed polyps were found in the distant sites of colon in cases of colorectal carcinoma.40 cases were include high grade dysplasia pattern,29 cases were include moderate grade dysplasia pattern,56 cases were include low grade dysplasia pattern and 46 cases had no dysplasia pattern. Conclusion: This study showed that SAs are characterized by a heterogeneous group of changes at the clinicopathologic changes. SSPs are distinct variants located in the proximal colon. Our future project is to detect the molecular changes of these lesions.

PP2-131
CORRELATION OF HISTOPATHOLOGICAL CRITERIA WITH CLINICAL PRESENTATION IN INFLAMMATORY BOWEL DISEASE (IBD): IS IT USEFUL IN DETERMINING THERAPY?

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Background: Patients with ulcerative colitis (UC) and Crohn's disease (CD) share many similarities, but there are also many significant differences that make a distinction necessary. Since different surgical approaches are being used for their treatment pathologic decision making can become very stressful. In order to overcome the difficulty in the diagnosis of IBD and also to predict patients who might need additional therapy it might be useful to assess the value of histologic parameters and colitis pattern in distinguishing UC from CD. Method: A total of 70 patients biopsied for suspected IBD were included in the study. Their demographic findings as well as clinical symptoms (diarrhoea, blood in stool, abdominal pain, resistance to therapy) were noted. Histologically the presence of ulcer, villiform surface, cryptitis, crypt abscesses and distortion, mucin depletion, lamina propria inflammation, basal lymphoplasmacytosis, granulomas, lymphoid and ganglion hyperplasia, submucosal extension of inflammation, dysplasia was evaluated and graded as mild, moderate and severe. Statistical analysis was performed using Fisher's exact, chi square and Mann-Whitney tests. Results: There were 53 UC and 17 CD cases comprising 30 males and 40 females with an age range of 17-70. The most commonly observed histologic finding was LP inflammation seen in 66 (94,3%) patients followed by crypt distortion (n=62; 88.6%), cryptitis and mucus depletion (n=58; 82.9%). No statistical difference was observed between UC and CD patients in terms of histologic parameters except LP inflammation ($p<0.05$) which was more common in UC compared to CD. Patients who had rectal bleeding showed significantly increased cryptitis ($p<0.05$), basal lymphoplasmacytosis ($p<0.05$) in their biopsies. In patients with abdominal pain ($p<0.05$) and tenderness ($p<0.05$), cryptitis and crypt abscesses were significantly increased. Crypt abscesses were significantly ($p<0.05$) less common in patients who had the sense of well being. Patients with frequent diarrhoea showed significantly ($p<0.05$) more common ulcer formation and crypt abscesses in their biopsies. No correlation was observed between resistance to therapy and histologic parameters. Conclusion: Our results suggest that histological changes in IBD may be grouped under clinically relevant categories that provide a framework for their interpretation. Histological criteria of disease activity seem to correlate with clinical presentation of the patients with IBD and, thus could be used to design appropriate therapy.

PP2-132
ISOLATED ICC HYPERPLASIA: PRECURSOR OR UNUSUAL FORM OF GIST?
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Background: The most common primary mesenchymal neoplasms of the gastrointestinal tract are gastrointestinal stromal tumors (GISTs). The vast majority of GISTs occur in the stomach and small intestine. Colorectal GISTs are rare, comprising less than 10%. Precursor lesions for sporadic GISTs have not been well defined. Hyperplasia of interstitial cells of Cajal (ICCs) may represent facultative precursor lesion for sporadic GISTs in a hyperplasia–neoplasia sequence. ICCs are intestinal pacemaker cells that initiate peristalsis in the stomach and intestine, and are considered to be cell of origin for GISTs. Diminutive lesions consisting of KIT-positive spindle cells have been recognized in rare cases of familial GISTs and in von Recklinghausen's disease and very rarely in the vicinity of sporadic GISTs. Isolated cases of ICC hyperplasia not related to any other clinical condition or GIST has not previously been reported. Case report: A 56-year-old woman suffering from constipation and abdominal pain was attended to emergency room. A diagnosis of intestinal obstruction was made and she underwent an abdominal surgery. No mass lesion was observed except for serosal thinning in the sigmoid colon and adherence of omentum to abdominal wall. Serosa of the sigmoid together with the bowel wall was resected and histopathology revealed a diffuse lateral hyperplasia of spindle cells located between the layers of the muscularis propria throughout sigmoid colon. Spindle cells were positive for c-kit and CD34 but negative for myogenic or neurogenic markers, supporting that they are ICCs. No mitoses or cellular atypia were seen. There was no other tumour in the resection specimen. Thus the case was considered as (isolated hyperplasia of ICC) microscopic c-kit positive stromal nodul (GIST?). On further clinical evaluation the patient did not have any related clinical condition such as von Recklinghausen's disease, Carney's triad or GIST elsewhere. Conclusion: Since no association with GIST or any other condition related to GIST was present in our patient, the case was considered as an isolated hyperplasia of ICC. Interestingly the lesion was located in the colon which is not the typical site for GISTs. Whether this condition is a precursor of GIST or an unusual form of GIST needs to be further evaluated.
Key Words: Cajal cell hyperplasia, GIST

PP2-133
SERRATED POLYPS: COUNTING OR NOT COUNTING THE HISTOPATHOLOGICAL CRITERIA?
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Background: The concept of "serrated neoplasia" pathway in colorectal carcinogenesis has introduced new precursor lesions with a generic name "serrated" polyps among which are hyperplastic polyps (HPs), sessile serrated adenomas (SSAs), serrated adenomas (SAs) and mixed polyps (MPs). SSAs are histologically similar to HPs but biologically comparable to adenomas. Though many histologic criteria have been proposed in the differential diagnosis of HPs and SSAs a consensus has not been reached yet. Therefore, we have decided to apply the proposed criteria to a series of serrated polyps in order to assess their discriminatory diagnostic value. Method: A total of 84 serrated polyps (38 HPs, 37 SSAs, 4 SAs, and 5MPs) were included in the study. There were 9 criteria comprising of exaggerated serration, papillary surface configuration, increased crypt branching, horizontally arranged crypts, basal crypt dilation, increased epithelium/stroma, mitosis and cytological atypia in the upper crypt zone, inverted crypts, and irregular distribution of goblet cells, used in the diagnosis. Each case was re-examined for the presence of these criteria and scored accordingly. Number of positive criteria and frequency of each criterion in each polyp group were evaluated in order to assess their diagnostic value. Results: In HPs, 78.94% of which had less than 3 criteria, the number of positive criteria ranged from 0 to 5 while 83.78% of SSAs had 4 or more positive criteria ranging from 3-7. Only 10.52% of HPs showed 4 or 5 criteria whereas 16.22% of SSAs had 3 criteria. MPs and SAs, all showed more than 4 criteria ranging from 4 to 7. Papillary surface configuration (50%) and crypt dilation (34.21%) were the most frequently observed criteria in HPs whereas SSAs showed irregular distribution of goblet cells (94.59%) and mitosis and cytological atypia in the upper crypt zone (89.19%) in a majority of the cases. Of the 5 SSAs with only three criteria, all showed mitosis and cytological atypia in the upper crypt zone and all but one had irregular distribution of goblet cells while HPs with more than 4 criteria did not demonstrate either of the latter two. Conclusion: Some overlap was observed between HPs and SSAa in terms of the number of diagnostic criteria found in both lesions. However, both mitosis and cytological atypia in the upper crypt zone, inverted crypts, and irregular distribution of goblet cells prove to be more suggestive of SSAs than HPs. Therefore, a qualitative approach rather than quantitation of criteria would aid in the diagnosis of serrated polyps.

PP2-134
PATHOLOGY TRAINEES: CAN THEY DIAGNOSE GASTROINTESTINAL EPITHELIAL DYSPLASIA AS WELL AS WE DO?
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Background: Diagnosis and grading of dysplasia in one of the most difficult tasks of a pathologist, mainly due to lack of objective criteria and a worldwide consensus among pathologists. Despite consensus meetings and classification systems, interpretation of biopsies showing dysplasia is still difficult even for experienced gastrointestinal pathologists as well as pathology trainees. We therefore decided to assess the diagnostic difficulties that pathology trainees experience in dysplasia interpretation in gastrointestinal specimens and also to evaluate interobserver aggreement. Method: Eight training pathologists and one expert gastrointestinal pathologist reviewed 20 gastric and 8 colonic lesions (11 endoscopic biopsies, 6 polypectomy, and 10 operation specimens). The specimens were evaluated according to a simplified classification scheme of five categories: i. regenerative changes, ii. low grade dysplasia, iii. high grade dysplasia, iv. intramucosal carcinoma, and v. carcinoma with submucosal invasion using both cytologic and architectural criteria reviewed elsewhere by each trainee separately. The trainees were further divided into three groups according to their level of experience in pathology as group 1: 2nd year trainees (n=3), group 2: 5th year trainees (n=2), and group 3: 8th year trainees (n=3). Kappa statistics and McNemar test were performed for statistical analysis. Results: According to the expert pathologist there were 1 regenerative change, 4 low grade, 5 high grade dysplasias, 17 intramucosal carcinoma and 1 invasive carcinoma diagnosis among the study cases. The agreement of the trainees on the

diagnoses of gastrointestinal specimens showed great variation with a kappa value of 0.250 to 1.000 while kappa value of agreement between expert and trainees ranged from 0.339 to 0.600. The trainees diagnosed all regenerative lesions and invasive carcinomas (100% agreement) whereas only 15.625% of cases with low grade dysplasia and 45% of high grade dysplasia were in agreement with the expert pathologist. Conclusion: Our results suggest that experience in pathology seems to be a major determinant of diagnostic accuracy even in the training period and also that gastrointestinal dysplasia remains to be the cause of diagnostic difficulty for both young and experienced pathologists. However, there is still much to be done to improve the diagnostic skills of pathology trainees and to standardize the criteria of gastrointestinal epithelial dysplasia.

PP2-135
AN UNUSUAL CASE OF GASTRIC ADENOCARCINOMA, LEIOMYOMA AND SIMULTANEOUS OCCURRENCE OF B-CELL SMALL LYMPHOCYTIC LYMPHOMA INVOLVING THE PERIGASTRIC LYMPH NODES AND SPLEEN

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Background: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a low grade indolent systemic neoplasm involves bone narrow and lymph nodes. Since it has a longstanding clinical presentation, it is expected having an higher risk of secondary cancers. We present here a rare case of a simultaneous occurrence of adenocarcinoma of the gastric cardia, submucosal leiomyoma and CLL/SLL involving the perigastric lymph nodes and spleen. Case: A 78 year old man underwent total gastrectomy, omentectomy and splenectomy because of the advanced cardia tumor. Macroscopically, the cardiac tumor was 2,7 cm in greatest dimension. There were multipl tumoral nodules in the omentum and hilar region of the spleen. Microscopically, the tumor composed of mucin producing atypical glandular and cribriform structures infiltrating the cardiac serosa. Non-tumoral antral mucosa showed hyperplastic polyp plus minute submucosal spindle cell tumor that was 5 mm in diameter. Six perigastric lymph nodes and splenic hilar lymph nodes revealed metastasis of adenocarcinoma whereas other nodes and white pulp of the spleen were diffusely infiltrated by uniform, small atypical lymphoid cells. Immunohistochemically, atypical lymphoid cells were strongly positive for CD20, CD5, CD23, and Bcl-2. Submucosal spindle cell tumor showed SMA, h-caldesmon and desmin positivity consistent with leiomyoma. Conclusion: This is the first case of unrelated simultaneously presented cardiac adenocarcinoma and leiomyoma in association with CLL/SLL infiltration in the perigastric lymph nodes and spleen in the literature.

PP2-136
GANGLIOCYTIC PARAGANGLIOMA OF THE DUODENUM: A CASE REPORT

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Background: Duodenal gangliocytic paraganglioma (GPG) is a rare tumor that characteristically occurs in the second portion of the duodenum and typically presents with gastrointestinal bleeding. Duodenal GPGs have an excellent prognosis after surgical resection but metastatic spread to regional lymph nodes and recurrence may rarely occur. We present here a rare case of duodenal GPG in view of the literature. Case: A 41 year old man underwent pancreaticoduodenectomy for carcinoma of the ampulla vater. Macroscopically, the tumor, 2,7cm in greatest dimension, located in the ampulla without infiltration of the pancreas. Histologically, the tumor composed of epitheloid cells, ganglion-like cells with abundant cytoplasm and vesicular nuclei, and spindle cells arranged in broad fascicles. Immunohistochemically, tumor cells were strongly positive for S100, pancytokeratin, chromogranin A and synaptophysin. There was no metastasis in the regional lymph nodes. Conclusion: Paragangliomas of the ampulla vater have malignant potential. We review the differential diagnosis of this infrequent tumor.

PP2-137
SMALL VESSEL CHANGES IN POSTTRAUMATIC ULCERS

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Background: There are many different risk factors for developing stress ulcers after sever trauma. Different investigations showed that stress ulcers are more often in older age. Some authors consider that pathological changes on small vessels involve in developing stress ulcers after severe trauma and may have main role. Method: Material for this study was collected in the Institute of Forensic medicine in Belgrade. All cases with trauma who had risk for stress ulcers (period of survival at least 24 hours) are observed. They were divided in experimental (with stress ulcers) and control group (no stress ulcers). Histological analyses of abdominal organs were done with special focus on small arteries and arterioles. We analyzed data obtained from autopsy protocols, heteroanamnestic data from deceased's family and medical documentation about treatment, too. The samples were statistically prepared (χ2-test, Fisher's test). Used staining were H&E and elastica van Gieson. Results: The vast majority of all analyzed cases were male (71,43% in the experimental and 85,71% in the control group). Age average was 59,00 (SD=21,64) in the experimental and 52,86 (SD=21,48) in the control group. Regarding age, stress ulcers were most frequently diagnosed among individuals in eight decade of life (6 or 28,57%). There is no significant statistical difference between experimental and control group when we compared age (χ2=0,268, p>0,05). The mean outliving period was 14,65 days (SD=12,94) in the experimental group and 8,76 days (SD=8,70) in the control group. Analyses was also performed in relation to the manner of death. The vast majority of persons in both group were injured in accidents (17 or 80,89% in both group), predominantly traffic acidents (13 or 61,90% in the experimental and 10 or 47,62% in the control group). The most freqently couse of death in both groups, was demage of vital brain centers with/or without complications. Atherosclerosis of abdominal aorta in the experimental group in 52% cases were difficult. There is no significant differences between experimental and control group when compare frequency of changes on small vessels (X2=0,102, p>0,05). Histological changes on small vessels of gaster are not significantly different in experimental and control group (P=0,232, p>0,05). In some cases without ulcers spasm of small arteries was found. Conclusion: Morphological changes of small vessels do not have main role in appearing posttraumatic stress ulcers.

PP2-310

IMMUNOHISTOCHEMICAL ANALYSIS OF GALEKTIN-3 EXPRESSION AND PROGNOSTIC FACTORS IN INVASIVE GASTRIC CARCINOMA

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Background: Gastric carcinoma is a common disease with different incidences in the countries.Nowadays gastric carcinoma is one of the most common neoplasm of all cancers. In gastric carcinoma, many studies has conducted for finding out the prognostic factors and various factor like the type of the tumor and its deepness, stage, metastas to lymph nodes, distant metastas were linked in the period survival. Galectin-3, a multifunctional B-galactocide binding lectin possibly participates in a variety of biological events including cell proliferation ,differentiation and apoptozis.The implication of galectin-3 during malignancy progression has been suggested in several cancers ,including colon ,prostate, thyroid and breast cancer,however ,scarce data are available in gastric cancer. Method: In our study, we investigated the expression of galectin -3 in gastric cancer progression and metastasis, 60 invasive gastric carcinoma cases were examined for galectin -3 expression by using immunostaining. Results: We found that cases with high grade gastric cancer expressed galectin-3 were slightly more than low grades. But we found the different galectin-3 expressions according to type of tumor. This was statistically significant. In conclusion: Our results seem to be irrelevant with current literature on the gastric cancers, but there are generally differences between galectin expressions in all organ cancer. The importance of galectin-3 in prognostic markers will be determined with many case series.

Uropathology

PP2-138

UP-REGULATION OF FIBROBLAST AND EPIDERMAL GROWTH FACTOR RECEPTORS, IN URINARY BLADDER CARCINOGENESIS IN HUMANS AFTER THE CHERNOBYL ACCIDENT

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Background. The morbidity of urinary bladder cancer in the Ukraine increased from 6.7 to 10.0 per 100 000 population between 1986-2006 after the Chernobyl accident. The present study was carried out in order to examine the molecular status of selected growth factor receptors (GFRs) in the urinary bladder lesions, recently described by our group as “Chernobyl cystitis”. Material and Methods. Fibroblast growth factor receptor 3 (FGFR3), epidermal growth factor receptor 1 (EGFR1), the member of the same family EGFR2neu, and Raf-1 serine/threonine kinase expression were immunohistochemically (IHC) evaluated in urinary bladder biopsies from 22 males with benign prostate hyperplasia (BPH) – group 1. For comparison, 16 analog males with BPH and 5 females with chronic cystitis living in non-radio-contaminated areas of the country were also investigated as controls (group 2). Additionally, 23 patients with primary urothelial carcinomas (UC), living in the radio-contaminated areas were included for carcinomas group. The current study was approved by the Institute of Urology Ethics Committee, Kiev, Ukraine. Results. Chronic proliferative atypical cystitis (“Chernobyl cystitis”) was observed in group 1 patients. Foci of dysplasia and carcinoma in situ (CIS) were found in 22 (100%) and 19 (86%) of the 22 cases, respectively; moreover, two pTa grade 1 Ucs were also incidentally detected. Greatly elevated levels of FGFR3, EGFR2neu, and to a lesser extent EGFR1 and Raf-1 expression in the urothelial dysplasia and CIS were evident for patients with BPH of group 1. Statistically significant differences in IHC scores for FGFR3, EGFR1 and Raf-1 were observed between groups 1 and 2 ($p < 0.0001$; 0.001; 0.005, respectively) and between groups 1 and carcinomas group ($p < 0.0001$; 0.005; 0.0001, respectively), including EGFR2neu ($p<0.005$). Conclusion. Our findings suggest that FGFR and EGFR signaling pathways, associated with Raf-1 activation, may contribute to multi-stage urothelial carcinogenesis, through autocrine or paracrine growth stimulation.

PP2-139

NON-TUMORAL PARENCHYMA IN LEYDIG CELL TUMORS: PATHOGENETIC CONSIDERATIONS

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BACKGROUND: Little is known about the pathogenesis of Leydig cell tumors (LCTs) of the testis. Interest has grown in studying male reproductive tract disorders based on a new concept on this field: the testicular dysgenesis syndrome, which comprises a variable spectrum of clinical manifestations, such as cryptorchidism, hypospadias, impaired spermatogenesis and testicular germ cell neoplasms. The observation of several associated dysgenetic features in the non-tumoral parenchyma and in the contralateral testes of men with testicular germ cell neoplasms has served as the basis to propose that there may be a common mechanism for different male reproductive disorders.

However, the possible relationship between LCTs and other testicular lesions has not been explored. Here we describe the presence of primary lesions in the non-tumoral parenchyma of testes with LCT, from which we try to establish possible pathogenetic associations. METHOD: We studied the non-tumoral parenchyma adjacent to 16 LCT specimens. Parameters as Leydig cell hyperplasia (LCHY), qualitative evaluation of the germinal epithelium and spermatogenesis, presence of Sertoli cell-only tubules (SCOT), and Sertoli cell nuclear morphology were consistently assessed in all cases. RESULTS: SCOT associated with Sertoli cell dysgenetic morphology was the most frequent finding, present in 50% of cases. Another interesting finding was the presence of LCHY in 4 cases (25%). Abnormal spermatogenesis was found in 81.25% of cases, and it consisted of lesions in the adluminal or basal compartments of seminiferous tubules. CONCLUSION: Although the pathogenesis of LCTs is unknown, their association with other primary testicular abnormalities may be relevant to improve our understanding of their development. The present work delineates the spectrum of morphological findings in the adjacent parenchyma of LCTs. The occurrence of either dysgenetic Sertoli cells and/or LCHY adjacent to LCTs could represent primary anomalies resulting from a common insult, also involved in tumorigenesis. However, the abnormalities in spermatogenesis observed here are likely to represent consequences of either tumor compression or abnormal hormonal production. The significance of these associations merits further investigation regarding a common pathogenesis.

PP2-140

THE ROLE OF DNA PLOIDY AND PROLIFERATION MARKERS (MIB-1 AND "S-PHASE) AS PREDICTIVE FACTORS OF TUMOR VOLUME, STAGE OF TUMOR AND PRESENSE OF POSITIVE MARGINS AFTER RADICAL PROSTATECTOMY

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Backgrounds: DNA ploidy has consistently been found to be a correlate of prostate cancer patient outcome. However, a minority of studies have used pretreatment diagnostic material. The aim of this retrospective study was to evaluate correlations between DNA ploidy, proliferation markers (MIB-1, S-phase), Gleason score, presence of perineural invasion, volume of tumor on biopsy and Gleason score, volume of tumor, stage, presence of positive margins, extraprostatic extension and seminal vesicle invasion after radical retropubic prostatectomy (RRP). Patients and Methods: 212 patients undergoing needle biopsies were diagnosed with cancer between January 2005 and April 2006. All patients were treated with RRP without preoperative adjuvant hormonal therapy. Based on the needle biopsies, DNA ploidy, S-phase and MIB-I immunostaining were measured. These data were combined with the Gleason score, percent cores and percent surface area positive for carcinoma, and status of perineural invasion in multivariate models using tumor volume and risk of extraprostatic extension at radical retropubic prostatectomy. Results: On the basis of DNA content measurement, 145 patients were classified as diploid, 67 patients were classified as non diploid. In non diploid group the mean volume of tumor after radical prostatectomy was 5.04 cm3, percentage of cases with positive margins was 30.9% versus 2.23 cm3 and 12.0% in diploid group. Predictors of tumor volume, presence of positive margins and extraprostatic extension were DNA ploidy (P, 0.0001), MIB-1 positivity (P, 0009), the percent nuclei classified by DNA quantification to be in the "S-phase" category (P 0.04), the percent surface area positive for carcinoma (P, 0.0001). Predictors of coincidence of Gleason score on biopsy and radical prostatectomy was DNA ploidy (P, 0.0001). Conclusions: DNA ploidy and proliferation markers appear to be an interesting feature at diagnosis. DNA ploidy and MIB-1 makes it possible to predict a T3-T4 stage disease and confined tumor.

PP2-141

PRIMARY COMBINED TUMOR OF BLADDER: SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA COLONIC TYPE.

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Introduction. The most common histological type of bladder cancer is urothelial carcinoma which comprises 90-95% of bladder cancer. Squamous cell carcinoma (SCC) of the bladder is much less frequent. Worldwide it constitutes about 1,3% of bladder tumors in males and 3,4% in females. Primary adenocarcinoma (AK) of bladder account for less than 1% of all bladder malignancies. Of these 20-30% are urachal in origin. The most important risk factors for SCC and AK of non urachal origin are tobacco smoking, occupational exposures and schistosomiasis. Prognosis: these tumors are almost radioresistant and the treatment is primarily surgical with extended partial or total cystectomy, in case of urachal AK excision of the urachal mass, urachal tract and umbilicus advocated. Clinical case. A 53-years old man chronic tobacco smoking and with occupational exposure presented macroscopic hematuria. Ultrasound examination, cystoscopy and CT scan showed a solid mall with wide basement on the right bladder wall in the greatest dimension 5x4 sm. The TUR biopsy of tumor was done. Histological examination of the tumor indicated two differentiations of malignant cells: well differentiated AK colonic type and well differentiated SCC. In order to exclude metastases from GI rectoscopy, colonoscopy, gastroscopy were done/ No malignancy in GI found. Diagnosis of primary combined SCC and AK of bladder was performed. The partial cystectomy, lymphadenectomy were done. The was no connection between urachus and tumor. Nevertheless excision of urachus was performed. Macroscopically tumor was presented by solid bulky mass with necrotic masses. Histologic examination of the tumor indicated from well to moderate differentiation SCC and from well to poor differentiation AK of colonic type. Metastases were found in 3 lymphatic nodes. One metestases was presented with poor differentiated AK, and two with moderately differentiated SCC. Conclusion. We report one case of a very rare combined tumor of bladder with considered a highly aggressive behavior. Prognosis in this case is not clear due to the absent of such cases in the literature.

PP2-142

ROLE OF ULTRASOUND-GUIDED CORE BIOPSY IN THE DIAGNOSIS OF RENAL MASSES

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Background: Ultrasound-guided core biopsy has being increasingly used in the last years in the diagnosis of renal masses, but its performance has not still reached a generalised acceptance among radiologists and pathologists. The purpose of this study is to investigate its diagnostic usefulness in a series of renal masses. Material & Methods: Over a 7-year period (2000-2006), a total of 64 renal masses in 63 patients were biopsied under ultrasound control with 18G BioPince® needles. The cases specifically selected for puncture belonged at least to one of the following groups: a) patients with complicated clinical situations that contraindicated surgery, b) tumours with atypical radiological presentation, and c) evaluation of renal involvement in systemic diseases. Surgery confirmed the diagnosis of carcinoma in 8 cases. The clinical context and previous history served as diagnostic controls in the remaining patients. Results: Males predominated in the series (46M/17F), and the average age was 58.14 (range 1-92). Right kidney was slightly more affected (34R/30L). One patient showed bilateral masses. Ablation with radiofrequency was performed in 22 cases. 49 cases (76.56%)

were neoplastic. Diagnostic categories included renal cell carcinoma (34 cases), oncocytoma (6 cases), malignant lymphoma (3 cases), Wilms tumour (2 cases), carcinoid tumour (1 case), cystic nephroma (1 case), spindle cell sarcoma (1 case), and metastatic squamous cell carcinoma (1 case). Five cases (7.8%) were pseudotumours (3 renal cysts and 2 chronic pyelonephritides). Finally, normal renal tissue (6 cases) and fibroadipous tissue (3 cases) were also diagnosed. There were no major diagnostic discrepancies in the series, and only minor changes in assigning the histological variant of renal cell carcinoma were detected in 2 cases. No major complications related to the technique were detected Conclusions: Ultrasound-guided core biopsy is a very useful and reliable method in the diagnosis of renal masses and should be included within the first-line tools in its initial management.

PP2-143
HOW OFTEN ARE CK 5/6, 34βE12, P63 AND P504S USEFUL IN SUPPORTING THE DIAGNOSIS OF MINIMAL CANCER IN PROSTATE BIOPSIES?
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Background: Prostate pathology is quite heterogeneous and the diagnosis of cancer in core biopsies is not always easy. Main difficulties are related to the identification of minimal cancer and its distinction from cancer mimickers. Immunohistochemistry appears as a highly recommended tool to resolve these diagnostic dilemmas. The aim of this study is to critically evaluate the usefulness of this diagnostic method in a large homogeneous series. Methods: A series of 46 minute prostate carcinomas (unique focus of cancer of 0.5 mm or less) have been tested retrospectively with CK5/6 (Dako, 1:100), 34βE12 (Dako, 1:100), p63 (Dako, 1:100) and p504S (Dako, 1:100) antibodies to evaluate the reliability of these markers in supporting the diagnosis of cancer vs. benign lesion. Results: Gleason index (GI) distribution in the series was as follows: <7 (39 cases, 84.8%), 3+4 (3 cases, 6.52%), 4+3 (1 case, 2.17%), and >7 (3 cases, 6.52%). Cancer remained minute in 22 cases (47.8%) when additional cuts were done for immunohistochemistry and grew to become non-minute in 7 cases (15.3%). Interestingly, the minute focus of cancer utterly disappeared in 17 cases (36.9%) making impossible the immunohistochemical evaluation. Basal cells indicative of benignity were better identified with CK 5/6, who showed intense immunostaining in 40 cases (87%) and moderate in 6. On the other hand, 34βE12 displayed intense reaction only in 11 cases (24%), being moderate in 14 and mild in 19. Nuclear staining with p63 offered even poorer results, with only 26 cases (56.5%) being positive in unequivocal basal cells. p504S showed positive immunostaining in the minute cancer of 25 cases (54.3%), but was also positive in benign foci of 4 cases. Conclusions: The diagnosis of minute prostate cancer relies mainly on H&E sections by recognizing the classical morphologic criteria. Serial section intended for immunohistochemistry may loose a significant number of cases. CK 5/6 shows the more intense immunostaining of basal cells. p504S confirm the diagnosis of malignancy in half of the cases and may show false positive results. Immunohistochemistry must be interpreted in the particular context of every patient.

PP2-144
BILATERAL LEYDIG CELL TUMOR AND MALE INFERTILITY: A CASE REPORT
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Background: Leydig cell tumor is a rare form of testicular neoplasm which comprises 1-3% of all testicular tumors and only about 3% of these tumors are bilateral. A few Leydig all tumor have been described in patients with klinefelter's syndrome so far. Case: The patient described in this case report was a 24 year-old man with chief complaint of infertility for one year. Physical examination, semen analysis, testes sonography and hormonal assay were done for him. Right side simple orchiectomy was performed for patient. Conclusion: This tumor is always benign in children and approximately 90% are benign in adults. Clinical presentation is testicular enlargement, gynecomastia, sexual activity disturbances such as decreased libido, infertility and azoospermia. We recommend complete exam and karyotype in patients with infertility and azoospermia. Key words: Leydig cell tumor, Infertility, Azoospermia.

PP2-145
EXTRAGONADAL GERM CELL TUMOR AND MALE INFERTILITY
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Background: Over recent decades a possible decrease in sperm quality and an increase in the incidence of testicular cancer have been reported in many populations. Some recent findings, as cohort studies, showed an increased risk of testicular cancer in men with abnormal semen analysis. Case: A 30 years old man referred to our clinic with chief compliant of infertility for 3 years. Spermogram revealed azoospermia and right extratesticular intrascrotal mass was detected by ultrasound examination. Right inguinal surgical approach showed intact small sized atrophic right testis and an intrascrotal mass. In microscopic examination of the mass mixed germ cell tumor with teratoma, yolk sac and embryonal components were reported. Conclusion: Extragonadal germ cell tumors, like their testicular counterparts are associated with primary germ cell defects. The higher incidence of antecedent infertility suggests that either congenital or acquired primary germ cell defect contributes to defective spermatogenesis and therefore, there is higher risk of cancer development in incompletely migrated germ cells. We recommend complete evaluation of cancer in patients with infertility and azoospermia. Key words: Germ cell tumor, Extragonadal, Azoospermia

PP2-146
CAN CELL CYCLE REGULATORY PROTEINS p53, Ki67 AND bcl-2 PREDICT BIOCHEMICAL RELAPSE AFTER RADICAL PROSTATECTOMY: A TISSUE MICROARRAY STUDY
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Background: Prognostic value of cell cycle regulatory proteins in prostatic adenocarcinoma has been under intense investigation. To analyze p53, Ki67 and bcl-2 expression in our series and their predictive role in biochemical recurrence after surgery, we performed an immunohistochemical study in malignant and non-neoplastic prostate tissue. Methods: 71 patients who had undergone radical prostatectomy between 1992 and 2001 with follow-up data available were selected. Tissue microarrays were constructed from their radical prostatectomy specimens. They contained four cores from neoplastic and additional four cores from corresponding non-neoplastic regions. Gleason score ranged from 6-9, and pathological stage ranged from T2N0Mx to T3BN1. Staining for bcl-2 was scored visually taking percent negative, weak, moderate and strong positivity into consideration. Strong immunoreactivity was considered positive for p53. Ki-67 index was measured as the percentage of positive nuclei among tumor cells. Results: Age of the patients ranged between 48 and 75 (median 62). Follow-up period extended from 4 to145 months, except four males who were lost to follow-up immediately after the surgery. %56 had no evidence of disease, %44 were alive with disease. Tissue microarrays contained normal epithelium in 63, PIN in 3 and tumor in 69 cases. The expression of p53, Ki67

and bcl-2 in tumors were 39%, 76%, 5% respectively. While the secretory layer showed negative or weak bcl-2 staining in most cases, expression in basal cells was often stronger. Pairwise analysis revealed differences in staining between normal and carcinoma for all 3 markers. There was no correlation between stainings and the presence of or time to biochemical relaps. On the other hand, cases with higher Gleason sum showed the tendency for overexpression of p53, Ki-67 and bcl-2 although the differences were not statistically different. Conclusion: The proteins bcl-2, p53 and Ki-67 were expressed at a different rate in normal and neoplastic prostate tissue. Bcl-2 was mainly expressed by basal cells in normal glands. p53 and Ki-67 expression were increased in most prostate carcinomas. But overall expression levels did not correlate with biochemical recurrence in this study.

PP2-147

PLASMACYTOID UROTHELIAL CARCINOMA: PATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILE OF FOUR CASES

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Background: plasmacytoid urothelial carcinoma (PUC) is a very rare variant of Transitional cell carcinoma (TCC). The few previously reported cases were aggressive, high grade neoplasms, with a high stage at initial diagnosis and a poor prognosis. Its clinical presentation is frequently late due to the frequent absence of haematuria and indurated mucosal surface at cystoscopy. Materials and Methods: All cases of high grade urinary bladder TCC were reviewed for a period of 8 years in our hospital between years 1999 and 2006, to identify tumours with plasmacytoid differentiation. Clinical data were obtained from the clinical records, and representative sections from each case which was reported as TCC with plasmacytoid differentiation were examined, and tested with AE1/3, S-100, CD45, EMA, CAM5.2, CK7, CK20, CEA, CA19-9, beta-HCG, Ki-67 antibodies. RESULTS: There were a total of 4 cases of PUC out of 720 cases of high grade TCC (0.55%). The average age was 73 years (range 70-75). Haematuria was the main clinical presentation. Three cases showed distant metastases at time of diagnosis, two of them with peritoneal carcinomatosis, and the third one with soft tissue metastasis in left thigh. Radiological and cystoscopic examination revealed diffuse thickening in the urinary bladder. All cases showed local recurrence after a complete course of chemotherapy. Histopathological examination of bladder biopsies showed common papillary high-grade TCC with invasive areas of PUC in three cases, one of them showed areas of micropapillary TCC. One case showed pure plasmacytoid differentiation. The PUC component showed loose myxoid stroma infiltrated by non-cohesive tumour cells with round borders, abundant deeply eosinophilic cytoplasm, and small, hyperchromatic, eccentrically placed nuclei that closely mimicked plasma cells. Mitotic figures were frequent in the invasive component. Some typical signet ring cells were also present. Immunohistochemical profile of the plasmacytoid areas showed positivity for AE1/3, EMA, CK7 in all cases, with variable Ki-67 positivity (range 40% - 90%). CK20 was positive in 2 cases, CAM5.2 in 3 cases, CEA in one case, CA19-9 in two cases. All cases were negative for CD54, S-100, and Beta-HCG. CONCLUSIONS: PUC resembles plasma cell myeloma and is often confused with haemopoietic malignancies secondarily involving the urinary bladder. When plasmacytoid features are observed in TCC, it should be confirmed by immunohistochemical techniques, and should be reported to aid correlation with prognosis and distant metastasis.

PP2-148

PRIMARY MEDIASTINAL SEMINOMA: A COMPREHENSIVE ASSESSMENT INTEGRATED WITH HISTOLOGY, IMMUNOHISTOMISTRY, AND FLUORESCENCE IN SITU HYBRIDIZATION FOR CHROMOSOME 12P ABNORMALITIES IN 23 CASES

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BACKGROUND: Accurate diagnosis of mediastinal seminoma is critical because of its favorable response to radiation therapy and/or cisplatin-based chemotherapy. However, discrepancies between the genetic alterations and immunoprofiles of mediastinal and testicular seminomas have been reported, raising the question of whether techniques that are useful in the diagnosis of gonadal seminoma are applicable to its mediastinal counterpart. METHODS: The present study was conducted to evaluate the morphological and immunohistochemical characteristics and chromosomal abnormalities of 12p in 23 primary mediastinal seminomas and to compare their applicability as diagnostic tools. RESULTS: Lymphocytic infiltration was found in all 23 cases (100%). The incidence of other histological characteristics were as follows: fibrous septa/stroma (21 cases, 91%), prominent tumor cell nucleoli (21 cases, 91%), clear tumor cell cytoplasm (20 cases, 87%), distinct tumor cell borders (20 cases, 87%), granulomatous inflammation (17 cases, 74%), necrosis (8 cases, 35%), cellular pleomorphism (8 cases, 35%), prominent cystic change (2 cases, 8%), intercellular edema (1 case, 4%) and syncytiotrophoblasts (1 case, 4%). Moderate to strong nuclear OCT4 staining were identified in all 23 cases (100%). 17 tumors (74%) showed membranous expression of c-kit. Weak to moderate staining intensity staining for PLAP was identified in 10 cases (43%) with occasional background staining artifact. The incidences of positive staining were 43% for AE1/3, 39% for HMWCK, 48% for Cam 5.2, 39% for CK7 and 9% for EMA respectively. In most cases these epithelial markers highlighted only a small proportion of tumor cells. CD30 and CK20 immunostaining was completely negative in all seminomas. 22 seminomas (96%) revealed chromosome 12p abnormalities, including 12p amplification in 20 cases (87%) or i(12p) in 15 cases (65%). CONCLUSIONS: Lymphocytic infiltratis the most common histological feature observed in primary mediastinal seminoma and both OCT4 immunostain and FISH for 12p abnormalities can be very helpful in diagnosing mediastinal seminoma. The intense staining pattern of OCT4 and the high sensitivity of FISH make them superior to other auxiliary diagnostic utilities for detecting seminoma. In addition, the incidences of cytokeratin expression of primary mediastinal seminoma are similar to those of its gonadal counterpart and pathologists must exercise caution in the interpretation of epithelial markers in mediastinal neoplasms.

PP2-149

PURE LEIOMYOMA OF THE PROSTATE GLAND

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BACKGROUND: Spindle cell tumors and tumor-like lesions of the prostate gland are never-ending challenge with regard to the differential diagnosis. Although minute leiomyomatous sromal nodules are common in the setting of benign nodular hyperplasia, pure leiomyomas of the prostate are rare entities. METHOD: A 75-year-old man presented with acute renal insufficiency due to bladder outlet obstruction and urinary retention. The patient had a history of prostatism and few urinary tract infections. Digital rectal examination revealed extremely large prostate, certainly greater than 100 mL and it was of benign consistency. Computed tomography scan of the abdomen and pelvis showed bilateral hydronephrosis secondary to considerable prostatic enlargement. The patient underwent an open prostatectomy. RESULTS: Grossly, there was a sharply circumscribed and round nodule measuring 5 cm in greatest dimension with fine trabecular pattern on cut section. On microscopy, the nodule was composed of fascicles of spindle cells. There was no cytological or nuclear atypia and mitotic figures were absent. Immunohistochemical staining showed positivity for vimentin, desmin, smooth muscle actin and h-caldesmon. CONCLUSION: Because leiomyomatous stromal nodules of the prostate are frequent lesions in the background of benign nodular hyperplasia, certain critertia have to be met before a diagnosis of pure leiomyoma of the prostate is established. Other spindle cell tumors and tumor-like lesions must be considered in the differential diagnosis. Moreover, smooth muscle tumors of low or uncertain malignant potential have to be excluded on histopathological evaluation.

PP2-150

ROLE OF CYTOMEGALOVIRUS AND EPSTEIN-BARR VIRUS IN URINARY BLADDER CANCER: MICROBIOLOGICAL, MOLECULAR, PATHOLOGICAL AND ELECTRON MICROSCOPIC CHARACTERIZATION

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Bladder cancer complicating schistosomiasis ranks first among the reported malignancies in Egypt. Recently, it was correlated to viral infection with Epstein-Barr virus (EBV) and Cytomegalovirus (CMV). The present study was undertaken to determine the role of EBV and CMV in the etiopathogenesis of cancer bladder associated with urinary schistosomiasis and to study the correlation of viral genomic sequences in bladder tissue biopsies, serum and urine samples with serologic responses, electron microscopic studies (EMS) of the buffy coat cells and bladder tissue Cytokeratins (CK). Sixty inpatients in Urosurgery Department, TBRI with cancer bladder, 35 with schistosomiasis (group I) and 25 without (group II) and 20 clinically and pathologically proven non-cancer bladder controls (groupIII) were enrolled in this study. Midstream urine, blood and bladder biopsies were collected from all subjects. The following assays were performed; detection of EBV and CMV genomic sequences in bladder tissue biopsies, serum and urine specimens by the polymerase chain reaction (PCR), CMV Antigenemia test for detection of pp65 antigen in polymorphonuclear leucocytes (PMNLs), Enzyme Immunoassays (EIA) for detection of CMV IgG and IgM, EBV nuclear antigen (EBNA) IgG and IgM and EBV early antigen (EBEA) IgG in serum, EMS of the buffy coat cells of 10 patients of each group and Immunohistochemical (IHC) examination for detection of CK–5,6,8,17,19 in bladder tissue biopsies. Our results showed that viral sequences were detected in an overall of 33% of bladder tissue biopsies. From which both CMV and EBV sequences were detected in 80%. Significant values were found overall for viral infection in male (90%) patients with transitional cell carcinoma (TCC) (80%) of grade II (90%), with schistosomal affection (65%), with recurrence tendency (60%) with mixed infection with both CMV and EBV (80%). The results of all assays for detection of CMV or EBV in serum and urine were analysed against PCR on bladder tissue biopsies. Their diagnostic values were found to be unreliable. Cytokeratins were found to be significant.

PP2-151

TISSUE MICROARRAY ANALYSIS OF THE PROGNOSTIC VALUE OF RON AND MET AND HISTOPROGNOSTIC FACTORS IN UPPER URINARY TRACT TRANSITIONAL CELL CARCINOMA

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Objectives: RON and MET are members of the MET proto-ocogene family. RON (Recepteur d'Origine Nantais) is known to play a role in cell transformation and epithelial tumorigenesis. MET (c-met) encodes a receptor tyrosin kinase and intervenes in oncogenesis. Overexpressions of both genes have been demonstrated in human bladder cancer. As urothelium covers the whole urinary tract, we wanted to explore whether overexpression of both molecules plays a role in tumorogenesis in the upper urinary tract transitional cell carcinoma. We also wanted to define histoprognostic factors of these tumors, known to be of poor clinical outcome. Materials and Methods: 42 patients with upper urinary tract urothelial carcinoma have been explored. 24 had localisation in the renal pelvis, 18 ureteral localisation. (14 pTa low grade tumors and 2 pTa high grade tumors; 8 pT1, 6 pT2, 10 pT3 and 2 pT4). Immunohistochemistry for RON and MET was performed on tissue micro arrays taken from surgical specimen of all these patients. Results: Patients were aged from 46 to 100 years (mean 70.6 years). 23 (54%) patients died of disease. Eleven displayed association with carcinoma in situ. Overexpression of MET antigene was associated with higher risk to develop embols ($p=0.0002$), on the opposite, RON was not statistically significant ($p=0.5$). Univariante analysis showed that relapse was statistically significantly associated with ureteral localisation ($p= 0.02$), the presence of vascular invasion ($p=0.003$), high grade ($p=0.04$) and high stade urothelial carcinomas (0.02). The presence of vascular invasion, high grade and high stade urothelial carcinomas ($p<0.0001$ respectively) was also statistically significant. Superficial tumors displayed important relapse rate ($p=0.003$). Conclusion: Independent prognostic factors of relapse in urothelial carcinomas of the upper urinary tract are ureteral localisation, the presence of vascular invasion, high grade and high stade urothelial carcinomas. MET seems to influence one of theses prognostic factors, the development of vascular invasion via a mechanism, which is not exactly known yet. Nevertheless

association of MET overexpression and aggressive clinical behaviour in upper urinary tract carcinomas has not be shown before. In the opposite of bladder carcinomas, RON does not seem to have prognostic value in this kind of malignancies.

PP2-152
MICROVESSEL DENSITY IN PROSTATE CARCINOMA

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OBJECTIVES: Angiogenesis is believed to play an important role in tumor progression and metastasis. Previous studies have suggested that tumor angiogenesis (microvessel density [MVD]) may be of prognostic significance in patients with prostate carcinoma. The aim of this study was to assess MVD in patients with prostate carcinoma and its possible correlation with Gleason score and perineural invasion. MATERIALS AND METHODS: MVD was evaluated using the CD34 monoclonal antibody in archival paraffin embedded TURP (n=28) and radical prostatectomy (n=17) specimens from 45 patients. MVD was counted in areas with the greatest microvessel immunostaining which were designated “hot spots”. RESULTS: The microvessel density count in the tumor area significantly increased with increasing Gleason score (p=0,031) but it did not increase significantly with perineural invasion (p=0,068). CONCLUSION: Although problems exist in methods of counting, MVD, using CD34 monoclonal antibody may provide prognostic information for prostate carcinoma.

PP2-153
A CARCINOSARCOMA ARISING IN AN URINARY BLADDER DIVERTICULUM WITH BREAST METASTASIS IN 45-YEAR OLD MALE: A CASE REPORT

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A 45-year-old man with no previous history of genitourinary disease presented with dysuria and pain in the left groin. The urinalysis showed significant microhematuria, and urinary system ultrasound revealed a large urinary bladder diverticulum (UBD) in the left upper segment. An intravenous pyelogram showed UBD and significant medioposition of the left urether, but without signs of obstruction and secondary hydronephrosis. A cystoscopy examination revealed a large UBD, containing a tumor mass. Diverticulectomy and pelvic lymphadenectomy were performed. Microscopic examination revealed areas containing malignant neoplastic cells with an epithelial component of the transitional and squamous epithelium. Also, some neoplastic cells had morphologic characteristics of the mesenchymal component. Immunohistochemically, the reactions with antibodies against cytokeratin 8, cytokeratin HMV, neuron-specific enolasis and sarcomeric actin were positive, while negative results were obtained in reactions with antibodies against cytokeratin 20, PSA, PSAP, alpha-actin, S-100, CEA, PLAP and synaptophysin. The histopathological diagnosis was carcinosarcoma. Perineural invasion, invasion of the great vessel walls and infiltration of the fat and muscular tissue was observed. Lymph nodes were negative. Three weeks after the surgical procedure CT scan revealed thickness of the urinary bladder wall in the area of the resected diverticulum and one enlarged lymph node in the adipose tissue next to the urinary bladder. Irradiation therapy was started. Five months later the patient discovered palpabile node in the right breast. A fine-needle aspiration was performed and it revealed neoplastic cells with different grades of differentiation with some cells and clusters of cells showing the differentiation towards squamous epithelium. Systemic chemotherapy according to the M-VAC protocole and paliative radiotherapy of the right breast were started. The patient died twelve months after the diagnosis has been established. To our knowledge, we report the first case of UBD carcinosarcoma with breast metastasis.

PP2-154
ADENOMATOUS HYPERPLASIA OF THE RETE TESTIS. REPORT OF A NEW CASE

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Introduction: The great majority of the testicular neoplasms are germ cell tumors but pseudotumors of various types are also encountered and are often diagnostically challenging. Thus, adenomatous hyperplasia of the rete testis is an uncommon benign lesion. Here, we present the clinicopathological features and immunohistochemical findings occurring in a 45 year old man presenting with a grossly and echographical identifiable solid, hilar testicular area. Left orchiectomy was performed under the diagnosis of carcinomas of the rete testis. Macroscopically examination revealed a 5/4/ 3,5 cm testicule presenting on the cut surface a gray white translucide and firm hilar area. Material and methods: Many fragments of the tumor were fixed in formaldehyde 10%, included in paraffin and the sections were stained with HE, VG; immunohistochemically and histochemically : PLAP, CD117,PAS, Red Congo. Results: Microscopic examination revealed a diffuse rete testis tubulopapillary epithelial pattern with cystic dilation and the continuity with non-hyperplastic rete. The lining cells were cuboidal without mitotic figures. The seminiferous tubules surrounded showed atrophic changes. Immunohistochemical and histochemical tests done showed: PLAP and CD117 negative reaction, PAS and Red Congo negative reaction. Conclusions: These findings plead for the diagnosis of rete testis hyperplasia and differential diagnosis must be made with primary adenocarcinoma of the rete testis and metastatic adenocarcinoma of prostate.

PP2-155
ACTIVATED AKT EXPRESSION AND PROGNOSIS IN RENAL CELL CARCINOMAS

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Abstract Purpose: The phosphatidylinositide 3-kinase-protein kinase B (Akt) is involved in the control of apoptosis and cell proliferation therefore overexpression may contribute to tumour development and progression. In detail, a-AKT inhibits the pro-apoptotic activity of BAD, caspase-9 and the forkhead family, and activates several anti-apoptotic substrates. A mechanism of increased Akt activation has been shown to be frequent inactivation of the PTEN. Promising anti-cancer therapies increasingly focus on pathways involving phosphatidylinositide 3-kinase inhibitors as adjuncts to conventional chemotherapy in advanced renal cell carcinomas (RCC). Overexpression of a-AKT has a negative impact on prognosis in various types of malignancies. So the present study investigated the frequency of Akt activation and association with clinicopathological features and prognosis in renal cancer. Material and Methods:

Immunohistochemical analysis (p-Akt and PTEN) was performed on 386 tissue microarrayed specimens with renal cell carcinoma (clear cell n = 305, chromophobe n = 20, papillary n = 40, sarcomatoid n = 17) and tumor-negative renal tissue. Results: Increased a-Akt expression was seen in 158 (40.9%) of 386 RCC with strongest overexpression in the papillary (82.5%) and lowest overexpression in the chromophobe (10%) subtype. Clear cell carcinomas showed increased expression in 39 %. High stage and high grade carcinomas showed increased a-Akt in 45% which was more frequently expressed when compared with low stage and low grade carcinomas. Increased a-Akt expression was significantly associated with shortener survival in univariate and multivariate analysis. Conclusions: Increased expression of activated Akt is a prognostic marker in renal cell carcinomas and the overexpression in a considerable number of high grade and high stage tumors may provide a novel therapeutic option for advanced renal cell carcinomas which are often unresponsive to conventional treatment strategies.

PP2-156
MULTIPLE CARCINOMA OF THE URINARY TRACT

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Introduction: We present the case of a 69 year old man, smoker, hospitalized in the "Prof. Dr. Th. Burghele" Hospital in 2002 accusing right lumbar pains and intermittent haematuria accentuated one month before the event. Ultrasonography and the MRI performed for establishing the diagnosis have shown the presence of a tumoral mass placed at the upper pole of the right kidney. The complete nephrectomy was made. The macroscopic exam of the nephrectomy piece has shown a mass with tumoral aspect, 7/6,5/5 cm in size, yellowish with gray-white zones, hemorrhage and necrosis, apparently delimitated from the rest of the parenchyma, lying at the upper pole of the right kidney. Two years later the patient came back to the Hospital accusing disuria and hematuria. Ultrasonography and cystoscopic exam had show at the urinary bladder a tumoral mass with a cauliflower-like aspect, sessile, 2,5/2 cm. Transuretral resection of the tumoral mass was made and tissulary fragments were sent to the Pathology Laboratory for establishing the hystologic type, the tumor degree and invasion. Material and methods: Fragments of the tumors were fixed in formaldehyde 10%, included in paraffin and the sections were stained with HE and VG. Results: The microscopic exam (Nikon Eclipse 600) of the kidney mass revealed a proliferation composed of nest cell with clear cytoplasm and conspicuous membrane or eosinophylic cytoplasm with round central regular and irregular nuclei, increased size and macronucleoli. The nests were separated by a fine network with thin-walled vases, these features suggesting a clear cell renal carcinoma grade 2 Fürhman. The urinary bladder fragments presented an aspect of papillary urotelial carcinoma high-grade, infiltrating the muscular wall. Conclusions: The frequency of the synchronic neoplasms increase with the hope of life and the investigation methods improvement. The most frequent association of the multiple carcinoma of the urinary tract was urinary bladder and prostate carcinoma (in the radical prostatectomy pieces). Our association is rare but the clinicians must allow for the possibility of multiple synchronic and methachronic carcinoma with this location.

PP2-157
PROSTATE CANCER IN PATIENTS YOUNGER THAN 50 YEARS: COMPARISON WITH OLDER AGE GROUPS IN A CONTEMPORARY SERIES BASED ON 10-CORE BIOPSY

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Background: Prostate cancer is increasingly diagnosed in younger patients due to public awareness, prostate-specific antigen (PSA) testing, and practice of extended biopsies. We explored the clinical and the pathology findings in patients of different age groups in a larger contemporary study. Design: We retrieved clinical, biopsy, and radical prostatectomy (RP) data from our institutional database for patients who had biopsy and RP between 07/2000 and 06/2006. All men had 10-core biopsy and all RP were completely sampled. We compared the data of 1441 men in <50, 50-59, 60-69, >70 years age groups. Statistical analyses were performed using chi-square test for categorical variables and medians were compared for continuous variables with a nonparametric test for trend. Results: Age groups <50, 50-59, 60-69, >70, consisted of 141 (9.8%), 557 (38.6%), 645 (44.8%), and 98 (6.8%) men, respectively. Men <50 had median PSA of 4.1 ng/ml (range 0.5-17.6), which was lower than 5.3, 6.3, and 7.9 in age groups 50-59, 60-69, and >70, respectively (all medians, p<0.001). In 48.2% of men <50 PSA was [LTEQ]4. Patients <50 had median gland volume of 26.9 cc (range 7-75.1), vs. 32.6, 41.6, and 40.4, in men 50-59, 60-69, and >70, respectively (all medians, p<0.001). Ultrasound abnormalities were less common in men <50 (12.8%), than in older age groups (21.2%, 20.9%, 27.6% in 50-59, 60-69, >70, respectively; p=0.033). PSA density and abnormal rectal exam were not significantly different between the groups. On biopsy and RP, Gleason score (GS) 7 and GS [LTEQ]8 were less common in men <50. The proportions of Gleason score 3+4 on biopsy (91%, 79%, 79%, 89%) and RP (84%, 82%, 82%, 80%) were similar across the groups. Cancer extent on biopsy (core %) and tumor (TU) volume on RP did not differ significantly between the groups. Only 5% of men <50 had pT3 disease (vs 7%, 13.5% and 18.4% in men 50-59, 60-69, >70, respectively; p<0.001). Margin positive rate of 22.7% in men <50 was lower than 25%, 28.4%, and 41.8% in men 50-59, 60-69, >70, respectively (p=0.003). Seminal vesicle invasion, node positivity, and lymphatic/vascular invasion were not significantly different in all age groups. Conclusions: Men with prostate cancer <50 currently present with lower PSA, have smaller gland volumes, and lower GS on biopsy and RP, but TU volumes on biopsy and RP are similar as in older men. We document the lowest incidence of pT3 disease on RP in men <50, and a low incidence of adverse findings on RP in all age groups, which further documents the stage-shift of the disease.

PP2-158
ASAP IN NEEDLE CORE PROSTATE BIOPSIES IN MIGUEL SERVET UNIVERSITY HOSPITAL BETWEEN 2002 AND 2006

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Background: The term atypical small acinar proliferation (ASAP) comprises a broad group of lesions such as high grade PIN,

mimickers of cancer and reactive atypia. It also includes situations in which, after a review, it was seen that they were focal carcinoma, but lacked enough cytological and/or architectural atypia to establish a definitive diagnosis of cancer. Method: We will review needle core prostate biopsies performed in Miguel Servet University Hospital of Zaragoza (Spain) between 2002 and 2006. We will determine the incidence of ASAP among these biopsies, the average number of cores per patient, the number of patients with a diagnosis of ASAP who underwent repeated biopsies, and the time elapsed between the first and the following biopsies. We will also determine the percentage of carcinomas diagnosed in the first repeated biopsy. Results: Some provisional results of the study show an incidence of ASAP of 1,9% among 3600 needle core prostate biopsies performed in five years. All patients with ASAP were re-biopsied, 42% of them had a diagnosis of prostate adenocarcinoma in the first repeated biopsy, the predominant Gleason score being 6 (75%). Conclusion: An ASAP does not have defined morphological features, but reflects that a given acinar proliferation lacks definitive diagnostic criteria of carcinoma. Urologists must be aware that ASAP is not the same as high grade PIN, as the risk of diagnosing cancer in re-biopsies is much higher in ASAP, with an average of 40,2%. It has been described that around 90% of carcinomas following a diagnosis of ASAP are detected in the first repeated biopsy. All patients with a diagnosis of atypia suspicious for carcinoma or ASAP should undergo re-biopsy within 3-6 months. Some studies affirm that only 56% of patients with a diagnosis of ASAP are re-biopsied. We believe in the importance of performing repeated needle core prostate biopsies in patients with diagnosis of ASAP, since a high percentage of these cases are actually carcinoma.

PP2-159
URACHAL ADENOCARCINOMA CLINICAL PATHOLOGICAL CHARACTERISTICS: 20 YEAR EXPERIENCE AT THE HGM AND INCMNSZ

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Aim: To know the epidemiological, clinical and pathological characteristics of urachal malignant neoplasms Material and Methods: Cases diagnosed as urachal carcinomas, seen in the last 20 years were selected from the archives at the General Hospital of Mexico Pathology Unit and Pathology Department at the INCMNSZ. The macroscopic descriptions, photographs and histological slides were assessed in each case. Clinical information was gathered from each patient's chart. Results: Seven cases were found based on surgical samples and diagnosed as urachal adenocarcinoma. Average age of tumor presentation was 50.2 years. Five cases were in males and the remaining two in females. The most frequent symptom was hematuria, reported in six cases (85.7%) followed by mucous urination in 3 cases (42.8%), dysuria (42.8%), tenesm (28.5%) and a mucous discharge from the umbilical wound in only one case. Six cases had urachal remnants, 4 were of cystic appearance. Two cases had diffuse invasion of the bladder wall, while in two cases there was infiltration of the prostate. One case had regional lymph nodes metastases. The most frequent histological types were the mucinous type in three cases, two were colonic and two others were of the "ring stamp" variety. The last two were the most clinically advanced stages. Conclusions: Urachal adenocarcinoma is a rare neoplasm. Its incidence ranges between 0.34 and 0.7% of all bladder tumors. It is estimated that overall survival is 5 years, independent of surgical treatment, ranging from 43 to 50%. Due to its low frequency, prognostic factors have not been determined for this type of neoplasm.

PP2-160
EXPRESSION OF VARIOUS CELLULAR MARKERS IN GENITOURINARY RHABDOMYOSARCOMA: IMMUNOHISTOCHEMICAL STUDY USING TMA METHODOLOGY

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Background: Rhabdomyosarcoma (RMS) is the most common sarcoma in the pediatric age group, with 20% of the cases involving the genitourinary tract. These tumors are often characterized by rapid growth, local extension as well as by early metastatic spread. Multimodal therapy has therefore been introduced with ongoing efforts to improve characterization of various prognostic factors, in order to diminish the therapeutic burden and minimize side effects. In accordance, the use of immunohistochemical analysis of the expression of various cellular markers in tumoral cells may enable to better design the therapeutic schedule according to individualized risk-based data and may even define specific therapeutic targets in RMS. The aim of this study was to evaluate the extent of expression of various cellular markers in RMS, using tissue microarray (TMA) technique. Methods: TMA paraffin embedded block was constructed of 8 samples of RMS from different patients operated between 1995-2005. Each tumoral sample was presented in the block by several cores, 2 mm in diameter, containing tumoral tissue with adjacent transitional epithelium and normal detrusor which served as positive and negative controls. After serial slicing of 4 μm thickness, the histological slides were stained with hematoxylin&eosin (H&E) and immunostained with antibodies against Erb-B2, p53, c-kit, Ki 67, Caspase 3, EGFR, N-CAM, TOPO-II and BCL-10. The immunostaining was graded semiquantitatively by the percent of positive cells and the intensity of stain. Results: Four cellular markers were expressed in RMS including Erb-B2, Ki 67, EGFR and N-CAM. Significant positive staining (grades 2-3) for at least one of these markers were noted in 5 of the cases. Conclusions: The expression of Erb-B2, Ki 67, EGFR and N-CAM in genitourinary RMS may have potential therapeutic implications. Our preliminary results may promote further studies to assess the potential role of these markers to serve as therapeutic targets for specific therapeutic agents and thus decrease the overall treatment burden in children suffering from genitourinary RMS.

PP2-161
EXPRESSION OF VARIOUS DRUG RESISTANCE PROTEINS IN WILMS' TUMOR: IMMUNOHISTOCHEMICAL STUDY USING TMA METHODOLOGY

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Background: The multidrug resistance-related protein (MRP-1), lung resistance-related protein (LRP) and topoisomerase-II (TOPO-II) are associated with drug resistance against various chemotherapeutics and protect cells against toxic compounds. Their expression in cancer cells may result in altered membrane transport (MRP-1, LRP) and altered enzymatic activity (TOPO-II). The aim of this study was to evaluate the extent of MDR-related protein expression in Wilms' tumor (WT) using tissue microarray (TMA) technique. Methods: TMA paraffin embedded block was constructed from normal renal tissue, 14 samples of WT from different patients, and from xenografts derived thereof. Each tumoral sample was presented in the block by several cores, 0.06 mm in diameter. After serial slicing, of 4 μm thickness, the histological slides were stained with hematoxylin & eosin (H&E) and immunostained with antibodies against MRP-1, LRP and

TOPO-II. The immunostaining was graded semi-quantitatively by the percentage of the stained cells and the intensity of stain. Results: All the normal kidney tissue samples expressed MRP-1 and were weakly stained or negative for LRP and TOPO-II. Samples of WT were universally stained for MRP-1 (only in the tubular component of the tumor), no expression of LRP was detected and various distribution of TOPO-II was observed. The xenografts varied regarding MRP-1 and TOPO-II expression and exhibited weak or negative staining of LRP. Conclusions: Our study presents the expression of various multidrug resistance proteins in WT, indicating that only MRP-1 might have a potential clinical role. The differences between the expressions of those proteins in the authentic tumors and in their related xenografts might explain differences in response to chemotherapy comparing original tumors and related animal models.

PP2-162
SYSTEMATIC, STANDARDIZED PATHOLOGICAL EXAMINATION OF THE PROSTATE IN RADICAL CYSTECTOMY SPECIMENS RAISES THE DETECTION RATE OF PROSTATIC CARCINOMA IN THESE SPECIMENS

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INTRODUCTION The accepted treatment for patients suffering from urinary bladder cancer is radical cystectomy. Since the majority of these patients are males above 60 years of age, a high rate of coincidental prostatic carcinoma would be expected. Surprisingly, the normal pathological examination of these specimens, using random sampling, results in a lower than expected incidence of prostatic carcinoma. Revised, standardized protocols for sampling and microscopic examination of prostate specimens have recently been implemented in our institution. We here examine the effect of these new methods on the detection rate of prostatic carcinoma in radical cystectomy specimens. METHODS Pathological reports of radical cystectomy procedures in males conducted at the Sheba Medical Center between the years 1994-2004 were reviewed. Specimens examined during the years 1994 and 1998 were processed using a random sampling method; all but 5 of the cases examined between 2000-2001 were also processed using random sampling; and cases examined between 2003-2004 were all processed using the new standardized sampling protocol. The data collected on each case included: the patient's age, total amount of paraffin blocks per case, and amount of paraffin blocks for prostate samples per case. RESULTS 62 pathological reports were reviewed. In comparison with random sampling method, used previously, the detection of prostatic carcinoma increased drastically using systematic standartized pathological examination: 64,7% in 2003-2004 years and 22.2% in 2000-2001 yearsversus 5.6% in 1994 and 1998 y.y. Simultaneously the total number of paraffin blocks from prostate per case was increased from 4.4 blocks per case in 1994 and 1998 years to 13.8 blocks in 2003-2004. The data collected is presented in the table. CONCLUSIONS The results indicate a direct correlation between sampling methods and the detection rate of prostatic carcinoma found in male radical cystectomy specimens. The use of systematic, standardized sampling protocols when processing the prostate in these cases raised the rate of detection of prostatic carcinoma from 5.5% to 64.7% - a rate similar to that reported in the medical literature for the general population

PP2-163
CD24 EXPRESSION IN BENIGN PROSTATE HYPERPLASIA LOW AND HIGH GRADE PIN AND PROSTATE ADENOCARCINOMA

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Background: CD24 recently identified novel prognostic marker gene that consists of 27 amino acids and is attached to cell membrane by a phosphoatidylinositol anchor. It functions as an alternative ligand of P-selectin, an adhesion receptor expressed on activated endothelial cells and platelets, and could thus enhance the metastatic potential of CD24 expressing tumor cells. It has been implicated in metastatic tumor progression of various solid tumors. We aimed to investigate CD24 expression in prostate adenocarcinomas, low and high grade PIN's and benign prostate hyperplasias, and to evaluate its relationship with histopathological parameters, and serum PSA level. Method: We examined immunohistochemically the expression of CD24 protein in 76 prostate adenocarcinomas, 16 low grade PIN, 16 high grade PIN, and 14 benign prostate hyperplasias and correlated our findings with histopathological parameters, and serum PSA level. Results: While we detected CD24 expression in prostat adenocarcinomas and PIN specimens, there was no expression in benign prostate tissues. CD24 immunoreactivity was detected in 48 (63.2%) out of 76 prostate adenocarcinomas. In prostate adenocarcinomas cytoplasmic CD24 expression was significantly positively correlated with Gleason score ($p=0.000$, $r= 0.465$) and PSA level ($p=0.022$, $r=0.263$). Conclusion: Our findings demonstrated that positive CD24 expression occurs in prostate adenocarcinomas with high Gleason score, and high PSA level. We suggest that CD24 might constitute an important moleculer marker for prostate cancer biology. Key words: Prostate adenocarcinoma, PIN, CD24, immunohistochemistry

PP2-164
p53, Ki-67 AND CYCLOOXYGENASE-2 (COX-2) CORRELATION BETWEEN PAPILLARY UROTHELIAL CARCINOMAS OF LOW MALIGNANT POTENTIAL AND NONINVASIVE LOW GRADE UROTHELIAL CARCINOMAS

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Introduction : Difficulties can be seen at the histomorfological diagnosis of the low grade noninvasive papillary urothelial carcinomas (LG-PUC) and the papillary urothelial neoplasms of low malignant potential of the bladder (LMP-PUN) that have different prognosis. We studied p53, Ki-67 and cycloxygenase-2 (cox-2) markers for detecting the possibility of getting benefit at the differential diagnosis of these two subgroups. Materials and methods : We reviewed the cases diagnosed as urothelial neoplasias at the Urology Clinic of Taksim Eğitim ve Araştırma Hospital and applied transuretral resection (TUR) or biopsy. We re-evaluated the cases diagnosed as papilllary urothelial neoplasia of low malignant potential and low grade noninvasive papillary urothelial carcinomas by using WHO classification 2004. From the primary tumors we applied p53, Ki-67 and cox-2 immunohisttochemically to 22 LMP-PUN and 23 LG-PUC, totally 45 cases. Results : While statistically significant difference was observed between LMP-PUN and LG-PUC cases with p53 and Ki-67(lower at LMP-PUN), cox-2 was found to be oppositely related(higher at LMP-PUN). Commonly(both basal and

superficially) staining pattern found to remind LG-PUC with all the three antibodies. Cox-2 staining percentiles were significantly different between LMP-PUN and LG-PUC. Cox-2 is more, commonly stained at LMP-PUN then LG-PUC cases. Discussion : At our study we came to conclusion that immunohistochemical p53 and Ki-67 antibodies can be used to get benefitt of diagnosing the cases histomorfologically similiar to both LMP-PUN and LG-PUC. Moreover we decided that cox-2 inhibitors can be experienced at treatment and at preventing the progression of early stage bladder tumors. Key words : Bladder, LG-PUC, LMP-PUN, p53, Ki-67, Cox-2

PP2-165
IMMUNHISTOCHEMICAL STAINING WITH AMACR/ P63/HMWCK TRIPLE ANTIBODIES BY DOUBLE STAINING METHOD IN RADICAL PROSTATECTOMY SPECIMENS

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Introduction : Our study aims to find the staining percentage , staining density , sensitivity and spesifity of immunohistochemical markers for prostate carcinomas in one slide with three antibodies (AMACR/P504S, P63, HMWCK) together by double staining method . Materials and Methods : We search for 59 radical prostatectomy and 1 TURP materials from January 2002 to December 2005 in Istanbul Haydarpaşa Numune Hospital retrospectively. In our study we especially select low grade prostate carcinomas including wide HGPIN areas. In these cases , we get one slide that include both HGPIN and tumor areas and stain these with triple antibodies (AMACR/P504S, HMWCK, P63) by double staining method . Then we look for their staining intensities and percentages. Results: In our study AMACR expression in malign and HGPIN areas with 100 % specifity, sensitivity values for carcinoma areas as 65-85 %, for HGPIN areas as 98.2 %. In benign areas we don't see any AMACR expression. And also we find positive relationship between Gleason combined score of tumor, the carcinoma area and AMACR expression in the same slide. We see no staining in carcinoma areas with HMWCK and p63 antibodies so find HMWCK, P63 spesifity as % 100. HMWCK sensitivity in benign areas around carcinoma is 85.0 ± 10.33 and also in this area p63 sensitivty is 86.0 ± 9.6. HMWCK and p63 sensitivity and spesifity in HGPIN areas around carcinoma are % 100 for both. Conclusion : AMACR / P504S biomarker helps us to diagnose prostate carcinoma with high sensitivity and spesifity. Although today we look for values of PSA in serology for diagnose of prostate carcinoma, AMACR /P504S may be more spesific biomarker than PSA. We recommend new researches about this biomarker, AMACR / P504S.

PP2-166
IMMUNOHISTOCHEMICAL ANALYSIS OF TISSUE FACTOR EXPRESSION IN HUMAN BLADDER CARCINOMA: CORRELATION WITH ANGIOGENESIS, CLINICOPATHOLOGICAL ASPECTS AND SURVIVAL

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BACKGROUND: Recent studies have shown that tissue factor (TF), a primary physiologic initiator of coagulation cascade, may be involved in tumor angiogenesis and metastasis. This study was designed to assess whether TF expression correlates with microvessel density (MVD), clinical presentation, TNM and prognosis in bladder carcinoma. METHOD: Tissue samples were obtained from 67 specimens of transurethral resected bladder carcinoma. Clinicopathologic and follow-up data of patients were prospectively collected. Expression of TF was examined by immunohistochemical methods. Tumor MVD was evaluated using anti-CD34 as the endothelial marker. Statistical analysis comparing the TF expression with MVD, TNM and overall survival was performed. RESULTS: Forty-one (61.2%) patients had Ta tumors, six (9%) had T1 tumors and 20 (29.9%) presented T2 tumors. Thirty tumors (44.8%) were classified as Grade 1, 15 (22.4%) as Grade 2, and 22 (32.8 %) as Grade 3. Twenty-six specimens had less than 12 microvessels per high power field, and 41 had 12 or more. TF expression ranging from 0 to 25% of the tumor area were observed in 16 specimens (22.5%), 26 to 50% in 11 (16.4%), 51 to 75% in 33 (49.3%) and 76 to 100% in 7 (10.4%). Neither MVD nor TF correlated with tumor stage or grade. There was not a significant correlation between TF expression and MVD However, patients with lower TF expression had better overall survival than groups with TF expression ranging from 26 to 100%. CONCLUSIONS: Most bladder tumors from expressed TF and were highly vascularized. There was not significant correlation between TF and MDV. However, the group with less TF expression had better overall survival when compared with cases expressing an intensity of 26 to 100% of tumor area.

PP2-167
SPLENOGONADAL FUSION - A CASE REPORT

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Splenogonadal fusion is a rare congenital malformation, in which the spleen is abnormally connected to the gonads or mesonephric derivaties. Approximately 150 cases have been reported so far in the literature since the condition was first described by Bostroem in 1883. We report a case of a 23-year-old man who was admitted to the urology department of our hospital for evaluation of left cryptorchidism. CT scan revealed three abdominal nodules on the left and right solitary kidney. During the exploratory laparotomy, the left testis was found in the pelvis and was connected to the spleen with a reddish cord-like structure, which contained two nodules with diameter 0,9 cm and 2,7 cm. Gross examination identified that the testis had a diameter of 3,7 cm. Histological examination revealed that the testis was atrophic with small, thick-walled seminiferous tubules, germinal cell aplasia and mild hyperplasia of Leydig cells. A few seminiferous tubules contained psammoma bodies. The morphological and immunohistochemical findings (negative stain for PLAP-Placental alkaline phosphatase) ruled out in situ carcinoma. The other two nodules consisted of splenic tissue. The histological findings suggested the diagnosis of splenogonadal fusion. We present this case because of its rarity and we review the findings in the literature.

PP2-168
A NEW CASE OF TESTICULAR UNCLASSIFIED MIXED GERM CELL SEX CORD-STROMAL TUMOR

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Introduction: We present the morphological and immunohistochemical features of one case of testicular unclassified mixed germ cell sex cord-stromal tumor (MGSCST) of a 36 years old man hospitalized with a painful left testicular

mass, which appeared 3 months before. Ultrasound exam revealed a 30/20 mm testis with a hipoechogen parenchymal nodule, 8 mm in diameter, without vascularity. A radical left orhidectomy was performed. Macroscopic examination showed a parenchymal nodule, 10/10 mm, near the albuginea, partially circumscribed, grey-whitish coloured. Material and methods: Fragments from the tumor, testicular parenchyma, epididimys and spermatic cord were fixed in formaldehyde 10 %, then included in paraffin, stained with HE, VG and processed for immunohistochemical markers. Results: Microscopically examination revealed a tumoral proliferation of 2 components: epithelial and stromal. The epithelial cells were diffusely disposed in the center of the tumor and they formed tubular structures at the periphery of the tumor. They had abundant vacuolar, here and there eosinophylic cytoplasm; there were also scattered germinal-like cells, with round small nuclei, inconspicuous nucleoli and clear cytoplasm. The stroma was mainly fibrous. There was slightly nuclear pleomorphism, but no atypical mitosis. Some of the tumoral cells seemed to penetrate into the surrounding normal parenchyma. The other testicular structures were free of tumor. The immunohistochemical markers: VIM, S100 positive diffusely in the tumor, PLAP positive in some scattered cells of the tumor (dot-like pattern); inhibin , cromogranina and EMA negative, sinaptophisine weakly positive in tumoral cells. Conclusions: The pathological diagnosis was unclassified MGSCST. Testicular sex cord-stromal tumors are interesting neoplasms because they lead to many issues in differential diagnosis. Despite the negativity for inhibin in our case, this result is not an argument against the diagnosis of sex cord-stromal tumor. The positivity of PLAP favoured the diagnosis of MGSCST, excluding the other sex cord-stromal tumor with entrapped germ cells. Because of the small size of the tumor and the absence of atypical mitosis, we think that our case carry out a favorable prognosis.

PP2-169

RELATIONSHIP BETWEEN RENAL ARTERY CHANGES AND EXPRESSION OF VACULAR ENDOTHELIAL GROWTH FACTOR AND CD31 IN RENAL CELL CARCINOMA

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Background: Renal cell carcinoma (RCC) represents more than 90% of all malignancies of the kidney in adults. Main renal artery may show different lesions such as atherosclerosis or fibromuscular dysplasia (FMD). The aim of this investigation is to correlate expression of vascular endothelial growth factor (VEGF) and CD31 (microvessels density) with renal artery changes in RCC. Patients and methods: We analyzed consecutive series of 55 patients (M:F=35:20) with RCC who underwent nephrectomy. Patients were aging from 35 to 79 years (mean 59.8) and the tumor size was 2.5 to 16 cm (mean 7.1). Specimens were routinely processed and stained with hematoxylin and eosin, Mallory trichrome method and orcein. Immunohistochemical analysis was performed using primary antibodies to VEGF and CD31 (DAKO). Immunohistochemical reaction on VEGF was assessed as: 0–no positive tumor cells; 1–up to 10% positive tumor cells; 2- >10-50% positive tumor cells; 3- >50% positive tumor cells. Microvessels density was immunohistochemically highlighted with antibodies to CD31. The area of tumor that contains the maximum numbers of discrete microvessels and two adjacent areas which were counted have been identified by scanning entire tumor at low power field (X40). Microvessels were counted under high power field (X400) and cut off value was set at 40 microvessels per HPF. Statistical analysis was performed using $\chi 2$ test. The level of significance was set at $p < 0.05$. Results: Renal arteries of 23 (41.8%) patients showed no changes, FMD was found in 25 (45.5%) and atherosclerosis was observed in 7 (12.7%). VEGF expression was negative in 14 (25.5%) cases, 19 (34.5%) cases had less than 10% positive tumor cells, 13 (23.6%) cases had 10-50% positive tumor cells and 9 (16.4%) tumors expressed VGF in more that 50% tumor cells. Twenty (36.4%) tumors had microvessels density over cut off value and 35 (63.6%) had lower number of microvessels. Statistical analysis showed no connection between VEGF expression and microvessels density with T-stage, tumor grade, and renal artery changes ($p>0.05$) but showed correlation between VEGF expression and microvessels density ($p<0.05$). Conclusion: Renal artery changes, especially FMD, are very common in patients with RCC. Expressions of VEGF by tumor cells and microvessel density did not correlate with renal artery changes. Microvessels density correlated with VEGF expression despite of renal artery status. The cause of renal artery changes and their relationship to the tumor neoangiogenesis should be further analyzed.

PP2-170

EXPRESSION OF MAGE-A3/4 AND NY-ESO1 IN RENAL ONCOCYTOMA AND CHROMOPHOBE RENAL CELL CARCINOMA

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Background: The human genes MAGE-A3/4 encode tumor-specific peptide antigens, which are recognized by cytotoxic T lymphocytes. The antigens coded by those genes may be useful target for cancer immunotherapy. There is, however, little information on the expression of these genes and their proteins in renal tumors, especially renal oncocytoma (RO) and chromophobe renal cell carcinoma (CRCC). The aim of the study was to investigate the expression of MAGE –A3/4 and NY-ESO1 in RO and CRCC detected by immunohistochemistry. Methods: We randomly selected 20 consecutive tumors: 10 CRCC and 10 RO. Monoclonal antibodies MAGE-A3/4 and NY-ESO1 were used for immunohistochemical staining. Immunohistochemical staining results were expressed as follows: negative (-), (+) positive in up to 10% of tumor cells, (++) >10-50 % of tumor cells and (+++) >50% tumor cells. Results: Male/ female ratio for RO and CRCC was 3:7 and 2:8, respectively. The average age of patients with RO was 66,5 years (range 57 to 77). The size of tumor was from 0,9 to 8 cm (mean 3,6 cm). Strong cytoplasmic expression of MAGE-A3/4 was observed in RO in 4 cases (40%), moderately positive in 3 (30%) and slightly in 3 cases (30%). Positive cytoplasmic staining was also observed in adjacent kidney tissue. Strong NY-ESO1 expression was observed in 5 RO cases (50%), moderately positive in 2 (20%) and slightly positive in 3 cases (30%). The reaction in adjacent renal tissue was negative or slightly positive. The average age of patients with CRCC was 55,6 years (range 47 to 71). The size of tumor ranged from 2,7 to 17 cm (mean 5,4 cm). The expression of MAGE-A3/4 protein was negative (-) in 3 cases of CRCC, slightly positive (+) in 5 cases and in 2 cases moderately positive (++). Similar reaction was found in surrounding renal parenchyma. NY-ESO1 protein expression was not seen in 5 cases, slightly positive in 3, moderately and strongly positive in one case each. The expression found in surrounding renal parenchyma was stronger than in tumor tissue. Conclusions: This study documents strong immunostaining of MAGE-A3/4 and NY-ESO1 in RO, while staining of CRCC was weak and present in minority of cases. Positive staining for both antigens was

found in adjacent renal tissue. It seems that antigens may not be used as potential therapeutic target. Further studies are needed to explore potential value of MAGE - A3/4 and NY-ESO1 in the differential diagnosis between RO and CRCC.

PP2-171
BILATERAL GONADOBLASTOMA ADMIXED WITH COMPOSED MALIGNANT GERM CELL TUMOR ARISING IN CRYPTORCHID TESTIS: A CASE REPORT

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Gonadoblastoma is a tumor composed by germ cells associated to sex cord component. It usually arise in gonads in patient with chromosomal anomalies. The development of malignant germ cell tumors in gonadoblastoma impose a severe prognosis. We report a new case of patient with 16 year-old who consult for abdominal pain. Physical examination showed abdominal mass. Surgical investigation found 2 hypogastric masses with bowel malrotation. At gross, these masses were well circumscribed with 1200 and 800 g in weight and yellow at cutting with calcifications. Histologically, they shared the same feature of typical gonadoblastoma, associate to seminoma in the once and to seminoma and choriocarcinoma in the second. Adjacent testis tissue was found. The patient died in a context of lung metastasis a day after the surgery. We describe than, the histogenesis of gonadoblastoma, the criteria of diagnosis and the genotype context.

PP2-172
EPITHELIAL TO MESENCHYMAL TRANSITION IS OF STRONG PROGNOSTIC IMPORTANCE IN PROSTATE CANCER

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Cell adhesion molecules are of crucial importance in cancer invasion and metastasis. Epithelial to mesenchymal transition (EMT), characterized by reduced E-cadherin and increased N-cadherin expression, has been recognized as a feature of aggressive tumors, but the importance of this phenotype has not been settled in human prostate cancer. A consecutive series of 104 radical prostatectomies with detailed and long clinical follow-up was studied immunohistochemically for classical cadherins and catenins. In univariate survival analyses, cadherin switching (high N-cadherin and low E-cadherin) showed strong and significant associations with multiple end-points of progression and cancer specific death. In multivariate analysis of time to clinical recurrence, the EN-switch (HR 4.3; $P<0.0005$), had strong and independent prognostic impact, together with Gleason score. These novel data unravel the importance of EMT for prostate cancer progression, and a switch from E-cadherin to N-cadherin expression could have significant impact on the care of prostate cancer patients.

PP2-173
INVESTIGATION OF EPSTEIN-BARR VIRUS INFECTION IN PROSTATE CANCER IN TUNISIAN PATIENTS

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Background: Epstein-Barr virus (EBV) infection has been linked with several malignancies of lymphoid and epithelial cell origin. North Africa is considered as a high-risk area for two EBV-associated tumors: pediatric Hodgkin's lymphoma and nasopharyngeal carcinomas. It has been recently suggested that EBV may be involved in the pathogenesis of some cases of prostate carcinomas. The purpose of this study was to evaluate the prevalence of EBV infection in prostate carcinomas in Tunisia. Methods: Sixty-three consecutive adenocarcinomas of the prostate were tested for the presence of EBV on formalin-fixed and paraffin-embedded tissue specimens. Detection of EBV genomic DNA was performed by polymerase chain reaction (PCR) using primers flanking a 122-bp sequence in the BamHIW repetitive sequence. The presence of EBV in prostatic cells was investigated by EBER1/2-in-situ hybridization and by immunohistochemistry for the detection of EBV latent membrane protein-1 (LMP-1) and Epstein-Barr-replication activator protein (ZEBRA). Results: PCR for the detection of a 268-bp sequence in the β-globin gene indicated the presence of amplifiable DNA in all cases. EBV-DNA-sequences were detected by PCR in 7 cases (11%). By in-situ hybridization, EBER1/2 expression was not detected in neoplastic cells in any of the 63 cases, but in 3 cases, scattered non-neoplastic lymphocytes were EBV-positive. Immunohistochemical study of LMP-1 and ZEBRA was negative in all cases. Conclusion: Given the sensitivity of the technique used, theses results do not support a relationship between EBV and prostate carcinomas in Tunisia.

PP2-174
ANALYSIS OF HUMAN PAPILLOMAVIRUS INFECTION IN BLADDER CARCINOMAS IN TUNISIA

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Background: Human papillomavirus (HPV) infection has been associated with a variety of benign and malignant epithelial lesions. The association of HPV with a subset of bladder carcinomas has been reported but remains controversial. The aim of this study was to evaluate the frequency of HPV infection in bladder carcinomas in Tunisia. Methods: 125 consecutive bladder carcinomas (119 urothelial carcinomas, 5 squamous cells carcinomas and 1 adenocarcinoma) were investigated for the presence of HPV DNA sequences by polymerase chain reaction (PCR). DNA was extracted from formalin-fixed paraffin-embedded tumor tissues. HPV sequences were amplified by three different methods; these included the E1350L/E1547 primers amplifying a 180-bp sequence in a highly conserved region of a wide spectrum of anogenital HPV, the pU-1M/PU-2R primers specific for the oncogenic HPV types 16, 18, 31, 33, 52, 58, and the PU-31B/PU-2R primers for low-risk HPV types 6, 11. Results: PCR for the detection of a 268-bp sequence in the β-globin gene demonstrated the presence of amplifiable DNA in all cases. No HPV-related DNA sequences were identified by PCR in any case of bladder carcinomas tested. Conclusion: This study indicate that bladder carcinomas are not etiologically related to the anogenital HPV tested in patients from Tunisia, however the possibility that other types of HPV contribute in the bladder carcinogenesis can not be excluded and remain to be clarified.

PP2-175
INVESTIGATION OF HUMAN POLYOMAVIRUS INFECTION IN PROSTATE CANCER IN TUNISIAN PATIENTS

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Background: Several investigations have suggested that infectious agents can be potential cofactors in prostate cancer development. Human polyomaviruses JCV and BKV represent viruses with proven linkage to different human cancers. To determine whether these viruses contribute to prostate tumorigenesis in Tunisian patients, we have carried out a polymerase chain reaction (PCR) screening in archival samples obtained during transurethral resection of the prostate. Methods: A group of 63 patients affected by primary prostate cancer randomly selected has been analyzed in comparison with a control group of 24 benign prostatic hyperplasia samples. The DNA of the polyomaviruses BKV and JCV has been searched on formalin-fixed paraffin-embedded prostate tissue specimens by PCR using specific primers. Results: PCR for the detection of a 268-bp sequence in the human β-globin gene demonstrated the presence of amplifiable DNA in all specimens. These samples were considered to have sufficient DNA quality and were therefore used for subsequent analysis. JCV was not detected in any of the 63 prostate cancer cases; whereas 4 benign prostatic hyperplasia cases (16%) have shown positive signals. BKV investigation was negative in all prostatic cancers and benign prostatic hyperplasia specimens. Conclusion: Given the sensitivity of the technique used, theses results do not support a relationship between polyomaviruses BKV and JCV and the pathogenesis of prostate cancer

PP2-176
A RETROSPECTIVE ANALYSIS OF 296 PRIMARY LOCALIZED UROTHELIAL CARCINOMA OF UPPER URINARY TRACTS AIMING ON DISEASE-SPECIFIC AND URINARY RECURRENCE-FREE SURVIVAL

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Background: Urothelial carcinoma (UC) of the upper urinary tract (UUT) is relatively uncommon. It usually pursues aggressive clinical course and shows high frequency of urinary bladder (UB) recurrence and some with metastases even after radical surgery. Only few series on UUT-UC had prudently addressed what parameters could affect final outcomes and urinary bladder recurrence by using large-scaled survival analyses. Methods: Two hundred and ninety-six primary localized UUT-UC surgically treated through a 14-year span were retrospectively analyzed. Patients with preceding UB tumors or metastatic disease and presented as pT4 tumors were excluded. The clinical endpoints evaluated were disease-specific survival (DSS) and UB recurrence-free survival (UBRFS). Follow-up was obtained in all cases which ranged from 1 to 140 months. Results: There were 142 males and 154 females. There were 119, 135, and 42 tumors involved renal pelvis, ureter, and both sits, respectively. Concomitant UB tumors were observed in 52 cases. The primary tumors were non-invasive in 97, T1, T2, and T3 in 72, 48, and 78, respectively. Thirteen cases were of well-differentiated, 136 and 147 were of moderately and poorly differentiated, respectively. For DSS, tumor involving both ureter and renal pelvis ($p=0.0018$), high pT status ($P<0.0001$), a predominant papillary configuration ($p<0.0001$), marked nuclear pleomorphism ($p=0.0006$), high mitotic count (>=5/10 HPFs) ($p=0.0006$), perinurial invasion ($p<0.0001$), the presence of vascular invasion ($p<0.0001$), as well as squamous differentiation ($p=0.0013$), high histological grade ($p<0.0001$) and presence of tumor necrosis ($p<0.0001$) were significant survival predictors. For UBRFS, only the presence of concomitant urinary bladder tumor was significant predictor ($p=0.0045$). Conclusions: Among the clinicaopathologic parameters, high pT status, predominantly papillary growth pattern, perineurial and vascular invasion, accompanied by high histological grade and presence of tumor necrosis are strongest survival determinates for DSS in primary localized UUT-UC. While UBRFS is very difficult to predict and is only associated with the presence of concomitant UB tumor.

PP2-177
PROTEIN EXPRESSION AND MUTATIONAL ANALYSIS OF EPIDERMAL GROWTH FACTOR RECEPTOR IN RENAL ANGIOMYOLIPOMAS

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Renal angiomyolipoma (AML) is a benign but progressive tumor which occasionally requires nonsurgical therapy and there appears to be a possibility that epidermal growth factor (EGF) is associated with pathogenesis of renal AML. The response to gefitinib, anti- epidermal growth factor receptor (EGFR) agent and a prime example of target therapy, reportedly has been correlated with the presence of mutations within the tyrosine kinase (TK) domain of EGFR or the expression of its truncated form, EGFR variant III. Therefore we investigated EGFR protein expression and gene mutations in exons 18, 19 and 21 in 40 renal AMLs. No EGFR gene mutations of TK domain were detected in any of the 40 cases studied and strong immunostaining was found in 5% of the renal AML cases. Our findings indicate that in renal AML, anti-EGFR treatment may not be promising but that there is a possibility that EGFR is associated with renal AML pathogenesis.

PP2-178
INCIDENCE OF FOCAL CARCINOMAS AND ATYPICAL GLANDULAR PROLIFERATIONS (AGP) IN 1000 NEEDLE PROSTATIC BIOPSIES

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Introduction: with the routine use of prostatic specific antigen blood determination, sextant biopsies and immunohistochemical studies, focal carcinomas and atypical glandular proliferations (AGP), limited to isolated histologic fields are being detected more frequently. The recognition of these lesions is important since many of them correspond to potentially curable cancers or to benign lesions that mimic carcinoma. Materials and Methods: 1000 consecutive prostatic biopsies performed during the period of 2000-2007 were reviewed. 42 focal prostatic lesions were retrieved. We reassessed the original HE slides. Additional histologic sections and immunohistochemical studies were performed in those cases with uncertain diagnoses in order to clarify the nature of the lesions. Results: Thirteen (1.3%) lesions corresponded to focal carcinomas and 29 (2.9%) to AGP. Immunohistochemical studies and the analysis of additional histologic sections allowed the classification of 17 cases of AGP into specific categories (prostatic adenosis, atrophy, atypical basal cell hyperplasia and seminiferous ducts). Only three of the 13 patients diagnosed with focal carcinomas were subjected to radical prostatectomy in our institution; all of them had disease limited to the prostatic gland. Of the 29 AGP, additional biopsies

were taken in four cases and in one of them prostatic carcinoma was the final diagnosis. Conclusions: A significant number of AGP biopsies could be sub classified in to specific diagnostic categories with the use of immunohistochemical studies and additional HE levels. The frequency of focal carcinoma and AGP in our material was similar to other series; however in many of the AGP cases additional biopsies were not performed despite its high predictive value for carcinoma. Increase use of follow-up biopsy procedures is needed in order to detect early potentially curable lesions.

PP2-179
LIPOSARCOMA OF SPERMATIC CORD

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Introduction: We present the case of a 57 years old man admitted to our hospital for the presence of a posttraumatic right scrotal mass. Ultrasound exam showed a large solid, inhomogeneous scrotal tumor which pushed downward the testis and epididym. The MRI exam revealed no inguinal and abdominal adenopatic masses. A radical right orhidectomy was performed. Macroscopic examination showed a normal testis and epididym; the spermatic cord presented in the distal portion a large multinodular tumor (12 cm maxim diameter), with gray-whitish, yellow and gelatinous areas. Material and methods: Fragments from the tumor, spermatic cord, testicular parenchyma, epididimys were fixed in formaldehyde 10 %, then included in paraffin, stained with HE, VG and processed for immunohistochemical markers. Results: Microscopic examination revealed a tumoral proliferation composed of myxoid areas with fine arborizing vascular pattern, signet-ring lipoblasts, small cells with uniform nuclei, mature fatty tissue areas, colagenic areas. There were slightly cellular pleomorphism and rare mitosis. No necrosis was found. The parenchymal testis and the epididym were not involved by the tumor. Immunohistochemical markers: VIM, S100 diffusely positive in the tumor, ACT, CD34 positive in blood vessels, PCNA 10-15% positive in the tumor. Conclusion: The morphologic and immunohistochemical diagnosis was liposarcoma with areas of myxoid and well differentiated liposarcoma. Liposarcomas of spermatic cord are rare, comprising approximately 5-7% of all spermatic cord sarcomas. Due to the fact we hadn't any data about the resection margins, local radical excision seems to be insufficient because local recurrence is a major problem, occurring in up to 50% of the patients.

PP2-180
INTRATUBULAR GERM CELL NEOPLASIA IN ADULTS: RETROSPECTIVE EVALUATION OF 446 ORCHIECTOMY CASES.

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Background: Intratubular germ cell neoplasia (ITGCN) is a precursor lesion for certain types of invasive germ cell tumors (IGCT) in testes. We present a demographic profile of 61 ITGCN cases. Method: Data of 544 orchiectomy cases were retrieved from the archive of GATA Haydarpaşa Training Hospital between 1989 and 2006. Orchiectomies were performed because of maldescent testes in 284 cases, germ cell tumors (GCT) in 152 cases, trauma in 21 cases, and prostate cancer in 87 cases. ITGCN diagnosis was confirmed on second views. Microscopic diagnosis of ITGCN was based on H&E, PAS and PLAP staining results. ITGCN rate was calculated for each group. Age range of was 16 to 74 in maldescent group (average: 26,7 median: 23), 16 to 68 in GCT group (average: 20,3 median: 20), 54 to 89 in 87 prostate cancer group (average: 66,5 median: 65), and 16 to 74 in maldescent group (average: 26,7 median: 23). Results: Of 284 maldescent testes, 13 revealed ITGCN (4,5 %). Associated ITGCN was found in peritumoral testicular parenchyma in 93 of 152 GCT cases (61,1 %). In none of the prostate cancer group testes and trauma group testes were ITGCN found. Conclusion: ITGCN rate in our series is found similar to the previously reported incidence ranging between 2-4% for maldescent testes, and 63-99% for association with GCT. This study supports the high ratio coincidence of ITGCN and GCT in the same testes. In addition, recent studies also denote shared molecular alterations supporting the continuity between ITGCN and GCT. ITCGN is considered to be the preinvasive phase of testicular GCT with the exceptions of spermatocytic seminoma, pure yolk sac tumor, and mature teratoma.

PP2-181
COMPARISON OF IMMUNOREACTIVITIES OF P53, E-CADHERIN AND PTEN IN PATTERNS OF ACINAR PROSTATIC ADENOCARCINOMA

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BACKGROUND: Prostate cancer is the most common invasive malignancy in men. Despite many studies, etiology of prostate cancer remains unclear. Lots of genetic alterations have been reported but any specific genetic alterations or an alteration that is seen in most of the patients have not been identified yet. Prostate cancer differs from other epithelial tumors by its intratumoral heterogeneity and multifocality. METHOD: In this study, p53, PTEN, and e-cadherin immunoreactivities were examined in different histological patterns of the prostate cancer that are known to have prognostic significance. With this aim, 40 prostatic adenocarcinoma specimens having different Gleason patterns were selected from the paraffin-embedded archival blocks from patients that had underwent radical retropubic prostatectomy in GATA Haydarpaşa Training Hospital between 1997 and 2006. In all of the patients, p53, PTEN, and e-cadherin expressions were evaluated by immunohistochemistry in areas of different Gleason patterns. Also the relationships between these 3 markers and well known prognostic parameters of prostate cancer including extraprostatic involvement, lymph node metastasis, seminal vesicle infiltration, positive surgical margins, and tumor volume were examined. RESULTS: Between areas with Gleason grades 3 and 4, there was not any difference in p53 immunoreactivity, but loss of PTEN and e-cadherin expressions were found to be more common in grade 4 areas. In clinicopathologic variables, an association was observed between tumor volume and p53 immunoreactivity, also loss of PTEN expression was associated with seminal vesicle infiltration and positive surgical margins. We have not seen a relationship between loss of e-cadherin expression and chlinicopathological variables that we have investigated. Interestingly, loss of e-cadherin expression in patients that showed p53 immunoreactivity tends to be associated with positive prognostic parameters. CONCLUSION: These results show that alterations in p53 gene occur at an early stage but alterations of the PTEN and e-cadherin genes appear with the progression of the disease. We have not seen a case that shows abnormal expression of any of the three markers in low grade areas without abnormal expression in high grade areas. This finding suggests that the multifocality of prostate cancer results from the intraprostatic spreading of a monoclonal lesion rather than different primary tumors. As number of cases in our study were limited, for this purpose new studies with larger series are required.

PP2-182
MOLECULAR ALTERATIONS OF EGFR IN PROSTATE TUMOURS

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Background: The epidermal growth factor receptor (EGFR) is involved in a high variety of processes, mainly proliferation and apoptosis modulation. Recently, point mutations, small deletions, and amplification of this gene have been reported in lung, colo-rectal, head and neck, and many other tumours. These genetic changes seem to correlate with response to kinase inhibitor drugs. Most studies in prostate cancer have dealt with immunohistochemical overexpression of the protein but little is known about EGFR mutations in this tumor. The purpose of this study is to investigate EGFR gene alterations in prostate adenocarcinoma. Material and Methods: 69 prostate tumours and 2 cell lines (DU145 and PC3) have been studied. The tumours were 47 clinically significant (biopsy or prostatectomy) cases, 14 autopsy tumours, one lymph node and 7 bone metastases (42 Gleason ≤7 y 27 >7). DNA was extracted by manual microdissection from formalin-fixed, paraffin-embedded tissue. Exons 18 to 21 were studied by DNA amplification (PCR) with intronic primers and direct sequencing. Protein expression was studied by immunohistochemistry (Clone H11, Dako, Glostrup, Denmark) in a subset of 28 cases. Results: 9/69 (13%) tumours showed EGFR mutations: in 7 cases, all of them Gleason >7, mutations involved exon 21, while in the remaining 2 cases, with Gleason 6, mutations were identified in exon 20. The number of mutations was significantly different between low and high Gleason grade tumours, the latter including metastases (Exact Fisher's test, p=0,026). No mutations were found in the cell lines. The mutations resulted in 5 different protein sequence changes, the most frequent being V834A, in 4 tumours, and I821T, in 2 tumours. 7/28 tumours showed immunohistochemical expression of EGFR; 2/7 (28%) positive and 3/21 (14%) negative cases had mutations in the gene. Conclusions: EGFR gene mutations are found in 13% of prostate tumours, most of them with Gleason grade >7. There is a statistically significant trend towards higher mutation rates in high grade, high stage tumours. The mutation frequency is higher in tumours showing protein expression but the number of cases is low. These results must be compared with gene amplification analysis by FISH. This study has been funded by grants FIS/ISCIII 06/1411 and Asociación Española Contra el Cáncer-Barcelona-2006.

PP2-183
C-ERB-B2, AURORA-A, MIB-1 AND P53 HAVE PREDICTIVE VALUE FOR VASCULAR INVASION AND RELAPSE IN UPPER URINARY TRACT TRANSITIONAL CELL CARCINOMAS

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Objectives: Upper urinary tract transitional cell carcinomas (UUT-TCC) are rare but aggressive. Today's main prognostic factors are tumor stage and grade. Vascular invasion, a factor for predicting relapse and metastatic disease is not always seen on surgical paraffin embedded samples, although they might be present. We wanted to evaluate an immunohistochemical profile to be able to predict vascular invasion and relapse in UUT-TCC. Materials and Methods: 41 patients with UUT-TCC have been explored. 18 had ureteral localisation, 23 in the renal pelvis. (13 pTa low grade tumors and 2 pTa high grade tumors, 8 pT1, 6 pT2, 10 pT3 and 2 pT4). Immunohistochemistry for c-erb-b2, Aurora-A, p53 and MIB-1 was performed on tissue micro arrays taken from surgical specimen of all these patients. Fluorescence in situ hybridization (FISH) was made on samples with immunohistochemical overexpression. Results: Patients were aged from 46 to 100 years (mean 70.6 a). Eleven patients (27%) showed association with carcinoma in situ, 14 (34%) of patients displayed vascular invasion in surgical specimen, 25 (61%) developed relapse, 23 (54%) died of disease. Vascular invasion was associated with overexpression of c-erb-b2 ($p<0.001$), Aurora-A ($p= 0.0034$), p53 (0.0082) and MIB-1 (0.0017). Relapse was associated with high expression of c-erb-b2 ($p= 0.019$), Aurora-A ($p= 0.17$), MIB-1 (0.067) and p53 (0.047). c-erb-b2 results have been confirmed by FISH. Conclusion: Independent prognostic factors of vascular invasion and relapse in UUT-TCC are important to be established. Immunohistochemistry is easily available in pathologists daily routine, and it is important to find profiles on protein level to predict bad clinical outcome for a better patient's follow-up. c-erb-2 is an important prognostic factor and its overexpression has been observed in high grade and high stade bladder tumors. Few data are available on UUT-TCC, our study demonstrates clearly implication of this molecule. Aurora-A plays a role in cell division and in maintaining genomic integrity. It is linked to clinical aggressiveness and poor overall survival of the patients in bladder cancer. Our study is the first to show implication in UCC-TCC. Major implication p53 and MIB-1 in UCC-TCC are known to be prognostic factors of bad clinical outcome. Our data confirm these findings of the literature. We propose an immunoprofile of c-erb-b2, Aurora-A, p53 and MIB-1 to predict vascular invasion and relapse in UCC-TCC, considering that each marker has predictive power.

PP2-184
KLF6 AND P53 MUTATION IN PROSTATE CANCER

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Background: KLF6 is a zing finger transcription factor that reduces cell proliferation through up-regulation of p21 in a p53-independent manner. Different studies have proposed a high variability in the frequency of mutations (form 55% to 0%). The frequency of mutation of TP53 is also controversial ranging from 3% to 42%. The aim of our study is to investigate the role of the tumour supressor genes KLF6 and TP53 in a well-defined group of prostate tumours with different stages and Gleason scores. Design: The study is based on 78 cases of prostate cancer (Gleason 28 ≤6; 24 Gleason =7 y 26 ≥8), plus 16 from autopsy (8 Gleason ≤ 6; 3 Gleason=7 and 5 Gleason ≥ 8), 8 bone and 2 lymph node metastases and 2 cell lines (PC3 and DU145). DNA was extracted from formalin-fixed, paraffin-embedded tissue and manually microdissected. For KLF6 and TP53 exons 1-4 and 4-9 were amplified, respectively. All the changes found in a first round were confirmed or rejected by a second round of amplification and sequencing of the same DNA. Results: Only 1/102 (1%) mutation in KLF6 was identified. This was a

missense mutation (E227G, exon 3, Gleason 7). This was the only change of a total of 38 (3%) confirmed by a second amplification, the remaining 37 being fixation-induced artifacts. 5/102 cases (5%) showed mutations in TP53. They were located in exon 5 (L130H, 2 cases with Gleason 7-8 and one organ metastasis), in exon 6 (H214R, in a Gleason 9 tumour) and in exon 7 (Y234C, in a Gleason 6 tumour). For TP53, these 5 confirmed mutations represented 19% of the 27 changes found in the first amplification. Two previously reported mutations were found (P223L and V274F) in exon 6 and 8, in the DU145 cell line. Conclusions: Our findings indicate that KLF6 mutations are extremely uncommon in prostate cancer. TP53 mutations are also a rare event. The differences in the number of changes found in a first analysis and those confirmed in a second amplification can be explained by Taq polymerase artefacts in formalin-fixed material. Interestingly, if discarded changes are included, the figure approaches the mutation percentages reported by some authors that do not confirm their findings in a second amplification nor in a second DNA extraction. In conclusion, mutations must always be confirmed by a second test, to avoid misinterpreting artefactual changes as true, pathogenic sequence abnormalities. Supported by FIS 06/1411.

PP2-185
ESTROGEN AND PROGESTERONE RECEPTORS EXPRESSION IN PROSTATIC ADENOCARCINOMA – CORRELATION WITH C-ERB2 STATUS

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Background: The carcinogenesis in Prostate results as an imbalance between cell hyperplasia and apoptosis. This process can be influenced by age, diet, hormones and EGFR signaling. The exact role of estrogens in the development of prostate cancer remains unclear. Immunohistochemical expression of estrogen receptor alpha (ER-a) in prostatic cancer revealed conflicting results ranging from loss of up to increased receptor expression. Additionally, the potential implication of the progesterone receptor (PR) in prostatic carcinogenesis has been reported. The expression of c-erb2, a transmembrane glycoprotein similar to the EGF, has also been reported and has conflicting results. Method: In the present study, the expression of ER, PR and c-erb2 was examined immunohistochemically in 70 cases of prostatic adenocarcinoma and was correlated with Gleason's score and TNM stage. Results: ER-a and PR proteins were immunodetected in the nuclei of stromal cells, whereas c-erb2 expression was demonstrated in the cytoplasm of epithelial prostatic cells. The only statistically significant relationships found, in our group of patients, were between c-erb2 and PR expression ($p<0.012$) and between Gleason score and stage ($p<0.004$). Conclusion:Our data suggest that the estimation of hormonal receptor status on prostatic adenocarcinoma histopathological material could be used in order to apprehend the model of prostatic carcinogenesis and can be possibly used for a more effective therapy.

PP2-186
LATE METASTASES OF RENAL CELL CARCINOMA: THREE CASES

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Renal cell carcinomas (RCC) account for approximately 85 % of all malignant renal tumors. It has a high propensity for early metastasis. Most common sites for RCC metastasis are the lung (50 %), bone (33 %), liver, brain and skin. Approximately a third (4-50 %) of patients have metastatic disease at the time of diagnosis. Of these patients 1,6-3,6 % have solitary metastases. Metastases to the head and neck region include 8-14 % of all cases. Solitary soft tissue metastasis is extremely rare. We report three cases of late RCC metastatic to oral mucosa, cervical and gluteal soft tissue and larynx. Case 1: 60-year-old male patient underwent radical nephrectomy for grade III, stage III left RCC in 1997. He presented with right inferior gingival buccal RCC metastasis in 2004. He was treated with chemotherapy postoperatively. Case 2: 65-year-old male patient has been followed up for grade III, stage III left RCC since 2003. In 2005 cervical left soft tissue mass biopsy and in 2007 trucut biopsy of right gluteal soft tissue mass revealed RCC metastasis 4 years after radical nephrectomy operation. He underwent chemotherapy postoperatively. Case 3: 62 year-old male patient. He presented with left cruris mass in 2003. The incisional biopsy revealed RCC metastasis. On urologic and radiologic examination inoperable stage IV left renal mass and multiple bone metastases were diagnosed. He received both chemotherapy and radiotherapy after the diagnosis. In 2005 laryngeal mass biopsy showed RCC metastasis. Multiple hepatic metastases were radiologically demonstrated in 2007. All of the three cases were male. Metastases were seen in 4-10 years after the diagnosis of the primary tumor. All cases are alive at the present without further complications, the third case having tracheostomy. Histopathologic appearances of the metastases were consistent with clear cell carcinoma and identical to those of the primary tumors. Immunohistochemical studies demonstrated diffuse strong EMA and vimentin positivity. HMWC, CEA, S-100, CK 7 were negative. We present these three cases because RCC metastases to these regions are rare and also because of their late occurence. We discuss their histopathological and immunohistochemical findings.

PP2-187
ATYPICAL SMALL ACINAR PROLIFERATION IN THE PROSTATE: REVIEW OF A SERIES OF 64 PATIENTS

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Background: Since the initial pathologic diagnosis of atypical small acinar proliferation of the prostate (ASAP) not always ends in prostate carcinoma, we evaluate clinical and histopathologic factors in order to detect risk of cancer in patients with atypical small acinar proliferation (ASAP) on initial prostate biopsy. Method: From 2002 to December 2006, 51 out of 64 patients with a diagnosis of ASAP were rebiopsied, with a mean time between second and third biopsy of 6 months. In all cases we collected clinical urologyc data and serum prostate-specific antigen (PSA) level. Results and Conclusion: The mean age of patients were 67 years. All but two patients had elevated PSA levels (60.9% of them, between 4.1-10 ng/ml). ASAP was diagnosed in all patients: 53 in the initial biopsy,6 in the second one and 5 in the third one. After ASAP diagnosis, 51 patients were rebiopsied: Of these,5 again showed, 18 benign prostatic tissue and 28 had prostate cancer. We are not seen relations between age or clinical and laboratory data and final diagnosis of cancer.Radical prostatectomy was applied to 20 patients and their pTNM and Gleason grades was as varied as in with others radical prostatectomy specimens in our files. Since ASAP was associated with a definitive diagnosis of prostate cancer in 55% of cases, identification of ASAP warrants repeat biopsy to rule out concurrent invasive carcinoma

PP2-188
THE INCIDENCE OF PELVIC LYMPH NODE METASTASES IN PROSTATE CANCER PATIENTS WITH INTERMEDIATE VALUES OF PROSTATE SPECIFIC ANTIGEN

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INTRODUCTION: Feasibility of radical prostatectomy (RP) in patients with locally invasive prostate cancer (PC) is assessed according to standard parameters such as stage of the disease, serum Prostate Specific Antigen (PSA) and bioptic Gleason grade (GG). In PC the presence of metastases to the pelvic lymph nodes (PLN) is recognized widely as an unfavorable prognostic factor. Intermediate values of PSA are important in predicting the local confines of the tumor but can be detected in more than 30% of patients with locally advanced PC. AIM of this study was to find out the incidence of PLN metastases in PC patients with intermediate serum PSA in clinically localised stages of PC in whom RP was performed. MATERIAL AND METHODS: We used the biopsy cores and the tissue obtained after RP from 46 patients who had intermidiate PSA values which were defined as PSA from 2,6 to 10 ng/ml. In all patient clasical regional lymphadenectomy was performed. In all patients, preoperative stage was determined according to Partin tables (PT) from 2001. RESULTS: In 52,17% patients biopsy GG was ≤6, while it was 7 in 47,83%of patients. Clinically localised disease (cT2b) was found in 86,96 % of patients; other 13,04% of patients had locally advanced PC. After RP, the incidence of localised PC was 47,83%, while advanced disease was found in 52,17%. Metastasis in one or two regional lymph nodes (N1) were found in 10,87% patients with advanced PC. According to PT, expected incidence of positive PLN should have been 8% for pT3 stage. Positive correlation between assumed N1 stage according PT and N1 stage in our RP was statistically significant (p=0.012). CONCLUSION: In more than 50% of patients with intermediate values of PSA, locally advanced disease can be expected after RP. The use of Partin tables have important predictive value in assesing the local confines of the cancer and metastasis in regional lymph nodes. Recent RP series indicate PLN metastases to be less than 10%. We demonstrated detection of LN metastases (10.87%) in our RP series. Our results suggest that PLN should be performed even in patients with low-risk PC.

PP2-189
EXPRESSION OF PPAR GAMMA, KI-67 AND DNA PLOIDY IN UROTHELIAL CARCINOMAS AND CORRELATION WITH PROGNOSIS OF PATIENTS.

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BACKGROUND: Peroxisome Proliferator Activated Receptor gamma (PPAR gamma) is member of the Nuclear Hormone Receptor superfamily (NHR) of ligand activated transcription factors. Recent studies reported that PPAR gamma affects various pathways, important in tumorigenesis and tumor development in several types of cancer and revealed its potential antineoplasmatic role through regulation of the apoptotic mechanism and cell differentiation and inhibition of cell proliferation and angiogenesis. PPAR gamma was also studied in various organs and was correlated with the outcome of patients, although the results were controversial. The purpose of this study was to examine the expression of PPAR gamma protein, Ki-67, DNA ploidy, clinicopathological parameters and patients survival in urothelial carcinomas. METHOD: An immunohistochemical method was applied on paraffin-embedded tissue specimens from 117 urothelial carcinomas, 68 superficial (Ta-T1) and 49 invasive (T2-T4), in order to detect the PPAR gamma protein, Ki-67, mismatch repair genes (MSH6) and DNA ploidy. The above markers were assessed in combination with clinicopathological parameters. The results were statistically processed using chi-square test and overall survival distribution curve was assessed by Kaplan-Meier test. RESULTS: PPAR gamma protein was expressed in cytoplasm of cancer cells in 78.6% (92/117) of total cases. PPAR gamma was expressed in 91.2% (62/68 cases) and 61.2% (30/49 cases) of superficial and invasive carcinomas respectively. PPAR gamma was positively correlated with DNA ploidy (p=0.034) and MSH6 (p=0.036) and was inversely correlated with grade (p=0.002), invasive tumors (T2-T4) (p<0.001) and Ki-67 (p=0.042). Regarding survival, PPAR gamma was associated with improved overall survival (p=0,01), as shown by univariate analysis. CONCLUSION: Our study provides evidence that the expression of PPAR gamma is correlated with urothelial carcinomas of low grade of malignancy and low stage and also exerts favorable impact in the prognosis. These results are further supported by our findings regarding DNA ploidy and the inverse relation between PPAR gamma and Ki-67 and its own possible favorable role in overall survival.

PP2-190
INCIDENTALLY DETECTED PROSTATE CANCER IN CYSTOPROSTATECTOMY SPECIMENS

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OBJECTIVES: Bladder urothelial cancer and prostate cancers occur with increasing prevalence in the ageing population. To determine the incidence of adenocarcinoma of the prostate for patients undergoing radical cystoprostatectomy for bladder cancer.
METHODS AND MATERIALS: We retrospectively reviewed the pathology reports of 57 men who underwent cystoprostatectomy for bladder cancer. All patients had no evidence of prostate cancer on preoperative clinical assessment. The bladder was adequately sampled and the entire prostate sectioned at 5-mm intervals and examined histologically, in order to identify unsuspected prostatic cancer.
RESULTS: Nine patients (15.8%) were found to have incidental prostate cancer. All except one of incidental prostate cancers were organ confined and moderately differentiated. Prostate cancer was organ confined in 8 (88.88%). Capsular invasion was evident in 1 patient (11.11%). No patients exhibited nodal or distance metastases by the prostate cancer.
CONCLUSION: The incidence of prostate cancer in cystoprostatectomy specimens is high. We recommend digital rectal examination and Prostate Specific Antigen evaluation as part of the bladder cancer workup, and complete removal of the prostate at cystoprostatectomy to detect prostate cancer.

PP2-191
PROGNOSTIC SIGNIFICANCE OF MICROSATELLITE INSTABILITY DETERMINED BY IMMUNOHISTOCHEMICAL STAINING OF MSH2 AND MSH6 IN UROTHELIAL CARCINOMA

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BACKGROUND: Mismatch repair (MMR) genes (hMLH1, hMSH2, PMS1, PMS2, hMSH6) are involved in the recognition and repair of certain types of acquired DNA damage, arising during cell division, playing thus an essential role in preserving genetic stability. METHOD: An immunohistochemical method was applied on 130 bladder urothelial carcinomas (BUCs) specimens to detect hMSH2 and hMSH6 in order to investigate their clinocopathological and prognostic value as well as their relation to markers indicative of the tumor phenotype. Nonparametric statistics were performed to evaluate the association between hMSH2 and hMSH6 and the various parameters of interest. Survival distribution were assessed by both univariate and multivariate analysis. RESULTS: hMSH2 and hMSH6 proteins expression was absolutely detected in the nuclei of the malignant cells. Of the BUCs studied, 25,9% were negative for hMSH2 and 49,2% were negative for hMSH6, considered to depict microsatellite instability (MSI). The expression of hMSH2 protein was found to correlate with that of hMSH6 (p=0,028) and both proteins expression to correlate with the age of the patients (p=0,003 and p=0,041 respectively), whereas no association was found with patients' sex. Nuclear hMSH2 values were statistically lower in non-invasive UCs (Ta-T1) (p=0,013) as well as in carcinomas characterized by decreased p53 staining (p=0,04) and high Bax immunoreactivity (p=0,003). Furthermore, lower hMSH6 values were more often observed in well differentiated tumors (p<0,0001) and in tumors with low expression of the protein p53 (p=0,016). As far as the survival is concerned, both hMSH2 and hMSH6 negative immunoreactions were found to exert favorable impact on patient overall survival (p=0,041 and p=0,034 respectively), a finding further verified in the multivariate analysis, only for the hMSH2 protein expression (p=0,026). CONCLUSION: This is the first study to show that lack (and not reduction designated according to various cut points) of hMSH2 and hMSH6 proteins expression is of favorable prognostic significance and to correlate it with wild type p53 and increased Bax expression and therefore a possibly functional apoptotic pathway.

PP2-192
CD10 EXPRESSION IN UROTHELIAL BLADDER CARCINOMAS AND RELATIONSHIP WITH CLINICOPATHOLOGIC PARAMETERS: PRELIMINARY STUDY

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Background: CD10 is a cell surface metalloprotease that inactivates various bioactive neuropeptides such as inflammatory mediators and vasoactive peptides. It is expressed by hematopoietic cells and their neoplasms and in variety of nonhematopoietic tissue and neoplasms. In colorectal and breast carcinomas, it was reported that CD10 positivity was associated with invasion and metastases. CD10 has been suggested as a useful marker for urothelial carcinoma, but the few studies of CD10 in urothelial tumors have shown varying results. The aims of this study were to investigate the expression of CD10 in urothelial bladder carcinomas and to clarify its association with clinicopathologic parameters. Materials and Methods: A total of 50 urothelial bladder carcinomas were selected from archival material. All cases were reevaluated histopathologically and graded according to the WHO/ISUP 1998 systems. The TNM system was used for their pathological staging CD 10 immunohistochemical staining was performed in selected slides. Results: The age of the patients ranged from 23 to 89 (mean± SD 67.2±12.1) years. The cases consisted of 46 men (92%) and 4 women (8%). According to the histological grade, 23(46%) cases had a low grade and 27(%54) cases had a high grade. 35 of the 50 patients (70%) showed positive CD10 immunostaining: +1, +2 and +3 reactivity in 8(16%), 21(42%) and 6(12%) patients, respectively. The remaining 15 cases (30%) were negative for CD10. We observed a cytoplasmic diffuse or granular immunostaining pattern in tumor cells. In addition, rare stromal cells were also positive for CD10 in only a few cases.There was a statistically significant positive correlation between low grade and high grade tumors in the relation to the CD10 expression (P=0.0001). No association was detected between CD10 expression and pathological stage and other clinicopathologic parameters. Conclusions: According to our findings, the CD10 expression in high grade carcinomas showed a higher level than that in low grade carcinomas, and it is positive correlated with the histological grade. CD10 may play an important role in the progression and differentiation of bladder urothelial carcinomas. We believe that further studies will clarify the significance of CD10 expression in bladder urothelial carcinomas.

PP2-193
CORRELATION OF GLEASON SCORES BETWEEN NEEDLE BIOPSY AND RADICAL PROSTATECTOMY SPECIMENS IN PROSTATIC ADENOCARCINOMA

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BACKGROUND: The Gleason histologic grade of prostatic adenocarcinoma is one of the most powerful predictors of prognosis and plays an important role in the treatment decision. The objective of this study was to compare the Gleason scores of prostate needle biopsies with subsequent radical prostatectomy specimens. Possible factors affecting the accuracy of needle biopsy diagnosis and Gleason score (GS) were investigated.
METHOD: The case records of 51 patients with localized prostate cancer who underwent radical prostatectomy between 1998 and 2003 were reviewed, comparing the primary, secondary grade and Gleason score of needle biopsy and radical prostatectomy specimens. The concordance between the biopsy and the prostatectomy Gleason score was then established according to 4 groups as well-differentiated (GS 2-4), moderately differentiated (GS 5-6), moderately to poorly differentiated (GS 7) and poorly differentiated group (GS 8-10). The correlation of Gleason scores between needle biopsy and radical prostatectomy specimens was evaluated by analyzing parameters such as the patient age, preoperative PSA level, biopsy-core number, percent of biopsy tissue containing cancer and tumour heterogeneity.
RESULTS: Mean patient age was 63.9±4.87 years and mean PSA level was 13.94±12.04 ng/ml. Tumors in needle biopsies were mostly in the moderately differentiated (GS 5-6) group and in prostatectomy specimens in the moderately to poorly differentiated (GS 7) group. Comparing the Gleason scores, there was agreement between the biopsy and the prostatectomy specimens in 15 (%29.4) patients. The biopsy Gleason score was lower in 16 (%31.3) and higher than prostatectomy specimens in 20 (%39.2) cases. The difference between the Gleason scores was

±1 for 24 patients, ±2 for 9 patients and ±3 for 3 patients. By classifying patients into 4 groups, the concordance increased from % 29.4 to %49. Statistically, the strength of agreement was considered to be fair and no significant corelation was found between grading discrepancies and the patient age, preoperative PSA level, biopsy-core number, percent of biopsy tissue containing cancer and tumour heterogeneity.
CONCLUSION: The classification of patients into 4 distinct groups increases the concordance between the biopsy and the subsequent prostatectomy Gleason score. Although prostatic needle biopsies may be associated with significant grading errors, they provide valuable information about the histologic pattern reflecting the biologic behavior of the tumor and, help predicting prognosis.

PP2-194
EVALUATION OF MVD IN BIOPSY SPECIMENS OF ATL PATIENTS AND COMPARISON WITH NORMAL CASES BY IMMUNOHISTOCHEMISTRY METHOD

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Abstract Background : adult T-cell leukemia-lymphoma (ATLL)is a rapidly progressive systemic illness with the causative agent ,the human T-cell lymphotropic virus type -1. It is endemic in the japan , Africa ,Brazil and recently north east of Iran ,particulary the region of mashhad. The role of angiogenesis is appreciated in development and prognosis of many malignancies. To examine a possible association between tumor angiogenesis and ethiopathogenic and prognostic role of it in ATL patients this study is performed. Material and methods: This study included 20 ATL patients between 2000-2006. Micro vessel density (MVD) were identified immunohistochemically , using monoclonal CD34 antibody .the MVD of tumoral tissues was defined by counting the number of microvesseles in three hot spots , and the mean values indicated the Micro vessel density (MVD). Normal tissues as control group are noticed. Results: There was statistically significantly increased MVD in tumoral tissue of ATL patients (Mean =17,78)comparison to normal tissues(M =3,54).[p=0,000] Conclusion : Intratumoral Microvessel density quantification in histologic specimens of ATL patients reflects the biologic malignant potential and may be valuable in stratifying patients in planning appropriate adjuvant and antiangiogenesis therapy.

PP2-195
CASTLEMAN'S DISEASE AFFECTING FOCALLY THE CERVICAL REGION. APROPOS OF AN UNUSUAL CASE

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BACKGROUND: Castleman's disease is an unusual lymphoproliferative disease presenting with two different clinical forms, the localized and the multicentric. In the localized form which is curable, 70% of the cases concern the mediastinum and 14% the retroperitoneal space. The multicentric form on the other hand, progresses aggressively and complete remission is achieved only with prednisone or chemotherapy, provided that they are given at the time of diagnosis. CASE REPORT: We present the case of a 43 year old male who presented with an asymptomatic cervical mass measuring 4 cm in diameter, reporting a rapid increase of its volume during the last month. His physical examination and biochemical tests were unremarkable for pathological findings. Chest X-ray and thoracic computed tomography scan showed no significant lesions. The mass was surgically excised and three years after surgery, no sign of recurrence has been recorded. RESULTS: Macroscopy revealed the presence of a swollen lymph node with lobular shape, dishomogeneous texture and whitish cut surface. Histological examination showed a severe lymphadenitis with significant angiofollicular hyperplasia. The latest consisted of nodular masses with proliferating vascular spaces, prominent endothelia and a mixed lymphoid population with histiocytic involvement, infiltrating in a hyalinized stroma. Plasma cells were immunohisochemically identified as polyclonal, exhibiting though an IgG predominance. Lymphoid cells of T-cell origin showed no, or minimal cytological atypia, and were also polyclonal. No vessel distraction or angiocentricity were noted,

nor any viral infection was detected. CONCLUSIONS: Based on the above findings and despite the unusual location of involvement, we diagnosed this case as Castleman's disease, with histological features more compatible to the hyalino-vascular type. Although the disease remains of unknown aetiology, an inflammatory theory with reactional hyperplasia of the beta-lymphocytes has been proposed, being some of the times a result of viral infection. Angioimmunoblastic lymphadenopathy however should always be included in differential diagnosis, especially in cases of multicentric involvement.

PP2-196

QUANTITIVE MICROSCOPIC MEASUREMENTS IN BONE MARROW BIOPSIES. A PROPOSAL FOR A NEW MORPHOMETRIC METHOD

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BACKGROUND: Morhometry in bone marrow biopsies is a field of increasing interest in Haematopathology. It usually focuses on the cellular or angiogenic bone marrow quantity in several diseases, as follow-up or for prognostic evaluation of patients. Based on the Weinder's method for angiogenic estimation and taking in consideration the special characteristics of bone marrow, we developed a new morphometric technique for such measurements. METHOD: The technique is based on the adjustment of an ocular millimetric grid so as to screen the marrow surface and measure within it the studied cells or structures. The grid also provides the potential of simultaneous evaluation of the actual dimensions of cells or vessels, as well as their density in the marrow surface. Measurements are performed at 400X magnification, in whole the cellular marrow, while soft tissues and bone cartilage are excluded. The number of cells or vessels found for each case is finally divided to the number of the high power fields used for screening the whole marrow surface. This division erases the differences of uneven marrow distribution that cells or vessels might manifest. Furthermore, since every parameter of interest is studied both comparatively and statistically, with the findings in normal bone marrow biopsies, the final division outcome, represents the marrow quantity in mean values for every parameter studied. The results are finally expressed as density of cells or vessels per square millimeters. RESULTS: We have so far used this method for measuring the microvascular and mast cell density in the bone marrow of patients with Waldenstrom's disease and multiple myeloma, as well as the density of blasts in patients with myelodysplastic syndromes ranging from RAEB to MDS/AML. The results from applying this method are discussed through a comparative study of relevant references in literature. CONCLUSIONS: This we believe is a reliable method, cost effective and easily accessible for routine work, since it requires no special equipment or training skills.

PP2-197

IGM MYELOMA: REPORT OF A RARE CASE AND REVIEW OF THE LITERATURE

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BACKGROUND: IgM myeloma is a rare plasma cell neoplasm, with an estimated incidence of 0.5% in patients with multiple myeloma. It is estimated that 2 to 3.3% of IgM monoclonal gammopathies are IgM myelomas. Furthermore both IgM myeloma and Waldenstrom's macroglobulinemia may display similar clinical and histological features that make differential diagnosis very difficult. CASE REPORT: We present the case of a 65 year old female patient, who came up with multiple bone lesions and general deterioration. Hepatomegaly, splenomegaly, lymphadenopathy, hemorrhagic diathesis or neurologic involvement were not noted. Laboratory investigation revealed anemia, hypercalceaemia, as well as elevated IgM serum levels (33 g/l). RESULTS: The histological examination of the bone marrow biopsy performed, showed an extensive infiltration of the marrow spaces, reaching up to 75% of the cellular marrow, by neoplastic plasma cells with minimal cytological atypia. Neoplastic cells infiltrated the marrow spaces in an interstitial and diffuse pattern and were immunohistochemically positive for CD45, CD45RA, CD20, plasma cell and cyclin D1 antigens. The investigation for heavy and light immunoglobulin chains revealed IgMκappa clonality. The patient was treated with VAD/thalidomide and eight months later developed bacterial pneumonia and deceased. CONCLUSIONS: Since cases of IgM myelomas are very rare and sporadically reported in literature, no consensus has been achieved so far, regarding the criteria for considering this malignancy as a distinct clinical entity in the spectrum of B-cell neoplasias. Within this concept, we discuss the clinical, histological and immunohistochemical findings of the present case, in contrast with relevant bibliographic references.

PP2-198

MYELOID SARCOMA OF THE SMALL INTESTINE IN AN ALEUKEMIC PATIENT PRESENTING AS SMALL BOWEL OBSTRUCTION

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Myeloid sarcoma (MS) is a rare localized extramedullary tumor composed of immature cells with myelogenous differentiation. It usually develops either concurrently with acute myeloid leukemia (AML) or after remission. On rare occasions, it may be the initial manifestation of AML, occurring most commonly in bone, soft tissue, lymph node and skin. Primary gastrointestinal localization is very rare. A review of the literature revealed that only 15 cases of MS in the small intestine have previously been reported in aleukemic patients. We report the case of a 60-year-old woman who was admitted in our hospital with acute abdominal pain, vomiting and distention, symptoms suggestive of small bowel obstruction. On physical examination, there was no hepatoslenomegaly, peripheral lymphadenopathy, gingival hypertrophy or skin lesions. Her complete blood count was normal. A CT-scan of the abdomen and pelvis revealed a large solid mass in the terminal ileum. She underwent an exploratory laparotomy with complete resection of the tumor. Macroscopically, the tumor had maximum diameter 4 cm and on cut sections it was whitish and elastic. Microscopically, there was a dense and diffuse infiltration of the small intestinal mucosa, submucosa, muscularis propria, serosa and the adjoining adipose tissue, by medium or large neoplastic cells, having a small rim of cytoplasm and enlarged atypical nuclei with fine chromatine and small nucleoli. Immunohistochemically, the neoplastic cells were positive for myeloperoxidase, lysozyme, CD15, CD34, CD43 and CD68 whereas, they were negative for CD20, CD79a, CD3, CD45RO, CD30, CD99, CD117, pancytokeratin and HMB45. So, the diagnosis of MS, blastic type was made according to the WHO classification. The patient received systemic chemotherapy and 6 months later she is in a good condition without evidence of hematological disorders. In the absence of any initial hematological abnormality, the diagnosis of MS remains very difficult. In the present study we report an extremely rare case of

isolated MS of the small intestine in an aleukemic patient presenting as small bowel obstruction. Recognition of this rare entity is important, because early, aggressive induction chemotherapy can improve prognosis.

PP2-199

INCIDENTAL FINDING OF MYELOID SARCOMA IN AN ABDOMINAL LYMPH NODE DUE TO ACUTE APPENDICITIS

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Myeloid sarcoma (MS) is a neoplasm of immature myeloid cells involving an extramedullary anatomic site, that is usually associated with acute myeloid leukemia (AML). Any extramedullary site can be involved by myeloid sarcoma, although most commonly occurs in bone, soft tissue, lymph node and skin. We report the case of a 64-year-old man who was admitted in our hospital due to abdominal pain in the right lower quadrant and fever of approximately 24 hours duration. From his past medical history he was receiving treatment for hypertension, heart insufficiency and type 2 diabetes. All previous hematological tests were normal. On physical examination there was rebound tenderness of the right lower quadrant and rigidity on palpation. There was no hepatoslenomegaly or peripheral lymphadenopathy. His white blood count was 23.000/μL with 46% neutrophils, 4% eosinophils, 9% lymphocytes, 40% monocytes and 1% basophils. Haemoglobin measured 13 g/dL, and the platelet count was 150.000/μL. The peripheral blood film revealed 30% myeloblasts and AML was diagnosed. An abdominal and pelvic ultrasound identified an enlarged appendix with features of gangrenous appendicitis. Because the clinical examination was highly suggestive of acute appendicitis, an appendectomy was performed. During the operation enlarged mesenteric lymph nodes were noted and one of them was excised. Histological examination of the appendix showed a gangrenous appendicitis. The lymph node biopsy revealed effaced lymph node architecture due to diffuse infiltration by a relatively uniform population of medium-sized and large neoplastic cells with a small rim of cytoplasm and enlarged atypical nuclei. Moderate numbers of mitoses were present. Immunohistochemically, the neoplastic cells were positive for myeloperoxidase, lysozyme, CD15, CD34, and CD68 whereas, they were negative for CD20, CD3, CD99, pancytokeratin and HMB45. So, the diagnosis of MS, blastic type was made. The patient died 3 days after the operation due to heart failure. In the present study we report an extremely rare case of MS in an abdominal lymph node, developed concurrently with AML, as an incidental finding due to acute appendicitis.

PP2-200

PRIMARY BILATERAL ADRENAL NON-HODGKIN'S LYMPHOMA PRESENTING AS ADDISON'S DISEASE

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Adrenal masses are usually metastases from malignant tumors, most often from bronchial carcinomas. Non-Hodgkin's lymphoma (NHL) affecting the adrenal glands is usually secondary, associated with other sites of disease, most usually the retroperitoneal lymph nodes and ipsilateral kidney. Adrenal involvement in widespread NHL occurs in 4% of cases. Bilateral primary adrenal NHL is even rarer with only a few previous cases reported. Adrenal insufficiency is a common manifestation in bilateral primary adrenal NHL, occurring in half of the reported cases. We report the case of a 78-years-old woman presented with a 4 month history of nausea, anorexia, weight loss, weakness, night sweats and hyperpigmentation of the skin and the buccal mucosa. Addison's disease was diagnosed based on the clinical presentation and laboratory findings of low cortisol and high adrenocorticotropin levels. Computerized tomography (CT) of chest and abdomen revealed solid, large, bilateral adrenal masses. There was no enlarged lymph nodes or hepatosplenomegaly. A needle biopsy of the left adrenal mass revealed diffuse infiltration by large neoplastic cells characterized by enlarged atypical nuclei with prominent nucleoli and scant cytoplasm. The mitoses were numerous. Immunohistochemically the neoplastic cells were positive for CD20, CD79a, LCA and Vimentine whereas they were negative for NSE, Synaptophysin, Chromogranin, pancytokeratin, UCHL-1 and CD30. So, the diagnosis of diffuse large B-cell lymphoma was made according to the WHO classification. A metastatic work-up, including body CT-scan and bone marrow aspiration and biopsy revealed no evidence of disease, suggesting a stage I NHL. After corticosteroid replacement therapy, the patient received 7 courses of systemic chemotherapy with CHOP and rituximab and 14 months later, both adrenal masses decreased dramatically and the patient is in an excellent condition. Primary adrenal NHL is a rare entity and must be included in the differential diagnosis of bilateral adrenal masses. Histological examination with needle biopsy is very important in order to reach the correct diagnosis, avoid an unnecessary operation, apply the appropriate treatment and improve the survival.

PP2-201

DIAGNOSTIC VALUE OF COMBINING IMMUNOSTAINING FOR CD3 AND NUCLEAR MORPHOMETRY IN MYCOSIS FUNGOIDES

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Background: Mycosis fungoides (MF) represents the most common skin lymphoid neoplasm. In initial stages, differential diagnosis of MF with other benign dermal lymphoid infiltrates (BDLI) may be impossible on morphological basis alone. In previous studies by our group, only deletion of CD7 in MF proved to be of diagnostic help, but not the ratio between immunoexpression of CD4 and CD8. In the present study, 30 cases of MF and 11 cases of BDLI were analyzed, in order to compare morphometric parameters, which could be of diagnostic aid. Methods: Immunohistochemical detection of T-cells was made using an antibody to CD3, on paraffin-embedded tissue from all 41 patients (30 MF and 11 BDLI). Images of 100 positive cells per case in both groups were captured and analyzed using a simple computer program (Imagelab®) for nuclear perimeter (P), area (A), diameters (D) and nuclear contour index (NCI). Results: All parameters showed statistically significant higher values for MF. Area was the variable with the strongest discriminating power between the two groups of patients. Even if morphologic evaluation is not accurate to distinguish benign versus malignant dermal lymphoid infiltrates, due to the variability of size and shape of these cells, a more sensitive method promptly shows this difference. Conclusion: Our results suggest that morphometry of CD3-positive lymphoid cells may add valuable information in the differential diagnosis of MF and benign dermatoses. (Support: Conselho Nacional de Pesquisas Científicas-CNPq; Fundacão de Amparo à Pesquisa do Estado de São Paulo-FAPESP; Fundo de Apoio à Pesquisa-FAEPEX, Unicamp).

PP2-202
DETERMINATION OF ABSOLUTE CD4 LYMPHOCYTE COUNTS IN HIV/AIDS PATIENTS

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Introduction: Human immunodeficiency virus (HIV) infection is increasing at an alarming rate worldwide. The HIV affecting CD4 lymphocytes, finally leads to sharp weakening of immunity (AIDS) and an organism destructions. The CD4 lymphocyte count remains the strongest short-term predictor of risk of AIDS. Obtaining accurate, reliable and affordable CD4 lymphocyte counts is essential in determining disease stage, progression and when to start or change antiretroviral therapy (ARVT) and whole monitoring ARVT. Aim and methods: The objective of this study was to study of CD4 lymphocyte count at early stages of HIV type 1 infection, using the manual magnetic bead method (Dynabead). Blood samples were collected in EDTA Vacutainer containers from each of 200 HIV-positive patients (disease stage I and II). This method requires a three-step technique: depletion of monocytes, isolation of CD4 cells and CD4 lymphocytes counting. After isolation, the CD4 cells were lysed and the liberated nuclei counted in a conventional light microscope after staining with Sternheimer-Malbin solution. Nuclei stained with this staining solution appear blue under white light microscope. The number of cells counted in the haemocytometer is converted to the concentration of CD4 lymphocytes in whole blood by the special formula. Results: Our investigation showed differences in total CD4 lymphocyte counts by stage of the HIV infection. In the patients with HIV infection (stage I) CD4 cells count were about 320-1185 cells/mm^3. In HIV infection, stage II patients CD4 lymphocytes count ranged from 200 - 600 cells/mm^3. Conclusion: The results of this study demonstrated tendency to progressive loss of CD4 lymphocytes at early stages of HIV infection. (This work is supported by Grant A-9-154 RUz).

PP2-203
MYCOSIS FUNGOIDES: IMMUNOHYSTOCHEMICAL ANALYSIS OF LYMPHOID AND MICROENVIRONMENT CELLS BY MACROTISSUE ARRAY

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BACKGROUND: Cutaneous lymphomas (CL) represent a unique group of lymphomas and are the second most frequent extranodal lymphomas. CL probably are the result of a multifactorial and multistep process. Firstly an inflammatory reactive process is developing secondary to various chronic stimuli that may be genetic, environmental, infectious and immunologic. Consequences are the negative effects in cell regulation and deregulation of oncogenes and/or suppressor genes that later promote transition from pre-neoplastic conditions to neoplasia. Detailed molecular expression analysis of cutaneous T-cell lymphoma is not available. Some oncogenic alterations have been demonstrated, such as functional inactivation of the Fas receptor, constitutive activity of STAT 3, or the inactivation of the p16 gene via deletion or promoter hypermethylation. Objective: To study the expression of p16, c-myc, Ki67, bcl-2, CD1a, CD123, TCL1, CD68, STAT 3, STAT 4 and MAL1 in an homogeneous group of CL diagnosed in a terciary Hospital in order to know more about the characteristics of this neoplastic process. METHODS: We have studied 30 CL diagnosed consecutively as Mycosis Fungoides (14 early and 16 advanced stages) between January 2005 and December 2006. By macrotissue array techniques and immunohistochemistry protocol with p16, c-myc, Ki67, bcl-2, CD1a, CD123, TCL1, CD68, STAT 3, STAT 4 y MAL1 was applied in all paraffined histological samples. RESULTS: In 28 samples (92%) we have observed p16 positive overexpression, the two negative samples corresponding to early affectation. In 14 samples from early stages (48%) c-myc was negative. In 29 samples (96%) CD1a was positive in dermal and epidermal layer. CD123 (interleukine 3 receptor) was negative in 16 samples (52%) and TCL1 was positive in 12 cases (39%) in small cells with oval and cleaved nucleus and scarce cytoplasm. Overexpression of MAL1 was observed in advanced patients with aggressive CL. CONCLUSIONS: In our study, overexpression of p16 is observed in the majority of cases and high c-myc expression is seen in advanced stages. Probably dendritic plasmacytic cells are involved in the pathogenesis of skin lymphoproliferative disorders with cutaneous T cell infiltration. MAL1 could be a predictor of aggressive CL, but more studies and more cases are necessary in order to confirm it.

PP2-204
EXPRESSION OF BCL-2 PROTEIN AND AMPLIFICATION OF C-MYC GENE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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BACKGROUND: Chronic myeloid leukemia (CML) represents the malignant myeloproliferative disease developed out of pluripotent hematopoietic stem cell that contains fusion bcr-abl gene. The mechanisms that lead to these changes at molecular level are still unknown as well as the mechanisms that increase proliferative capacity of these cells. Disorders that occur in the process of apoptosis represent one of the possible molecular mechanisms that bring about disease progress. Within our study we carried out the analysis of the presence the amplification of C-MYC oncogene as well as the analysis of the changes in expression of Bcl-2 in patients with CML. METHODS: Our study included 30 patients with CML (23 in chronic phase, 7 in blast transformation). Using immunohistochemical APAAP method we analyzed the expression of Bcl-2 protein in mononuclear bone marrow cells of 30 CML patients. By differential PCR method we followed the presence of amplified C-MYC gene in mononuclear peripheral blood cells. RESULTS: The level of expression of Bcl-2 protein is considerably higher in bone marrow samples of patients undergoing blast transformation of disease. The amplification of c-myc gene has been detected in 30% patients in blast transformation of disease. CONCLUSION: The results of this work have shown that expression of Bcl-2 protein and amplification of C-MYC gene are in correlation with disease progression.

PP2-205
HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF THE PLASMA CELL DYSCRASIAS

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Aim: The aim of this paper is to show the role of the immunohistochemical technique in differentiation of the reactive from malignant lymphoproliferative processes. 3 cases of a plasma cell dyscrasias from the autopsy material at the Institute of pathology in Skopje, differentiated with the usage of the histological (HeEo, Giemsa) and immunohistochemical analysis are shown. Material and Methods: The method of Streptavidin Biotin Peroxidase Technique using a panel of monoclonal antibodies: kappa, lambda, and antibodies for IgA, IgG, IgM, plasma cell IgG (PC), LCA, CD20, and Ki67 were performed. The patients were 2 males and a female at the age of 65, 50 and 57 respectively. Standard autopsy technique and vigorous histological and immunohistochemical analysis were used. In all cases the basic pathological finding was proved to be plasma cell dyscrasia: immunocytoma (case No I), plasmacytoma (case No II) and myeloma multiplex (case No III). Results: The diagnosis was confirmed with immunohistochemical analysis where atypical lympho-plasma cell infiltrates in the tissue specimens taken from the visceral organs were with monoclonal nature: kappa -, lambda + (case No I, and case No III), and kappa +, lambda - (case No II). Besides, the staining for the other markers showed the following results: IgG+, CD10+, CD56+, and PC+. Conclusion: Immunohistochemical technique is important for differentiation of the monoclonal from polyclonal lympho-plasma cell dyscrasias, since they may appear in chronic inflammatory processes, too. The confirmation of the monoclonal characteristics of the same implies the neoplastic proliferation in these 3 cases.

PP2-206
CYCLIN D1 EXPRESSION IN NODULAR LYMPHOCYTE PREDOMINANT HODGKIN'S LYMPHOMA
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BACKGROUND: Hodgkin's lymphomas are divided into two main groups: Classical Hodgkin's lymphomas (cHL) and nodular lymphocyte predominant Hodgkin's lymphomas (NLPHL). Although these two diseases share morphological properties, it has been shown that these two entities have distinct origins. The differential diagnosis of Hodgkin's lymphoma (HL) from various types of non-Hodgkin lymphoma including T-cell rich B cell lymphoma (TCRBCL) can be challenging. Cyclin D1, a member of G1 cyclins, is a cell cycle regulator protein which has a major role at the G1 and S checkpoint, thus overexpression may result in the disturbance of cell-cycle regulation and apoptosis. Expression of cyclin D1 in HL has not been well characterized. In a few series overexpression of cyclin D1 in only 2-19 % of cHL has been reported. In contrast, expression in NLPHL has not been well characterized systematically. METHODS: Eleven NLPHL, 9 TCRBCL and 12 cHL (6 mixed cellular and 6 nodular sclerosing type) cases were culled from the archives of Hacettepe University Faculty of Medicine, Department of Pathology. Cyclin D1 expression was evaluated immunohistochemically (monoclonal antibody; clone SP4, Neomarkers). RESULTS: Strong cyclin D1 expression was seen in 6 (50%) of 12 NLPHL, in contrast to 3 (25%) of 12 cHL, in which few neoplastic cells expressed Cyclin D1. In TCRBCL group, only 1 case showed cyclin D1 expression. Interestingly, this case was a monomorphic posttransplant lymphoproliferative disorder with EBV positivity. CONCLUSION: Cyclin D1 can play a role in the pathogenesis of NLPHL and could be used as an additional marker in the differential diagnosis of LPHL.

PP2-207
BCL-1 POSITIVITY IN NON-MANTLE CELL LYMPHOMAS- REPORT OF TWO CASES
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Bcl-1/PRAD gene rearrangement involving the cyclin D1 gene is a feature of mantle cell lymphomas, virtually all of which show this rearrangement by flourescein in situ hybridisation and immunohistochemistry. Bcl-1 gene disregulation can also rarely be found in other B-cell neoplasias. We report two cases of Bcl-1 positivity in non-mantle B-cell lymphomas: CASE 1: Axillary lymph node excision was performed on a 58 year-old male patient. Macroscopic examination revealed a conglomerate lymph node of 6.5x4.5x3 cm with a multinodular cut section. Microscopic examination revealed a lesion which showed alternate follicular patterns and diffuse infiltration. The tumor was composed of two types of cells: The small to medium sized with cleaved nuclei, and the other having small nucleoli and pale cytoplasm. Immunohistochemically, the tumor cells expressed Bcl-1, Bcl-2, CD20 and CD10. Based on these morphologic and immunohistochemical features, the lesion was diagnosed as "Follicular Lymphoma". CASE 2: A 24-year-old male patient was presented with a pelvic mass. The macroscopic examination revealed a conglomerate lymph node with the greatest diameter of 6 cm. In the microscopic examination, the lymph node structure was replaced by diffuse neoplastic infiltration. The tumor was composed of large cells with pleomorphic, hyperchromatic, vesicular nuclei and conspicuous nucleoli. Immunohistochemically; the tumor cells expressed LCA, CD20, CD43, CD10, CD79a and Bcl-1. Based on these features the lesion was diagnosed as "Diffuse Large B-Cell Lymphoma".

PP2-208
INCIDENCE OF TRANSLOCATIONS INVOLVING THE T-CELL RECEPTOR AND TCL1 GENES IN PERIPHERAL T-CELL LYMPHOMA USING FLUORESCENCE IN SITU HYBRIDIZATION SCREENING OF TISSUE MICROARRAYS
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Background. The genetics of peripheral T-cell lymphoma (PTCL) remain poorly understood. Translocations involving the T-cell receptor (TCR) and TCL1 genes have been described in T-cell lymphoblastic and prolymphocytic lymphomas, respectively, but their incidence in other types of T-cell lymphomas is not well defined. Methods. Tissue microarrays (TMAs) were constructed using triplicate cores from paraffin-embedded tissue blocks in 104 cases of PTCL seen at Mayo Clinic between 1987 and 2006. Diagnoses were classified according to current WHO criteria, and included 19 angioimmunoblastic, 12 anaplastic large-cell (ALCL), 57 unspecified-type (PTCL-U), and 16 other T-cell lymphomas. Home-brew, two-color breakapart fluorescence in situ hybridization (FISH) probes that flanked the gene loci of the T-cell receptor (TCR) alpha, beta, and gamma genes (14q11, 7q35, and 7p15, respectively) and the TCL1 gene (14q32) were used to screen TMAs for abnormalities involving these gene loci. Results. We detected translocations involving TCR-alpha in 2 of 94 informative cases (2.12%), and involving TCR-beta in 1 of 66 (1.52%). No translocations involving TCR-gamma or TCL1 were detected (87 and 72 cases, respectively). One patient with PTCL-U had translocations involving both TCR-alpha and TCR-beta. One patient with ALK-negative ALCL had a translocation of TCR-alpha; further studies suggest the partner gene resides on chromosome 5q. Conclusions. Though rare, translocations involving TCR loci occur in peripheral T-cell lymphomas and can be detected by FISH. These findings may shed light on the genetics and pathogenesis of some cases of PTCL, including a potentially novel translocation involving TCR-alpha and chromosome 5q in a patient with ALK-negative ALCL.

PP2-209
P53 AND P27 EXPRESSION IN DIFFUSE LARGE B CELL LYMPHOMA AND SMALL LYMPHOCYTIC LYMPHOMA: IMMUNOHISTOLOGY WITH PROGNOSTIC SIGNIFICANCE?

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OBJECTIVE: The heterogeneity of non-Hodgkin lymphoma is highlighted by the variable expression of a variety of molecular aberrations, some of which have been shown to be predictive of outcome. The p27/Kipl gene inhibits a variety of cyclin-cyclin-dependent kinase complexes and regulates cell growth. The p53 gene acts as a tumor suppressor gene, controlling entry into the S-phase of the cell cycle. The purpose of the present study was to investigate alterations of cell cycle-related molecules, including p27,and p53, in diffuse large B cell lymphoma and small lymphocytic lymphoma, and their relation to conventional clinical prognostic parameters. METHODS: The expression levels of p27 and mutant p53 proteins in 39 diffuse large B cell lymphomas and 26 small lymphocytic lymphomas were analyzed by immunohistochemistry. The clinicopathological variables, recurrence and disease-free interval were assessed. Meanwhile, the clinical significance of them was analyzed combining with the clinicopathological factors and followup data. RESULTS: In diffuse large B cell lymphoma cases, p53 and p27 nuclear reactivity was seen in 30 (%76.9) of 39 and 7 (%18.4) of 39 cases, respectively. In small lymphocytic lymphoma cases, p53 and p27 were seen in 20 (%76.8) of 26 and 17 (%65.4) of 26 cases, respectively. There were statistically significant differences in p27 expression between the two lymphoma groups. The p27, and p53 protein expression in diffuse large B cell lymphoma were not associated with age, stages, lymph node localization, B symptoms, extranodal extension, relaps, drug resistance, and mitosis. p53 protein expression in small lymphocytic lymphoma was also not associated with age, stages, lymph node localization, B symptoms, extranodal extension, relaps, drug resistance, but p53 protein expression was associated with mitosis ($p=0.039$). No significant association was found between p27 status and clinicopathological parameters. p53, and p27 expression was not associated significantly with overall survival rate ($p>0.05$). CONCLUSION: The authors concluded that p53, and p27 protein expression was not related to the clinicopathologic parameters, and survival in two lymphoma groups. Further studies are needed to evaluate the predictors of outcome in non-Hodgkin lymphoma, considering a variety of prognosticators.

PP2-210
HUMAN IMMUNODEFICIENCY VIRUS-NEGATIVE PATIENT WITH COEXISTENT INTESTINAL KAPOSI'S SARCOMA AND ANAPLASTIC LARGE CELL LYMPHOMA

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BACKGROUND: Classical form of Kaposi's sarcoma is known to mainly affect elderly and visceral involvement is not often in HIV-negative patients. Here, we present a case of multifocal intestinal Kaposi's sarcoma in a patient who also had a coexistent anaplastic large cell lymphoma. CASE REPORT: A 41-year-old woman with no prior medical history was admitted to the emergency department with abdominal distention and pain. A preliminary diagnosis of mesenteric vascular occlusion was made and the patient underwent laparatomy. Segmentary small intestinal resection was performed. The patient deteriorated and died two weeks after the operation. No mass lesion was noted on the resection specimen, however the serosa and mucosa was brown, fragile and edematous. The mucosa was randomly sampled and mesenteric lymph nodes measuring upto 1 cm were dissected from the mesocolon. Microscopically, infiltration of lamina propria with multifocal spindle cell proliferation positive for CD31, CD34, factor 8 and thrombomoduline and negative for c-kit, S100, HMB45 and melan-A were seen. Additionally, on the serosal surface of the intestine and in mesocolonic lymph nodes, an infiltration by a different group of large, pleomorphic neoplastic cells positive for LCA, CD30, EMA, ALK, granzyme B and negative for CD3, CD20, CD79a and CD138 were noted. The patient was proven to be HIV-negative both by PCR and serologically. To further support the diagnosis of visceral Kaposi's sarcoma in an HIV-negative patient, immunohistochemical detection of HHV-8 infection was done by the use of antibody directed against LANA (latent nuclear antigen). Neoplastic lymphoid cells were found to be negative while nuclear positivity was detected in the neoplastic spindle cell proliferation. CONCLUSION: It was concluded that, this was a rare case of multifocal intestinal Kaposi's sarcoma with coincidental ALK- positive anaplastic large cell lymphoma in an HIV-negative patient. Although HHV-8 associated lymphomas have been reported to take anaplastic large cell morphology, association between these two entities could not be presented in this case as HHV-8 could not be detected in the latter.

PP2-211
B-CELL LYMPHOMAS WITH TRANSLOCATIONS INVOLVING THE CDK6 GENE LOCUS REPRESENT A DISTINCT CLINICOPATHOLOGICAL ENTITY.

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Reentry and progression through the G1 phase of the cell cycle is driven by cyclin-dependent kinases (CDK) in cooperation with cyclins, and alteration of this pathway is an important mechanism in oncogenesis. In lymphoid neoplasms, this pathway has been best studied in mantle cell lymphoma, in which CDK/Cyclin interaction is deregulated by translocations involving cyclins, most commonly cyclin D1. In this study, we describe clinicopathological and genetic features of lymphomas with genetic alterations affecting the CDK arm of this pathway. Four cases of B-cell lymphoma showing cytogenetic abnormalities of the 7q22 region where CDK6 gene is located were identified in the Mayo Clinic Cytogenetic database. Clinical and pathological data were reviewed. The karyotypes were studied by G-banding, and breakapart and dual-fusion fluorescent in situ hybridization (FISH) strategies using probes targeting CDK6, IGH , IGK and IGL loci were applied on metaphase spreads and paraffin embedded tissue. Three patients were male (age: 56, 66, 77) and one was female (age 68). All patients presented with peripheral lymphocytosis and three had systemic lymphadenopathy and splenomegaly. Peripheral blood morphology showed small to intermediate cells with atypical nuclear features. The bone marrow contained nodular and interstitial infiltrates of small lymphocytes. The spleen histology was available in two cases and showed features most consistent with a splenic marginal zone lymphoma. Immunophenotypically the neoplastic cells expressed CD20 and weak/partial CD5 but not CD23, CD10 or cyclin D1. Genetic analysis revealed that three cases had fusion of the CDK6 and IGK gene loci. The fourth case had a translocation involving CDK6 and an unknown partner gene on chromosome 21. Limited clinical follow-up data implied an indolent clinical course. Our findings suggest that B-cell neoplasms with CDK6 abnormalities have unique clinicopathological features overlapping with mantle cell lymphoma and splenic marginal zone lymphoma and may represent a distinct entity.

PP2-212

EPIDEMIOLOGIC, DEMOGRAPHIC DATA OF 212 NON-HODGKIN MALIGNANT LYMPHOMA AND MORPHOLOGICAL / IMMUNOHISTOCHEMICAL STUDY ACCORDING TO WHO CLASSIFICATION

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Aim: According to 2001 WHO- REAL classification; distribution of the subtypes of malign lymphoma differs all around the world. The frequency of B cell lymphomas is higher in western countries whereas T cell lymphomas are more common in asian countries. Our aims are; 1. To examine the morphology and immunohistochemical profile of non-hodgkin lymphoma cases (NHL), subclassify them according to WHO classification and to obtain demographical and epidemiological data by evaluating the diagnoses with clinical features. 2. To compare our data with the others reported in different parts of the world and improve epidemiological studies. Material-methods: Retrospectively, we examined 212 non-hodgkin lymphoma cases presenting between january 2003- december 2004 in Ankara Oncology Hospital, Pathology Department. The cases have been evaluated immunohistochemically (ALK, BCL-1, BCL-2, BCL-6, CD3, CD4, CD5, CD8, CD10, CD15, CD20, CD23, CD30, CD43, CD45, CD45RO, CD56, CD79a, EMA, Tdt, Granzym-B and EBV), clinical and morphological and than classified as B and T cell neoplasms according to 2001 WHO classification. Results: Of 212 non hodgkin lenfoma cases 195 (%92) are B cell, 17 (% 8) are of T cell origin. Most common subtype is Diffuse large B cell lymphoma (DLBCL)(125 cases, % 59). Other subtypes are respectively; Follicular lymphoma(%10,4), Small lymphocytic lymphoma (% 9.9), Mucosa associated lymphoid tissue-lymphoma (% 4.2), Mantle cell lymphoma (% 2.8). Uncommon subtypes are; lymphoplasmacytic lymphoma (% 2.4), nodal marginal zone lymphoma (% 1.4), Burkitt lymphoma (% 0.9), precurser B lymphoblastic lymphoma (0.5), intravascular large B cell lymphoma (% 0.5). The most common T cell lymphoma subtypes are respectively; peripheral T cell lymphoma (% 2.8) and anaplastic large cell lymphoma (% 1.9). Precursor T lymphoblastic lymphoma is more common than precursor B lymphoblastic lymphoma (% 1.4 and % 0.5). In our series there is small number of Mycosis fungoides, NK/T cell and primary cutaneous anaplastic large cell lymphoma cases. Gastrointestinal localisation is the most common extranodal lymphoma, following in order; head and neck, skin, extradural. Most common extranodal lymphoma subtype is DLBCL but skin lymphomas are of T cell origin. Conclusion: In our series immunophenotypic results of NHL subtypes are compatible with other reports in literature. When diagnosing and subtyping NHL cases, clinical, morphological and immmunohistochemical results should be evaluated together

PP2-213

FOCAL ADHESION KINASE (FAK) IN NORMAL AND NEOPLASTIC LYMPHOID TISSUES

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Background: Focal adhesion kinase (FAK) is a protein tyrosine kinase that plays a major role in the focal adhesion complex. FAK is essential for intracellular regulatory events such as cell growth, differentiation, cell migration and tumor metastasis. FAK expression has been reported to correlate with clinical behavior in several types of solid tumors such as neoplasia of brain, breast, thyroid, prostate, and colon. The aim of this study was to analyse the expression of FAK protein in a series of normal and neoplastic lymphoid tissues. Method: A polyclonal anti-FAK antibody was used to study by immunohistochemistry the protein expression in paraffin-embedded sections of normal haemato and (reactive lymph node, tonsil, thymus, spleen, bone marrow and lymph node with Toxoplasmosis infection), non haematolymphoid (skin, muscle, breast, thyroid, gallbladder, liver, stomach, pancreas, kidney, adrenal, endometrium, placenta, ovary, prostate, testis) tissues as well as in neoplastic non-haematolymphoid (fibroadenoma, breast carcinoma, thyroid carcinoma, adenocarcinoma of the colon, prostate, cervix, endometrium, ovary) neoplasias, and in a total of 453 lymphomas (including B, T cell non-Hodgkin lymphomas, Hodgkin's lymphomas and myelomas). Results: In normal lymphoid tissue, the strongest expression of FAK was detected in germinal centre and marginal zone B cells, positive staining was also found in mantle zone B cells and plasma cells. In human lymphomas, FAK was expressed mostly in B cell lymphomas. T cell lymphomas were predominantly negative with the exception of two cases of peripheral T cell lymphomas. In Hodgkin's lymphomas (HL), FAK was found only in the neoplastic cells of lymphocyte predominant Hodgkin lymphoma whereas the tumour cells of the classical form were FAK-negative. FAK that was expressed in normal plasma cells was negative in 9 out of 10 cases of myeloma. Conclusion: We demonstrate for the first time the expression of FAK in paraffin-embedded lymphoid tissue sections. FAK was expressed in many B-cell lymphomas whereas in T cell lymphomas FAK was negative in almost all instances. In HL, the highest expression of FAK was seen in the tumour cells of lymphocyte predominant, whereas in all cases of classical Hodgkin, the neoplastic cells were FAK-negative. Its differential expression may be of relevance for some B cell lymphomas e.g. it can be used as an additional marker to distinguish B from T lymphoblastic lymphoma/leukemia, or to distinguish lymphocyte predominant from classical Hodgkin lymphoma and normal from neoplastic plasma cells.

PP2-214

PROGNOSTIC SIGNIFICANCE OF MICRO VESSEL DENSITY IN DIFFUSE LARGE B CELL LYMPHOMAS

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Angiogenesis and angiogenic growth factors have a major role in the pathogenesis of malignancies. Studies related to non Hodgkin lymphomas and angiogenesis are relatively limited and have conflicting results. We investigated the role of angiogenesis in diffuse large B cell lymphomas (DLBCL) and its relation with clinical prognostic indicators. In this respect, We assessed microvessel density (MVD) in pretreatment lymph node biopsies of 54 previously untreated patients with DLBCL using anti CD34 immunohistochemical staining. All patients were treated with CHOP or R-CHOP. Mean age was 50,9 and median survival time was 83 months. The interobserver reproducability of microvessel

assesment was high. MVD was compared with age, gender, Ann-Arbor stage, WHO performance,IPI-score,bulky disease,bone marrow involvement, extra-nodal involvement, LDH level, type of the treatment, respons to first-line treatment and overal survival. We did not find a significant relationship between MVD and patient characteristics and response to treatment. In univariate analysis, survival correlated with Ann-Arbor stage, presence of B-symptoms and IPI-score. Response to treatment correlated with age, Ann-Arbor stage and the numbers of extranodal involvement. Larger series of patients are needed to determine the prognostic value of angiogenesis in NHL.

PP2-215
T-CELL LYMPHOMA WITH STRONG CD20 EXPRESSION ACQUIRED IN A RELAPSE: A CASE REPORT

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Background: Immunophenotypical lineage infidelity in mature non-Hodgkin lymphoma is a well-known phenomenon. The expression of T-cell marker CD5 in B-cell lymphoma is notoriously known and is regularly used in a diagnosis of CLL and MCL. On the contrary, a subset of T-cell lymphomas with the expression of some panB-cell markers (either CD20 or CD79a, usually not both at the same time) is also described. Such cases are explained by the origin of tumor cells from a subset of bone marrow CD20-positive T-lymphocytes. Another possibility may be an aberrant inconsistent expression on neoplastic cells. We describe a case of T-cell lymphoma, which acquired a CD20 positivity during the course of the disease. Case report: 70 year old female presented with peripheral lymphadenopathy and systemic symptoms. Peripheral T-cell lymphoma of angioimmunoblastic type (AILT) was diagnosed. The tumor cells were CD45+ CD45R0+ CD3- CD4+ CD8- CD10-/+ CD56- CD30- Ki67+ (approx. 50 %). The patient with aaIPI 1, stage IV B disease received 5 cycles of PACEBO chemotherapy. The treatment was well tolerated and complete remission was reached according to a PET/CT scan. The disease recurred after ten months. The tumor cells were CD45+ CD45R0+ CD3- CD4-/+ CD8- CD10-/+ CD56- CD30+ Ki67+ (more than 80 %). A surprising finding was a strong membrane expression of CD20; CD79a and BSAP (PAX5) were negative. No such aberrant expression in a primary tumor was present. The PCR examination using BIOMED-2 primers in both tumors revealed oligoclonal population with TCR gamma rearrangements of the same size. IgH or IgL were not clonally rearranged. Conclusion: There are cases of diffuse large B-cell lymphoma evolution in an AILT described in the literature. This possibility was excluded by PCR results. We conclude that the expression of panB-cell marker CD20 on T-cell lymphoma in the presented case is an example of an aberrant immunophenotype in therapeutically influenced tumor cells. Because the finding of CD20+ CD3- tumor cells may be a source of a misdiagnosis, our case stresses the importance of using the broad panel of antibodies rather than single lineage-specific markers. In cases with aberrant immunophenotype it is necessary to apply molecular methods to disclose clonality. The diagnosis of a lymphoma even in a relapse is a complex and multidisciplinary process which can bring surprising results. This study was supported by Research Project FNM MZO 00064203 n. 6704.

PP2-216
DIAGNOSTIC ROLE OF RET, PGP 9.5, PPAR GAMMA, CK19, HBME-1, GALECTIN-3, AND KI-67 EXPRESSIONS IN THE ONCOCYTIC THYROID NEOPLASMS

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Background: Thyroid carcinoma with oncocytic changes may sometimes cause diagnostic difficulties. The purpose of the current study was to investigate the expression and diagnostic role of RET, PGP 9.5, PPAR gamma, cytokeratin 19, HBME-1, Galectin-3, and Ki-67 expressions in oncocytic thyroid neoplasms. Methods: We investigated the expression of RET, PGP 9.5, PPAR gamma, cytokeratin 19 (CK19), HBME-1, Galectin-3, and Ki-67 using immunohistochemistry in 30 cases with oncocytic variant of thyroid carcinoma. The study group comprised of 18 cases of oncocytic variant papillary carcinoma and 12 cases of oncocytic variant follicular carcinoma. Expression score and correlation between immunohistochemical markers were analysed. Results: The expression of HBME-1, Galectin-3 and CK19 were found statistically significant in oncocytic papillary carcinoma group compared to oncocytic follicular carcinoma. RET oncoprotein staining was present merely in three cases of papillary carcinomas, whereas PPARγ synthetase positivity had an equal distrubution in both types of tumours. PGP 9.5 had no different staining patern in both of the groups. Oncocytic variant follicular carcinomas had lower Ki 67 proliferation indices compared to those of papillary carcinomas. Conclusion: Although oncocytic change creates morphological diagnostic problems, this study shows that oncocytic transformation does not indicate a spesific immunohistochemical expression profile. A panel of HBME-1, Galectin-3 and CK19 antigens is still useful to differentiate the oncocytic variant of papillary carcinomas from follicular neoplasms similar to their conventional counterparts.

PP2-217
GALECTIN-3 AND CD44V6 EXPRESSION IN THYROID LESIONS

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Background: Diagnostic difficulties are at times encountered with follicular thyroid lesions, usually attributed to the presence of variably developed nuclear features of papillary thyroid carcinoma (PTC). Such observations led to the introduction of the term “well differentiated tumor of uncertain malignant potential” (WDT-UMP). Galectin-3, a protein involved in numerous biological pathways and affecting cell apoptosis, proliferation, adhesion and neoplastic transformation, has been proposed as a useful marker for the diagnosis of PTC. CD44 variant domain 6 protein (CD44v6), a type 1 transmembrane glycoprotein involved in cell-cell and cell-matrix interactions, has been implicated in tumorigenesis, tumor invasion and metastasis and has been a target for antibody-guided therapy in various epithelial malignancies. CD44v6 has been reported to differentiate benign from malignant epithelial thyroid lesions in both cytologic and histologic specimens. The purpose of this study was to examine the usefulness of galectin-3 and CD44v6 in distinguishing follicular thyroid lesions. Method: Seventy nine thyroid lesions, including 15 nodular goiters (NGs), 25 PTCs, 20 follicular adenomas (FAs), 11 follicular carcinomas (FCs) and 8 WDTs-UMP, were studied. Immunohistochemistry was performed using commercially available monoclonal antibodies against galectin-3

(clone 9C4) and CD44v6 (clone VVF-7). Galectin-3 positive cases were considered those that displayed nuclear and cytoplasmic staining of any intensity in ≥25% of cells while membranous immunostaining for CD44v6 in ≥1% of cells was interpreted as positive. Results: Nuclear and cytoplasmic expression of galectin-3 was observed in 1/15 (7%) NGs, 22/25 (88%) PTCs, 2/20 (10%) FAs and 1/8 (12%) WDTs-UMP. FCs were negative for galectin. The sensitivity, specificity and predictive value of positive and negative test were 88%, 93%, 85% and 94%, respectively. Membranous expression of CD44v6 was observed in 2/15 (13%) NGs, 1/25 (4%) PTCs, 1/20 (5%) FAs, 2/11 (18%) FCs and 5/8 (63%) WDTs-UMP. CD44v6 was mainly observed in neoplastic cells entrapped within sclerotic foci of PTC. Conclusion: The results of the present study show that galectin-3 is highly expressed in PTCs, representing a useful marker in differentiating follicular variant of PTC from FA. CD44v6 highlights WDT-UMP, although this association needs to be further investigated in larger numbers of WDT-UMP.

PP2-218

THYROID MALIGNANCIES AND RENAL FAILURE.

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Introduction: Findings suggest that the incidence of nearly all malignant tumors is increased in immunosuppressed organ transplant recipients. Parathyroid and thyroid dysfunctions are detected in some of patients with chronic renal failure undergoing hemodialysis. Thyroidectomy and parathyroidectomy may become necessary in some of these patients. The aim of this study is to investigate the relative incidence of thyroid nodules in patients with on dialysis or renal transplant in comparison with the general population. Material and Methods: In 41 renal transplant recipients with thyroid dysfunction, thyroid FNAB findings, primary renal disease and the period of immunosuppression were evaluated retrospectively. Thyroidectomy specimens were also examined when available. These findings were compared with those of 230 cases in heamodialysis programme and with 221 patients without renal failure. Results: In transplant group the immunosupression period was about 5.5 years. Only one case had papillary carcinoma and one case had follicular neoplasia. Fifty of the 230 patients (21.7%) on hemodialysis who had thyroid FNAB underwent thyroidectomy. Papillary thyroid carcinoma was seen 4.3% of patients on hemodialysis. Conclusions: The most common abnormalities in transplant recipients' thyroid FNAB were amyloidosis (9.7%), and papillary thyroid carcinoma (2.4 %). The carcinoma rate between three goups were not significantly different (Transplant: %2.4, dialysis: %2.2, control: %1.4) ($p>0.05$). The frequency of amyloidosis was significantly higher in the transplant group (%9.8) in comparison to both the hemodialysis group (%3.5) and the control group (%0.9) ($p=0.005$). Our findings do not suggest on increase in the incidence of thyroid malignancies after renal transplantation. This may conceivably be due to closer examination of patients on dialysis resulting in detection and removal of nodules in thyroid and parathyroid.

PP2-219

HISTOPATHOLOGICAL ASSESSMENT AND DIAGNOSTIC IMAGING APPROACH TO GASTRO-ENTERO-PANCREATIC CARCINOMAS OF NEUROENDOCRINE ORIGINE - SINGLE NET CENTER EXPERIENCE IN POLAND

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Background The recent WHO classification confirmed GEP/NETs into four categories according to gross and histopathological features. The microscopic type of tumor and a multimodality approach in imaging for detection the primary tumor and metastases decide of a treatment. The aim of the study was to review the current diagnostic approach, based on the experience of one center in Poland performed during a 4-year period. Methods All of 134 patients with histologically confirmed GEP were subjected to clinical, biochemical and imaging examinations performed as routine clinical work-up. The imaging techniques consisted of anatomical (CT, EUS) and functional approaches (SRS, mIBG and FDG PET). Results The clinical classification divided the primary origin of the tumor as follows: 49% -foregut tumors, 44%- midgut, and 7% of tumors of unknown origin. All of patients had malignant advanced disease. Group of patients with WHO 2, diagnosed as well differentiated endocrine carcinoma, consisted of 98 (73%) subjects. CT and SRS imaging were used in each case, and additionally EUS in foregut tumors, to localize the primary tumor, and assess tumor extent. Overall sensitivity of CT considering the active disease amounted to 96%, while specificity-75%. Sensitivity of SRS was 97%, while specificity- 85%. Second group of patients diagnosed as WHO 3, poorly differentiated endocrine carcinoma, included 29 patients, 17 foregut and 9 midgut tumors, and 3 of unknown origin. Diagnostic imaging examinations consisted of CT. Standard SRS (sst2) was negative in most of cases. The aggressive behavior of this type of tumors was detected by means of FDG-PET. Sensitivity of CT amounted to 100%, and that of SRS- 44%. Specificity of CT amounted to 67% and that of SRS-100%. Third group of patients diagnosed as WHO 4, mixed tumor, consist 7 patients, 4 foregut and 3 midgut neoplasms. The imaging approach consisted of CT/MR and in 5 cases FDG-PET. Sensitivity of CT amounted to 100%. Only one patient presented with a SRS positive study. FDG- PET sensitivity amounted to 100%. Conclusion: A microscopic type of GEP-NET correlates with imaging methods. A new histological WHO classification to be very helpful in order to select patients towards the best treatment modality and further follow-up. Diagnostic imaging of GEP-NET, consider anatomical and functional techniques, which should be read together.

PP2-220

METASTATIC BREAST CARCINOMA INVOLVING THE THYROID GLAND AFFECTED WITH PRIMARY PAPILLARY CARCINOMA DIAGNOSED BY IMMUNOHISTOCHEMISTRY. CASE REPORT

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Background: Metastatic involvement of the thyroid is occurred to be uncommon (in 2, 2 % of female cases) and having poor prognosis. Thyroid affection in the patients with breast cancer is diagnosed many years after mastectomy. The combination of both the primary and secondary malignant tumours in the same thyroid gland is a great diagnostic problem hardly solved not only clinically but also microscopically. Design (Case report): The patient, female, white 45-year-old, underwent radical mastectomy (1991), histologically - invasive ductal carcinoma. 5 years later (1996) she underwent left semi-thyroidectomy because of the nodes in her thyroid; histologically the thyroid gland tumor was corresponding with metastasis of breast invasive ductal carcinoma. After 9 years (2005) she underwent right semi-thyroidectomy for the same reason. Histologically, in tumor nests there were area of different appearance; solid, cribriform and alveolar patterns were intermitted with foci of tubular-follicular pattern. Nuclear characteristics and features in these different types of tumor areas were also distinguished. To produce more accurate diagnostic definition immunohistochemical investigation with the wide panel of markers including ER, PR, GCDFP-15, TPO, TTF-1 was performed. Results: Tumor cells in solid, cribriform and alveolar patterns were strongly positive for ER,

PR, GCDFP -15, being negative for TPO and TTF-1. In contrary, the structures of follicular pattern were strongly positive for TPO, TTF-1 and negative for breast cancer IHC markers that confirmed the presence of concomitant thyroid papillary carcinoma (diffuse follicular variant). Conclusion: We presented the case report of the coincidence thyroid papillary carcinoma and its metastatic affection by breast cancer. IHC method afforded to demonstrate difference between the primary and the metastatic origin in the very uncommon combination of neoplasms.

PP2-221
TWO CASE REPORTS: MIXED MEDULLARY-FOLLICULAR CARCINOMA AND MIXED MEDULLARY-PAPILLARY CARCINOMA OF THYROID

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Mixed medullary and follicular carcinomas and mixed medullary and papillary carcinomas are tumors showing the morphological features of both medullary carcinoma and follicular carcinoma or papillary carcinoma, respectively. These tumors are also called composite carcinoma, biphasic carcinoma or compound carcinoma. These are very rare tumors and most have been described in single case reports. In mixed medullary-follicular carcinoma, the morphologic features of medullary carcinoma together with immunoreactivity for calcitonin co-exist with those of a follicular neoplasm together with immunoreactivity for thyroglobulin. Mixed medullary-papillary carcinoma contains intimately admixed populations of cells with the characteristic clear nuclei of papillary carcinoma and medullary carcinomas component. Our first patient was a 44 year old woman, who had a mass in her right thyroid lobe and clinically had hoarseness and dysphagia. In her ultrasound, she had a 1 cm solid nodule on the right side of the thyroid. Right complete thyroidectomy and left nearly complete throidectomy had been performed and 1,3 cm nodule on the right lobe of the thyroid was diagnosed as Mixed Medullary-Follicular Carcinoma after performing calcitonin and thyroglobulin immunohistochemistry and follicles showing neoplastic features. Non-tumoral areas and left lobe were diagnosed as Adenomatous Hyperplasia and Lymphocytic Thyroiditis. Our second patient was a 23 year old man who had a painless mass in his right thyroid lobe. In his ultrasound, he had a 1 cm solid nodule which was a cold nodule on scintigraphy. Right complete thyroidectomy and right modified neck dissection had been performed. 1,2 cm nodule on the right lobe of the thyroid was diagnosed as Mixed Medullary-Papillary Carcinoma after performing calcitonin and throglobulin immunohistochemistry and classical histopathological features of papillary carcinoma. There were three metastatic lymph nodes in his right modified neck dissection and they were diagnosed as Medullary Carcinoma metastases. As mixed medullary-follicular (or papillary) carcinomas of thyroid are very rare neoplasms, every diagnosed case should be reported, in order to increase the data in the literature.

PP2-222
HASHIMOTO'S THYROIDITIS WITH PAPILLARY THYROID CARCINOMA LIKE NUCLEAR ALTERATIONS AND THE ROLE OF IMMUNHISTOCHEMISTRY IN DIFFERANTIAL DIAGNOSIS OF TRUE PAPILLARY THYROID CARCINOMA

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Hashimoto's thyroiditis (HT) is an autoimmune disease with widespread lymphocyte infiltration, fibrosis, and parenchymal atrophy in thyroid glands. In hemotoxyline-eosin slides; focal and incomplete papillary thyroid carcinoma (PTC)- like nuclear alternations – nuclear enlargement, overlapping, chromatin clearing, grooves and intranuclear inclusions- in thyrocytes may be described almost one-third of HT on routine microscopic examination. The incidence of carcinoma in HT has been reported wide range ratio between 0.5% and 30%. Although there is a high incidence of PTC in HT and common morphological features between these entities, the carcinogenetic steps between PTC and HT is not well understood. The aim of present study was to determine similar or different molecular expression patterns between PTC and HT. We selected four PTC-associated proteins and examined the expression of cytokeratin (CK)-19, fibronectin 1(FN1), galaktin 3, HBME1 and one proliferative activity marker-Ki-67- proteins by immunohistochemistry in PTC, HT, HT+ papillary carcinoma, HT+ follicular neoplasia cases. Fourty-one cases of HT, 15 HT+PTC, 7 HT + follicular neoplasia, 20 PTC, and 10 nodular goiters were selected from the archives of the department of pathology. Immunohistochemical staining was performed manually using the standard avidin-biotin peroxidase complex technique with DAKO (LSAB kit, DAKO, Denmark). The expressions of the proteins in malignant tumors were diffuse. There was no expression or focal staining in nonneoplastic thyroid tissue. Focal expression of galaktin 3, CK19, HBME1, FN1, and Ki-67 were seen in thyrocytes showing PTC-like nuclear alternations in HT. We could not demonstrate the expression of these five proteins in thyrocytes with no PTC-like nuclear alternations in HT. There was a high expression of both Galactin 3 and CK19 in true papillary carcinoma and PTC-like nuclear alternations in HT. In summary, PTC associated proteins are focally expressed in HT, especially in thyrocytes with PTC-like nuclear alternations. Similar molecular and morphological features between PTC and these thyrocytes suggest a common pathway for carcinogenesis.

PP2-223
IMMUNOHISTOCHEMICAL DETECTION OF P53 AND P63 IN THE CHRONIC LYMHOCYTIC THYROIDITIS WITH PAPILLARY THYROID CARCINOMA

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BACKGROUND: The pathologic mechanisms underlying papillary thyroid carcinoma are incompletely understood. Most models suggest that the origin of papillary carcinoma is the mature thyroid follicular epithelial cell. In recent reports, p63 was detected in papillary carcinoma, hashimoto's thyroiditis, in squamoid aggregates and solid nests. The aim of the study was immunohistochemical detection of p53 and p63 in chronic lymhocytic thyroiditis with papillary thyroid carcinoma. METHODS: Immunoperoxidase staining was performed in 10 slides that were prepared from archival blocks with the diagnosis of chronic lymhocytic thyroiditis with papillary thyroid carcinoma. p53 and p63 staining patterns in and around the tumor areas evaluated. RESULTS: Nine of the cases were female. The ages ranged from 20 to 60. P53 staining in tumoral areas was positive in 6(60%) cases with the ratio of 0 -80 %. Staining was also positive in thyroiditis areas in 6(60%) cases with the ratio of 0-40%. P63 staining in tumoral areas was positive in 5(55.5%) cases with the ratio of 0 -80 %. Staining was also positive in thyroiditis areas in 7 (77.8%) cases with the ratio of 0-20%. Both p53 and p63 staining were observed in squamous metaplasic focuses in tumoral areas. CONCLUSION: p53 and p63 expressions are parallel in the areas of papillary thyroid carcinoma and chronic lymhocytic thyroiditis. These finding suggests that papillary thyroid carcinoma and chronic lymhocytic thyroiditis may be associated in etiology.

PP2-224

HETEROTOPIC THYROID TISSUE PRESENTING AS A PORTA HEPATIS MASS

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BACKGROUND: Heterotopic thyroid along the course of thyroglossal duct or close to midline is a well known entity and particularly involves the base of tongue, submandibular region, larynx, trachea, mediastinum, and heart. It is extremely uncommon in the subdiaphramic area except those seen as a component of ovarian teratomas. In the literature, only five such cases have been reported in porta hepatis to date; the current case represents the sixth. CASE REPORT: A 60 year-old woman with a history of bilateral subtotal thyroidectomy due to multinodular guatr presented with acute cholecystitis underwent total cholecystectomy. Throughout operation a 5 cm mass at porta hepatis was detected and subsequently excised. RESULTS: On gross examination the mass was 5x3x3 cm with gelatinous cut surface and focal areas of calcification. Microscopically, follicles rich in colloid, arranged in nodular fashion some of which displayed cystic dilatation lined by oval to cuboidal epithelium were observed. No atypia or any other features suggesting neoplastic transformation including papillary structures and psammoma bodies were noted. Neither lymph node architecture nor teratomatous tissue elements were detected. The possibility of a metastatic origin from an occult thyroid carcinoma was excluded by the clinical and histopathological findings. Immunohistochemically, epithelium together with colloid was reactive for thyroglobulin. Accordingly, the lesion was diagnosed as an ectopic thyroid tissue with nodular goiter and hyperplastic changes. Both grossly and microscopically, cholecystectomy specimen was an ordinary one exhibiting acute inflammation with gall stones. CONCLUSION: Possible causes of accessory thyroid tissue include a component of teratoma; epithelial metaplasia; abnormal migration in embryogenesis; implantation due to trauma or operation; and metastasis from thyroid carcinoma with minimal atypia. Considering the current case, the heterotopic tissue appeared to be an individual nodule; no finding consistent with metastasis or a direct epithelial connection suggestive of metaplasia was revealed. Moreover, neither any other processes suggestive of implantation nor components of teratoma was perceived. Consequently, intra-abdominal heterotopic thyroid including those in porta hepatis as seen in the present case may be developmental in origin, and thus it might have developed during early embryonic stage before the complete development of diaphragm.

PP2-225

THE CHANGES IN THE INCIDENCE OF THYROID GLAND DISEASES: DATA FROM CUKUROVA REGION OF TURKEY

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BACKGROUND: The incidence of thyroid diseases differ in various geographical regions and it is effected by environmental conditions. The only established risk factor for differentiated thyroid cancer is ionizing radiation. The most dramatic effect of exposure to fallout from the Chernobyl accident on physical health has been the increase in thyroid cancer.The aim of this paper is to analyse changes in thyroid disease incidence trends in Cukurova region during the period of 1986-2006. MATERIAL - METHODS: All thyroidectomy cases were retrieved from the archives of Cukurova University, School of Medicine, Department of Pathology and classified according to the final diagnoses and other available information, retrospectively. The cases were evaluated in 3 periods: 1986-1990, 1991-1996 and 1997-2006. RESULTS: From 1986 to 1990, 21320 cases were registered to the Pathology Department. Hundred and seventy six (0.8 %) of them were thyroidectomy specimens. Overall histopathological diagnoses were nodular goiter (79%), adenoma (3.4%), fethal nodul (3.4%), papillary carcinoma (P.C) (8.9%), follicular carcinoma (F.C) (2.8%), and metastatic carcinoma (1.7%). Female/male ratio was 1.12 in all cases. A malignant tumor was seen in 13.4% of the thyroidectomy specimens. The average age was 42. From 1991 to 1996, 46120 cases were registered. Five hundred and eleven (1.1 %) of them were thyroidectomy specimens. Overall histopathological diagnoses were nodular goiter (79.5%), adenoma (3%), fethal nodul (1%), lymphocytic thyroiditis (0.4%), P.C (5.6%), F.C (2.2%), medullary carcinoma (0.8%). Female/male ratio was 1.58 in all cases. A malignant tumor was seen in 13.6% of the cases. Average age was 45. From 1997 to 2006, 220180 cases were registered. One thousand five hundred and twelve (0.6 %) of them were thyroidectomy specimens. Overall histopathological diagnoses were nodular goiter (66%), adenoma (12%), lymphocytic thyroiditis (3.5%), P.C (13%), F.C (2.7%), medullary carcinoma (1.4%), well differentiated papillary – folicular thyroid carcinoma (0.07%) and papillary + follicular carcinoma (0.007%). Female/male ratio was 1.163 in all cases. A malignant tumor was seen in 17.8% of the cases. Average age was 44. CONCLUSION: The mean ages and female /male ratio of the patients did not differ in three periods.The incidences of nodular goiter and follicular carcinoma were also similar. However FA, PC and lymphositic thyroiditis incidences seemed to increase from 1986 to 2006. Further and detailed investigations based on the population in Cukurova region are needed to explain these changes in the incidences.

PP2-226

CLINICAL AND PATHOLOGICAL FEATURES AND PROGNOSTIC FACTORS FOR PAPILLARY THYROID CARCINOMAS: A REVIEW OF 753 CASES.

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OBJECTIVES: The aim of this study was to present a descriptive frequency of papillary thyroid carcinomas in our department, using our registry data. From 2000 to 2006, Intraoperative and postoperative pathologic samples of 753 individuals were diagnosed papillary thyroid carcinoma at the Istanbul University Cerrahpasa Faculty of Medicine Department of Pathology. Pathologic samples of 414 of these 753 patients who underwent surgery and examined pathologically at the other hospitals were consulted for second opinion at our department. RESULTS: Of the patients evaluated, 81% were female and 19% male, with an average age of 48 (minimum 10- maximum 89). Of all the cases, 74,1 %(n=559) were of the usual type, whereas 25,9 % (n=194) showed complex histological features with different components present and 18% represented specific subtypes. Pathologic analysis revealed 753 papillary thyroid carcinomas, including 326 classic variant of papillary thyroid carcinomas (PTCs) (43.3%) and 250 follicular variant of PTCs (33.2%) and 127 sclerosing variant of PTCs (16.8%) and 9 cystic variant of PTCs (1,2%) and 8 tall cell variant of PTCs (1,1%) and 7 hurtle and oncocytic cell variant of PTCs (0.9%) and 7 low differentiated variant of PTCs (0.9%) and 6 classic variant of PTCs with Warthin type areas (0.8%) and 3 classic variant of PTCs (0.9%) with oncocytic cells and 2 follicular variant of PTCs with oncocytic cells (0.3%), 2 Warthin like follicular variant of PTCs (0.3%) and 2 trabecular variant of PTCs (0.3%) and 2 clear cell variant of PTCs (0.3%) and 2 dedifferentiated variant of PTCs (0.3%). Of the

surrounding non-tumor tissue, 6.0% (n=45) had hyperfunction signs; 2%(n=15) Hashimato thyroiditis; 4,2% (n=32) follicular adenoma; 1,2%(n=9) Hürthle cell adenoma; 2,8%(n=21) Hürthle cell metaplasia and 26% (n=198) non-specific lymphocytic thyroiditis. 72,6% (n=541) adenomatous hyperplasia. We found multicentricity 27,5% (n=207) of all cases. CONCLUSION: Histological assessment is the gold standard for detecting invasive thyroid cancer. Thyroidectomy is effectively a staging procedure by which the presence and extent of invasive disease can be established and subsequent management determined.

PP2-227
CLINICAL AND PATHOLOGICAL FEATURES AND PROGNOSTIC FACTORS FOR FOLLICULAR THYROID CARCINOMAS: A REVIEW OF 153 CASES

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OBJECTIVES: The aim of this study was to present a descriptive frequency of follicular thyroid carcinomas in our department, using our registry data. From 2000 to 2006, Intraoperative and postoperative pathologic samples of 153 individuals were diagnosed follicular thyroid carcinoma at the Istanbul University Cerrahpasa Faculty of Medicine Department of Pathology. Pathologic samples of 64 of these 153 patients who underwent surgery and examined pathologically at the other hospitals were consulted for second opinion at our department. RESULTS: Of the patients evaluated, 83% were female and 17% male, with an average age of 47 (minimum 14- maximum 83). Pathologic analysis revealed 153 follicular thyroid carcinomas, 10.4% (n=16) had 1 cm sized tumor or less; 89,6% (n=137) had larger than 1 cm tumor. Of all the cases, 81 %(n=124) were of minimally invasive type, whereas 19 % (n=32) showed widely invasive histological features. Vascular invasion was found 11,7% (n=18) of the patients. Of the surrounding non-tumor tissue, 3,9% (n=6) had hyperfunction signs; 11,7% (n=18) adenomas and 12,4% (n=19) non-specific lymphocytic thyroiditis. 56,8% (n=87) adenomatous and/or diffuse hyperplasia. Accompanying other thyroid carcinomas were also seen in 6,5% (n=10) of the patients. CONCLUSION: Histological assessment is the gold standard for detecting invasive thyroid cancer. Thyroidectomy is effectively a staging procedure by which the presence and extent of invasive disease can be established and subsequent management determined.

PP2-228
DNA PLOIDY ANALYSIS IN BENIGN AND MALIGNANT THYROID LESIONS

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In this study we investigated 112 benign and malignant thyroid lesions with image analyzer in order to identify the frequency and diagnostic importance of aneuploidy. All specimens were sent for intraoperative diagnosis and at that time touch imprints of the identified lesions were done. Slides were stained with the feulgen method for DNA ploidy analysis. Ploidy software of Samba image analyzer (Grenoble, France) was used for examination of slides in cytometric method. Of the 112 patients, two were diffuse goiter, sixty eight were nodular goiter, eleven were follicular adenoma, five were minimally invasive follicular carcinoma, twenty-four were papillary carcinoma and two were poorly differentiated carcinoma. DNA ploidy analysis revealed that the 112 cases 93 (83%) were diploid, 4 were (3.5%) tetraploid and 15 (13.4%) were aneuploid. Frequency of aneuploidy was 13.6 % in benign lesions and 12.9 % in malignant tumors. There was no statistically significant difference between benign and malignant lesions. The highest frequency of aneuploidy was observed in cases of nodular goiter with adenomatous hyperplasia (25%) followed by cases of follicular adenoma (18.2%), nodular goiter without adenomatous hyperplasia (11.7 %) and papillary carcinoma (16.7 %). These results were supported by the literature. But we did not find aneuploidy in 2 poorly differentiated and 5 minimally invasive follicular carcinomas in contrast to the literature. This may be explained by the fact that we did not have enough carcinoma cases for analysis. In conclusion, our results showed that aneuploidy is not helpful in differentiating benign and malignant lesions. However, in larger series, DNA ploidy analysis along with genetic and cytogenetic investigations may contribute to understanding the etiology and pathogenesis of thyroid lesions.

PP2-229
THE FOLLICULAR VARIANT OF PAPILLARY CARCINOMA – THE ANALYSIS OF CYTOLOGIC FEATURES

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Background:The follicular variant of papillary carcinoma (FVPC) is the second encountered form of papillary carcinoma (PC). Cytological findings in FVPC are variable and can be mistaken for follicular lesions (FL). Methods: 28 cases of FNA's from histologically confirmed FVPC were analysed. Several cytologic features of FVPC were evaluated in comparison with 9 PC and 10 benign FL. Results: Cellularity of FVPC was variable, in 11 cases (39%) was high. Follicle structures were present in all cases (100%), monolayered sheets in 13 cases (46%), papillary structures in 5 cases (18%). Nuclear grooves and powdery chromatin were present in all cases (100%), intranuclear inclusions in 20 cases (71%), predominant nucleoli in 9 cases (32%), in 5 cases (18%) psammoma bodies were present. There were similar cytologic features excluding large number of follicular structures in FNA of PC. There were no papillary structures, inclusions and abundant nuclear grooves in FNA of FL. Conclusions: The combination of cytologic features such as follicular structures, nuclear enlargement, intranuclear inclusions and grooves are significant for the follicular variant of papillary carcinoma diagnosis.

PP2-230
IDENTIFICATION OF DIFFERENTIALLY REGULATED GENES IN PAPILLARY THYROID CANCERS

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Background: Techniques designed to identify differentially expressed genes (DEGs) in tumors have become pivotal in modern pathology. Genefishing techniqueTM using the annealing control primer (ACP) system was recently developed by Hwang et al to screen for DEG transcripts in low concentrations. The technique that uses primers that anneal specifically to the template and allows only genuine products to be amplified, a process that eliminates false-positive results. Design: We have utilized a new differential display method, designated with ACP system, to analyze differentially expressed genes (DEGs) in seven cases of normal thyroid tissues and papillary thyroid carcinomas. The genes identified in this study should provide an insight into the carcinogenesis and progression mechanisms of papillary thyroid carcinomas. Results: With this method we detected 21 genes that are differentially expressed in PTCs and performed cloning with sequencing in 14 genes. We confirmed

the expression patterns of 2 DEGs by RT-PCR assay and identified same results in 7 PTCs. The two DEGs overexpressed in PTCs are confirmed for a DC-stamp and type I collagen A1. They are novel genes firstly identified in PTCs. Conclusion: These results suggest that this new PCR-based technique is a very useful tool for the identification of novel genes differentially expressed in PTC. This study was undertaken as a step toward identifying previously unrecognized molecular genetic mechanisms in PTC. Although the detailed functions of these two genes and their products remain to be determined, the genes identified here will provide insights into mechanisms of carcinogenesis or tumor progression in PTCs.

PP2-231
MULTIFOCALITY IN THYROID CARCINOMAS
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Histopathologic type, tumour size, extrathyroidal spread, metastasis and multifocality are risk factors that should be considered for therapeutic approach in thyroid carcinomas. The aim of this study is to investigate frequency of multifocality of thyroid carcinomas in thyroidectomy specimens. 140 patients who underwent thyroidectomy and diagnosed as malignant neoplasms were included in this study. A retrospective pathology report review was performed for all patients. Of the 140 thyroid carcinomas, 118 (84.2 %) were papillary carcinomas, 16 (11.4 %) were follicular carcinomas, 5 (3.5 %) were medullary carcinomas, and 1 was poorly differentiated thyroid carcinoma. 44 out of 118 papillary carcinomas were papillary microcarcinomas. Multifocality was observed in 29 cases (24.5 %). Malignancy was noticed out of nodule in 42 biopsies. Gross examination and frozen section of the thyroidectomy may be helpful identification of multifocality and some malignant lesions that were not defined before the surgery. This will prevent patients from the second operations.

PP2-232
INCIDENTAL GIANT MEDIASTINAL PARATHYROID ADENOMA: A CASE REPORT
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BACKGROUND: Parathyroid adenomas are unusual neoplasms, three times more frequent in women and occur at any age. In terms of location, 10% occur in anomalous positions and 70% of them are in the mediastinum. CASE/PATIENT: We report a case of a 56 year-old woman with metastatic malignant melanoma. PET-Scan (Positron Emission Tomography) was performed for staging the neoplasm and revealed an upper mediastinal mass suspicious for lung and pericardial invasion. A metastasis was suspected. No abnormalities were detected in blood chemical analysis, including calcium and phosphate levels. Thoracothomy and resection of the mass were done. RESULTS: The tumor was well circumscribed, envolved by a delicate capsule, weights 210g and measures 10,5 x 9,0 x 5,0 cm, it was solid, grayish brown with hemorrhagic areas. Microscopically the patterns of growth were diffuse, acinar and follicular, composed predominantly for oxiphylic cells with minimal nuclear atypia. Immunohistochemistry was performed and there was diffuse positivity for parathormone and positivity for chromogranin and Neuron-specific enolase; ki-67 labeling index was less than 1%. CONCLUSIONS: Considering the variety of aberrant locations in which ectopic parathyroid adenomas may be found, these neoplasms can be difficult to identify and treat surgically. They can rarely growth to a larger size. In the present case another difficulty is that the patient had already a diagnosis of malignant melanoma. Virtually all parathyroid neoplasms, including the rare parathyroid carcinoma, are functional. Despite the large size of this neoplasia, the patient hadn't symptoms of hyperparathyroidism or hypercalcaemia. To our knowledge this is the greatest parathyroid adenoma reported in the literature.

PP2-233
DIAGNOSTIC UTILITY OF TTF-2 (FOX-E1) AND PAX-8 IN THYROID EPITHELIAL NEOPLASMS
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Background: Thyroid-specific transcription factors, TTF-1, TTF-2 and Pax-8, are crucial for thyroid organogenesis and differentiation. Compared with TTF-1, the other two markers have not been extensively investigated in surgical pathology. The goal of this study is to evaluate the expression of these markers in thyroid tumors of the full spectrum of differentiation. Their sensitivity and specificity to thyroid tumors are studied by observing the expression in other tissues including lung carcinomas. Design: A total of 69 cases of thyroid neoplasms were studied: 16 papillary carcinomas (PCAs); 18 follicular adenomas (FAs); 16 follicular carcinomas (FCAs); 8 insular carcinomas (ICAs); 3 anaplastic carcinomas (ACAs); and 8 medullary carcinomas (MCAs). Immunostains for TTF-1, TTF-2, Pax-8 and thyroglobin were performed using tissue microarrays. In addition, TTF-2 and Pax-8 were applied on 77 cases of lung carcinomas, comprised of 73 adenocarcinomas and 4 squamous cell carcinomas, as well as normal tissue and malignant tumors from 16 other organs/sites. The extent of staining was graded as focal (<50%) and diffuse (>50%). Results: TTF-1 nuclear expression was seen in all PCAs, FAs, FCAs and ICAs in a diffuse fashion, whereas its expression in MCAs was variable. Two of the three ACAs demonstrated only a few positive cells. TTF-2 expression was diffuse in a majority of the PCAs, FAs, FCAs, and ICAs in a predominantly nuclear staining, and one ACA showed focal nuclear staining. TTF-2 reaction, however, was predominantly cytoplasmic in all MCAs. Pax-8 was diffusely expressed in a predominantly nuclear staining in all PCAs, FAs, FCAs, ICAs and ACAs except for a focal staining in one PCA and ACA, whereas the expression was predominantly cytoplasmic in all MCAs. Thyroglobin was diffusely expressed in FAs, and variably in PCAs, FCAs and ICAs, but it was negative in ACAs and MCAs. TTF-2 was negative in all other neoplastic and non-neoplastic tissue including those of the lung. Pax-8 was expressed in renal tubules as well as clear cell renal carcinoma, nephroblastoma, seminoma, ovarian serous carcinoma and diffuse large B cell lymphoma, but not in lung tissue/carcinomas. Conclusion: Pax-8 is a sensitive marker for thyroid lesions, but its specificity is compromised by the expression in lesions of metanephric and gonadal derivation, and B-lymphocytes. TTF-2 is a sensitive and specific marker for thyroid lesions. Both markers can be useful to differentiate between thyroid and lung carcinoma.

PP2-234
SURGICAL THYROID PATHOLOGY. PROTOCOL APPLIED IN THE INTERDISCIPLINARY THYROID PATHOLOGY STUDY GROUP IN THE LAST 20 YEARS
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Introduction: The sampling in well differentiated thyroid tumors and the nomenclature used in the thyroid tumors are in general matter of discussion in the literature. The Thyroid pathology study group of our hospital has made a pursuit of both parameters with the aim to classify them. Material and Methods**:** We have compiled the surgical thyroid pathology in the last 20 years (2431 studies). The standardized protocol and terminology established by consensus in the Thyroid Pathology Study Group, has been applied in our hospital during the last 20 years. Results: The results are the followings: Adenoma: 353; Papillary Carcinoma: 358; Follicular Carcinoma: 110; Poorly Differentiated Carcinoma: 21; Anaplastic Carcinoma: 2; Medullary Carcinoma: 21; Lymphoma: 7; The remaining ones were Hyperplasias and Thyroiditis. Conclusions: 1. In the interdisciplinary thiroid pathology study group meeting, after revision of this great number of cases, we have proved that it is necessary to follow a standardized protocol with the aim of diminishing diagnostic difficulties. 2. In the interest of adequate management of thyroid patients, the nomenclature must be consensuated with the work group. 3. When in frozen sections the diagnosis is delayed, it is necessary to issue the definitive diagnosis quickly (24 hours-4 days) to avoid surgical complications. 4. In our Hospital we have detected: a) great number of well differentiated carcinoma; b) many problems are made by a quick diagnosis of some bordeline tumors.

PP2-235
COX-2 AND INOS EXPRESSIONS IN THYROID NEOPLASMS

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Background: The aim of this study is to evaluate the differences in the immunohistochemical expressions of the Cox-2 and INOS proteins in papillary carcinoma (PC), follicular carcinoma (FC) and follicular adenoma (FA) cases of the thyroid. Cox-2 is highly inducible at inflammation sites and is associated with various types of human tumors. Cox-2 expression is also upregulated during increased proliferation of tumor cells, inhibition of apoptosis and neovasculogenesis. However INOS overexpression is related with chronic inflammatory diseases as well as human tumors. INOS plays a crucial role in tumorigenesis as a mediator of carcinogenic nitrosamine formation. Materials and Methods: Hundred and two cases were selected from the archieves of the Department of Pathology, Cukurova University Hospital. Of the total 102 cases; 63 were papillary carcinoma, 18 were folicular carcinoma and 21 were folicular adenoma. Histopathologic diagnoses were reviewed by two pathologists. Cox-2 and INOS expressions were examined by using immunohistochemical methods. Results: INOS expressions were detected weakly in 9 (14.3%), moderately in 9 (14.3%) and severe in 45 (71,4%) of the PC cases. In FC cases; weak, moderate, and severe stainings were observed in 9 (50,0%), 3(16,7%) and 6(33,3%, and in FA cases 8(38,1%), 6(28,6%) and 7(33,3%), respectively (p=0.002). Of the PC cases, 8(12,7) stained weakly, 12(19,0)stained moderately and 43(68,3)stained severe according to Cox-2 expressions. These results were 5(27,8), 4(22,2) and 9(50,0) in FC, and 10(47,6), 4(19,0) and 7(33,3) in FA groups, respectively (p=0.01). There were no significant differences between two carcinoma groups according to ratio of severe staining in Cox-2 but the ratio of severe expression is significantly higher in PC than FC according to INOS. A significant difference was observed between carcinoma and adenoma groups in Cox-2 expressions, but no difference was determined in INOS expressions. Conclusion: The present study showed that Cox-2 expression may serve as an useful marker to distinguish carcinoma cases from adenoma cases. These results are compatible with literature and suggest that Cox-2 expressions could be an important molecular event for the thyroid tumor progression. However, INOS expressions did not show any differences between these two groups. In addition, 1/3 of the FA cases were stained severe with INOS. In PC cases INOS expressions were detected higher than FC cases. For this reason INOS could be an important agent for devoloping papillary tumors of the thyroid.

PP2-236
CHRONIC THYROIDITIS AS A RISK FACTOR FOR MULTIFOCAL PAPILLARY THYROID CARCINOMA

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Background: Weather Chronic Thyroiditis (CT), particularly Chronic Lymphocytic Thyroiditis is associated with increased risk for the development of Papillary Thyroid Carcinoma (PTC) is controversial. Aim of this study is to explore any association of CT with PTC in our patient population, with special emphasis on multifocal PTC (MF-PTC). Methods: All thyroid resections performed at our institution from 01/2000 to 10/2006 were reviewed. Along with the primary surgical diagnosis any morphological changes in the non-lesional thyroid parenchyma were also collected and categorized into CT, Hyperplasia (including Graves Disease) and Normal. Multifocality was defined as 2 or more separate foci of same type of tumor. Clinical significance of this lymphocytic infiltration was beyond the focus of our study. Results: A total of 626 thyroid resections (512 females and 114 males) with 385 total/near-total thyroidectomies, 207 lobectomies and 30 lobe+isthmusectomies were identified in our database. Our study group consisted of 483 cases (77% of total), where background information was reported for non-lesional thyroid tissue. In this group, primary surgical diagnosis was PTC in 210 cases (43%), follicular/Hurthle cell adenoma in 90 (19%), follicular/Hurthle cell carcinoma in 15 (3%), other malignancies (medullary, poorly differentiated or anaplastic carcinomas, lymphomas, metastases, etc.) in 18 (3%) and benign (other than adenomas) in 150 (31%) cases. Overall, CT was reported in the background thyroid in 153 cases (32%), Hyperplasia in 172 (36%), and it was reported to be normal in 158 cases (33%). Of 210 patients with PTC, 118 were unifocal and 92 were multifocal. In unifocal PTC, the background showed CT in 37 (31%) cases, hyperplasia in 32 (26%), and unremarkable histology in 49 cases (42%). In multifocal PTC, the background showed CT in 46 (50%), hyperplasia in 14 (15%), normal in 32 (35%) cases. Conclusion: CT was reported in overall 153 (32%) of 483 thyroid resections as the background morphology and 40% of all PTCs. However, when PTCs are divided into unifocal and multifocal cases, UF-PTC (n=118) was associated with background CT in 31% of cases, patients with MF- PTC showed a markedly higher incidence of CT (50%) in the non-neoplastic thyroid as compared to other primary diagnoses ($p<0.05$). Any causal relationship between the chronic lymphocytic inflammation and PTCs or a common etiologic mechanism in pathogenesis of these two disorders should be considered.

PP2-237
HBME-1, CYTOKERATIN 19 AND GALECTIN 3 EXPRESSION IN THYROID CARCINOMAS

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AIM: The existence of HBME1, cytokeratin19 and galectin 3 expressions were investigated in different malignant tumors of thyroid in this study. MATERIAL AND METHOD: : 51 tissues of thyroid operation materials diagnosed as malignant thyroid tumors in our laboratory between 2003-2005 were selected and examined retrospectively. Immunohistochemistry was performed

for HBME-1 , cytokeratin 19, and galectin 3. RESULTS: 51 malignant thyroid tumors were examined. The diagnoses were as follows: 38 papillary carcinoma, 7 follicular carcinoma, 3 medullary carcinoma, and 3 poorly differentiated thyroid carcinoma. 33 cases of papillary carcinomas stained for HBME-1 diffusely, and 5 of them focally. Two of the follicular carcinomas expressed HBME-1 diffusely, 3 of them focally, and 2 of the cases did not stain. Two of the medullary carcinomas were stained focally, and one of them diffusely. One of the poorly differentiated thyroid carcinomas stained focally, one stained diffusely, and one case did not stain. 33 of the papillary carcinomas stained for cytokeratin 19 diffusely, and 5 of them stained focally. Three of the follicular carcinomas stained diffusely and 4 of them stained focally. Two of the medullary carcinomas stained diffusely and one of them stained focally. One of the poorly differentiated thyroid carcinomas stained diffusely, two of them stained focally. 29 of the papillary carcinomas stained for galectin 3 diffusely and 9 of them stained focally. Two of the follicular carcinomas stained diffusely and 5 of them stained focally. All three of the medullary carcinomas stained diffusely, one poorly differentiated thyroid carcinomas stained diffusely, and two of them stained focally. For staining of HBME-1, 31 cases of papillary carcinomas, showed membranous pattern, 6 cases showed both membranous and cytoplasmic pattern, and one case showed cytoplasmic pattern. CHI square test was performed statistically in the study. A considerable difference was observed in distribution and pattern of expression of HBME-1 in papillary carcinoma. CONCLUSION: There are immunohistochemically different markers supporting the diagnosis of thyroid cancers. Among them are HBME-1, cytokeratin 19, and galectin 3. Expressions of HBME-1, cytokeratin 19, galectin 3 and membranous staining pattern for HBME-1 were found significant for papillary carcinomas in this study.

PP2-238

SOLITARY FIBROUS TUMOR OF THE THYROID GLAND

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Introduction: Solitary fibrous tumor is an uncommon mesenchymal neoplasm that arises primarily from the pleura, but recognized also in other tissues. The clinical behaviour is usually benign, but the existence of aggressive cases has been documented both in the pleura and in extrapleural sites. Solitary fibrous tumors of the thyroid gland are rare; only nineteen cases have been reported in the literature. Case Report: A 65-year-old woman was admitted to hospital with a slow-growing thyroid mass. Ultrasonography revealed a solid intrathyroid nodule in the left lobe. The tumor was evaluated as a spindle cell neoplasm at intraoperative consultation. Bilateral total thyroidectomy was performed. Grossly, the tumor measured 6 cm in diameter and occupied almost the whole left thyroid lobe. The cut surface was white-tan and had a whorled appearance with occasional small cystic areas. Microscopically the tumor was well-circumscribed and was composed of a patternless proliferation of bland spindle cells, collagen bundles and entrapped thyroid follicles. Immunohistochemically tumor cells were positive for vimentin, bcl-2 and CD34. Discussion: Although solitary fibrous tumor was first described as a primary spindle cell tumor of the pleura, it may occur in a wide variety of sites, including the orbit, mediastinum, abdomen, retroperitoneum, extremities, and in almost any organ. With regard to the thyroid gland, 19 cases have been reported to date. Intrathyroid solitary fibrous tumor should be differentiated from similar cases located in the perithyroid soft tissue. The differential diagnosis of solitary fibrous tumor includes other benign and malignant mesenchymal tumors of the thyroid, spindle cell follicular adenoma, Riedel's thyroiditis, the spindle cell, and paucicellular variants of anaplastic carcinoma, and papillary thyroid carcinoma with nodular fasciitis-like stroma. Recurrence and metastasis has not been described in any reported cases, but the clinical behaviour of solitary fibrous tumor is unpredictable and requires long-term follow-up.

PP2-239

SCLEROSING PARAGANGLIOMA OF THE CAROTID BODY WITH CERVICAL LYMPH NODE METASTASIS: A CASE REPORT AND REVIEW OF THE LITERATURE

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Background: Carotid body paragangliomas have an unpredictable history with no correlation between histology and clinical behavior. Recently a distinctive variant of paraganglioma characterized by extensive collagen deposition resulting in a pattern of growth that resembled an invasive malignant neoplasm named sclerosing paraganglioma is described. Case: We present a 28-year-old woman presented with dysphagia with right sided neck mass Magnetic resonance imaging (MRI) showed 7x4x3cm bilobulated large tumour originating from right carotid bifurcation. Preoperative partial selective embolisation was done to provide an easy surgical excision. The patient underwent frozen section and surgical excision of this neoplasm. Carotid resection with reversed interposition saphenous vein graft was performed without complication. Histopathology showed a tumor with extensive collagenization seperating nests of tumor cells with a pseudoinfiltrative appereance. Tumor cells were large and round to polygonal, with moderately pleomorphic nuclei and abundant eosinophylic granular cytoplasm. Mitotic activity was low. Necrosis was not present. There was lymph node involvement. Tumor showed perineural infiltration. The tumor cells were positive for chromogranin A, synaptophysin and vimentin and negative for cytokeratin, TTF-1, CD34 or SMA. Sustentacular cells at the periphery of the tumor nests were positive for S-100 protein. Conclusion: Since the infiltrative nature of this lesion may lead to a misdiagnosis of medullary thyroid carcinoma, sclerosing paraganglioma should be included in the differential diagnosis of sclerosing lesions of the head and neck region. Appropriate immunohistochemical stains are useful for establishing the correct diagnosis.This is the first report of a metastatic sclerosing paraganglioma. This case demonstrates that sclerosing paraganglioma can be significantly symptomatic and might have a malignant potential requiring aggressive initial local treatment to include lymph node sampling and complete tumor resection. The patient is free of disease 12 months after its initial presentation.

PP2-240

C-KIT EXPRESSION IN GASTROENTEROPANCREATIC ENDOCRINE TUMORS

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We aim to investigate the c-kit immunoexpression profile of gastroenteropancreatic endocrine tumors (GEPET) and the relation between the c-kit expression and tumor localization as well as differentiation. Fifty five cases of GEPET were retreived from the archieve of the Department of Pathology, Istanbul

Faculty of Medicine, Istanbul University. Nineteen (34.5%) cases of pancreatic, 16 (29.1%) small intestinal, 8 (14.5%) gastric, 5 (9.1%) rectal, 5 (9.1%) appendiceal and two (3.7%) colonic GEPET were included in the study. The tumors were classified according to the WHO classification system as well-differentiated endocrine tumor-benign behaviour (n=15, 27.3%), well-differentiated endocrine tumor-uncertain behaviour (n=11, 20%), well-differentiated endocrine carcinoma (n=22, 40%) and poorly differentiated endocrine carcinoma (n=7, 12.7%). All tumors were immunostained for chromogranin A, synaptophysin, Ki-67 and c-Kit. The c-Kit immunoexpression was detected in 20% (n=3) of the well-differentiated endocrine tumor-benign group, 9.1% (n=1) of the well-differentiated endocrine tumor-uncertain behaviour group, 31.8% (n=7) of the well-differentiated endocrine carcinoma cases and 42.8% (n=3) of the poorly differentiated endocrine carcinoma cases. Rectal endocrine tumors showed the most frequent c-Kit immunoexpression followed by colonic and gastric endocrine tumors. Even though it was observed that the poorly differentiated tumors tend to express c-Kit more frequently, this result was not statistically significant. The c-Kit immunoexpression in GEPET ranged from 9.1-42.8% according to differentiation level and clinicopathological parameters of the tumors. In the differential diagnosis of the tumors of the gastrointestinal tract and pancreas, particularly with a poorly differentiated morphology it should be kept in mind that the GEPET may express c-Kit.

PP2-241

Ki67 EXPRESSION IN THYROID CARCINOMAS WITH LUNG METASTASIS: COMPARED WITH CLINICO-PATHOLOGICAL PARAMETERS AND FOLLOW-UP.

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BACKGROUND: Invasion to the surrounding tissues of thyroid and vascular involvement are still most important prognostic features in metastatic thyroid carcinomas. The aim of this study is to investigate expressions of proliferation marker Ki67 and also to compare other clinico-pathological parameters. METHODS: Fourteen cases were diagnosed as thyroid carcinoma with detected lung metastasis in 1995-2001 years, some of which were consulted to our hospital (Gulhane Military Medical Academy, Pathology Department) from different laboratories. Of 14 thyroid carcinoma cases, 12 were papillary carcinoma (1 tall, 1 columnar, 1 solid, 2 classic, 3 follicular and 4 oxyphilic variant) and 2 follicular carcinoma (1 Hurthle cell and 1 with insular component). All slides were reviewed and a representative block of each tumor was selected. Immunohistochemically, Ki67 was stained using avidin-biotin peroxidase technique by autostainer (Dako labvision). Positive nuclear Ki67 expressions were quantitatively counted by KS 400 software (computer assisted analysis system). Staining in invasive thyroid carcinomas without metastasis was also evaluated as control. The clinico-pathological parameters such as age (≤45 vs. >45 year), gender, tumor size (<3 cm vs. ≥ 3cm, invasion to surrounding tissues of thyroid, vascular involvement, presence of solid areas, differentiation (well or poor/ agressive variants) were evaluated compared with Ki67 expression (<5% vs. ≥ 5%). RESULTS: Invasive thyroid carcinomas without metastasis showed generally 1 or 2% Ki67 expression. Of 14 thyroid carcinoma with lung metastasis, 7 (50%) showed ≥5 % Ki67 expression (mean 10%), some of which were inside the proliferative cores. Others were around 1-2% similar to invasive thyroid carcinomas without metastasis. Vascular invasion was detected in 11 (78%) cases. Nine cases (64%) were more than 45 years old. Solid area was noted in 2 cases. There was invasion to surrounding tissues of thyroid in 7 cases (50%). CONCLUSIONS: In this series vascular involvement seems to be closely associated with lung metastasis in thyroid carcinoma. Ki 67 expressions may probably be potential prognostic value, especially because of the presence of proliferative cores with higher expression.

PP2-242

CONTRIBUTION TO THE DIAGNOSTIC ACCURACY OF HBME-1, GALECTIN 3 AND CYTOKERATIN 19 IN ENCAPSULATED (NONINVASIVE) FOLLICULAR VARIANT PAPILLARY THYROID CARCINOMAS

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BACKGROUND: The diagnosis of encapsulated (noninvasive) follicular variant papillary thyroid carcinoma (eFV-PTC) may be sometimes problematic and histologically mistaken by nonneoplastic (cellular thyroid nodules) and benign or malignant neoplasm (follicular adenomas and minimal invasive follicular carcinomas) thyroid lesions. The aim of this study is to investigate the contribution to eFVPTC diagnosis of immunohistochemical markers that are commonly used in papillary thyroid carcinoma. METHODS: Twenty-two cases were diagnosed as eFV-PTC in 2004-2007 years, some of which were consulted to our hospital (Gulhane Military Medical Academy, Pathology Department) from different laboratories. All slides were reviewed and a representative block of each tumor was selected. Immunohistochemically, HBME-1, Galectin-3 and Cytokeratin (CK) 19 markers were stained using avidin-biotin peroxidase technique by autostainer (Dako labvision). Intensity and distribution of expression in positive immunohistochemical staining was semiquantitatively categorized as mild, moderate and marked (intensity vs. distribution: +1, <25%; +2, 25-50, +3, >50, respectively). Staining in normal thyroid tissue surrounding tumor was also evaluated as control. RESULTS: Normal thyroid tissues showed generally focal-weak positivity (+1) with HBME-1, Galectin 3 and CK 19. However, these markers in eFV-PTC were moderate (+2) or marked (+3) intense staining in more than half of the cases. Of the 22 eFV-PTC cases, 16 were positive with Galectin-3 (72%), 21 (95%) HBME-1 and 22 (100%) CK 19. Combination of HBME-1(+)-CK19 (+) was positive in 21 (21/22 95%), Galectin-3 (+)-HBME-1 (+) or Galectin-3 (+)-CK19 (+) in 16 (16/22 72%) cases. CONCLUSIONS: The combination of HBME-1 and CK 19 positivity is highly sensitive in eFV-PTC diagnosis. However, Galectin-3 is not helpful marker in supporting the diagnosis in this series.

PP2-243
PREVALENCE OF HEPATITIS G EXPOSURE IN PATIENTS ON CHRONIC DIALYSIS

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Background: Hepatitis G virus (HGV) is a blood-borne virus. The predominant route of its transmission is parenteral. The aim of this study was to assess the prevalence of HGV exposure in hemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD) patients. Methods: This study was performed in a main dialysis centre in Iran. 77 Patients on HD and 13 patients on CAPD were enrolled in study. HGV exposure was detected serologically by presence of anti HGV envelope protein E2 (anti-E2) by an ELISA assay. Patients were also screened for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), and hepatitis C antibody (anti-HCV).In anti-E2 positive patients; HGV RNA was detected by RT-PCR using primers derived from the NS5A region of the viral genome. Results: Anti-E2 was positive in 3.89% of HD and none of the CAPD patients. Mean age of anti-E2 positive patients was 53.3+/-26.5 years. In none of them, HGV RNA was detected. 66.66% of them had received blood transfusion. The duration of dialysis in them was 68+/-64 months. Co-infection with HCV or HBV was not significant in these patients. Conclusion: HGV exposure rate was not high in dialysis patients in our study and was not different according to the dialysis modality. The appearance of anti-E2 was accompanied with clearance of serum HGV-RNA. No relationship was noted between HGV exposure and age, sex, history of blood transfusion, time on dialysis, HCV or HBV markers.

PP2-244
ULTRASTRUCTURAL FINDINGS COMPARED WITH CLINICAL AND LABORATORY PARAMETERS IN CASE OF ACUTE HEPATITIS C VIRAL INFECTION

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Background. A complex evaluation of clinical, laboratory and morphological findings provides an adequate approach useful for a positive hepatitis C viral (HCV) infection outcome, in general, and selection of therapy course, in particular. A spectrum of liver tissue injury, and its correlation with clinical and laboratory data in acute HCV is not completely understood. The present study aimed to investigate dynamics of clinical, laboratory and ultrastructural parameters from an acute onset of HCV infection and along a 6 months long patient's follow-up period providing a basis for better understanding of applications of antiviral therapy. Methods. Liver biopsies were obtained from 6 patients with an acute HCV infection aged from 22 to 41 year. All patients matched this study had consistent serum HCV RNA positivity. Ultrastructural data were assessed by transmission electron microscopy of liver biopsies. Five patients in the study group received an interferon therapy with interferons including pegylated ones, namely, 4 of them received realdiron, 1 – pegasys. Results. Four of the treated patients revealed rather stable laboratory and virological recovery along the duration of follow-up but 1 patient revealed sustained HCV RNA positivity without cytolysis. Outcome of the disease during the observation period varied and showed the following peculiarities: stable recovery in 3 treated cases, chronization in 1 case. There was an early laboratory and virological remission observed in one treated patient followed at the end of follow-up by HCV RNA positivity without cytolysis, and positive cytolysis dynamics revealing a HCV RNS negativity already after 3 months period in 1 untreated patient. Extensive apoptosis of intrasinusoidal lymphocytes and activation of Kupffer cells were constantly present ultrastructural findings. The hepatocyte ultrastructure showed a varying amount of mitochondria and lipid inclusions, changes of the nuclear chromatin and nucleoli. Changes of the cellular shape and cytoplasmic density, extensive blebbing suggested a presence of apoptotic cell death. A case with chronization course revealed a local perisinusoidal and pericellular accumulation of collagen microfibrils indicative of fibrosis. Conclusion. The present study showed that application of electron microscopy providing a better understanding of events along the liver cell death and early chronization of the process studied is a useful tool in selection of a strategy of antiviral therapy.

PP2-245
EFFECT OF ADAMANTYLAMIDE DIPEPTIDE AS ADJUVANT THERAPY TO PRAZIQUANTEL IN MICE INFECTED WITH DIFFERENT S. MANSONI ISOLATES

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This work investigated the possible use of AdDP as adjuvant therapy to praziquantel (PZQ) in miceinfected with PZQ-insusceptible Schistosoma mansoni isolate in a trial to increase the susceptibility of this isolate to the drug. Two batches of C57 BL/6 mice were infected with PZQ-susceptible and insusceptible S. mansoni isolates, and each batch was divided into five groups. Seven weeks postinfection, the experimental group received AdDP (5 mg/kg) in addition to PZQ in reduced dose (3X100/kg). Three of the remaining four groups were treated controls; they received AdDP, PZQ in reduced dose and in full dose (2X500/ mg/kg), and the fourth group was infected untreated. In mice infected with PZQ-susceptible or -insusceptible S. mansoni isolate, praziquantel alone, and in addition to AdDP, reduced worm and egg loads and increased percentage dead eggs. Also, they improved the histopathological changes (reduction in granuloma diameter, percentage fibrotic area with increased percentage degenerated eggs). Inducible nitric oxide synthase (iNOS), nitric oxide (NO) in culture of peritoneal macrophages, and number of CD68-positive cells were decreased with improved alanine amino transaminase. In mice receiving combined therapy AdDP-PZQ, the antischistosomal efficacy and the reductions in the inflammatory granulomatous reactions, NO in cultured peritoneal macrophages, percentage fibrotic areas recorded, were comparable to in mice receiving full dose of PZQ, with significantly higher reduction in CD68 cells denoting enhanced antischistosomal efficacy and healing of the inflammatory reactions in the liver.

PP2-246
UNDIFFERENTIATED EMBRYONAL SARCOMA OF THE LIVER: A CASE REPORT

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Background: Undifferentiated embryonal sarcoma of the liver (UESL), a primitive malignant neoplasm, is a rare pediatric malignancy. Case presentation: A 9-year-old girl presented with epigastric mass and severe abdominal pain. Abdominal computed tomography scan revealed a huge tumor in the left hepatic lobe which was hyperechogenic in doppler sonography. Subsequently, the patient underwent a left hepatic lobectomy. The specimen

showed a mass measuring 13X12X8 cm in the left lobe of the liver. The tumor was well circumscribed but incompletely pseudocapsulated. The cut surface revealed a gray-white soft mass with multilocular appearance together with extensive areas of hemorrhage, necrosis, and degenerative foci including a cystic space filled with gelatinous material. On microscopy the tumor showed proliferation of atypical spindle cells admixed with polygonal or round cells and multinucleated giant cells which were loosely arranged in a myxomatous background. The multinucleated giant cells often contained eosinophilic hyaline globules in the cytoplasm. In histochemical staining these globules were PAS-positive and diastase-resistant. In the tumor periphery some ducts were lined by a single layer of cuboidal cells resembling normal bile ducts showing strong reactivity with CK19. Immunohistochemical staining with vimentin, desmin, smooth muscle actin , S-100, AFP, alpha-1-antitrypsin (AAT), alpha-1-antichymotrypsin, CD68, CD31 and CK19 revealed that tumor cells express vimentin, CD68, AAT, alpha-1-antichymotrypsin. The patient remains well with no evidence of local recurrence or metastasis 10 months after the operation. Conclusion: Our case support that UESL might show a divergent differentiation toward histiocytic, fibroblastic, myoblastic, myofibroblastic, or lipoblastic lines. Moreover, the case presented here emphasizes that although there is no specific immunohistochemical marker for UESL, vimentin and histiocytic markers such as CD68, AAT is are useful markers in the diagnosis.

PP2-247

USEFUL IMMUNOHISTOCHEMICAL MARKERS IN DIFFERENTIATING HEPATOCELLULAR CARCINOMA AND METASTATIC ADENOCARCINOMA

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Background: Differentiating hepatocellular carcinoma (HCC) from metastatic adenocarcinoma (MA) in the liver can be difficult, especially if the tumor shows pseudoglands or poorly differantiated morphology in a patient with unknown primary tumor. For such cases, an appropriate immunohistochemical panel should be used to make the correct diagnosis. We aimed to examine the value of immunohistochemical panel composing Hepatosit Ag, pCEA, CD10, Villin, CD34, TTF-1, MOC-31, CK7 and CK20 in the differential diagnosis. Method: 68 HCC and 107 MA cases diagnosed at Ankara University, Department of Pathology between 1999 and 2006 were studied. 6 tissue microarray blocks (TMA) were built using manual microarrayer (Beecher's Instruments) and stained with the markers mentioned above. The canalicular staining was evaluated for pCEA, CD10 and Villin whereas, the presence of diffusely stained endothelial cells around the tumor aggregates were noted for CD34. Cytoplasmic and or membranous staining was assessed for other markers. Results: : Of the HCC cases 95,6% with Hepatosit Ag, 50,7% with CD10, 46,2% with pCEA, 42,6% with CD34, 26,5% with TTF-1, 20,6% with Villin, 19,1% with MOC-31 showed positivity. Specific canalicular staining pattern with pCEA, CD10 and Villin was observed in most of HCC cases. Of the MA cases 99,1% with MOC-31, 86,8% with pCEA, 81% with Villin, 36,9% with CD10, 7,5% with TTF-1, 1,9% with Hepatosit Ag showed positivity. With pCEA, CD10 and Villin cytoplasmic or membranous staining was observed. MA cases had no specific staining patterns for pCEA, CD10, Villin and CD34 observed in HCC cases. Both CK7 and CK20 were negative in 61,8% of HCC cases whereas, MA cases demonstrated different staining profile according to the primary tumor. Conclusion: With the staining of Hepatosit Ag in HCC, MOC-31 in MA and pCEA, CD10 and CD34 in both groups, we suggest that these markers should be included to the panel for the accurate diagnosis. CK7 and CK20 negativity would provide an additional information for the diagnosis of HCC.

PP2-248

HISTOLOGICAL CHARACTERISTICS OF CHRONIC HEPATITIS C ACTIVITY AND ITS IMPACT ON LIVER FIBROSIS DEVELOPMENT

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The number of patients with hepatitis C (CHC) is on the increase worldwide. Progression of the liver fibrosis as the main complication of the CHC may result in disability and death of the patients. The aim of the present research was to develop the morphological methods of defining the CHC histological activity and estimation of its impact on liver fibrosis progress. Forty-four CHC patients examined and diagnosed based on the complex of clinical-biochemical indices and excluding the viral hepatitis of the different etiology. Histological slides examined morphologically. There has been studied the impact of the histological activity and density of the lymphocyte and monocytes infiltration in various zones of liver lobes for the display of fibrosis in patients with CHC. Results showed that the most significant impact on the fibrosis progress in patients with CHC had a display of the porto-periportal necrosis and inflammatory infiltration of the portal tracts. There have been developed significant complex morphological criteria of CHC activity, which contain the average number of lymphocyte and monocytes infiltration cells of 1000 sq mkm of biopsy and average area of an infiltration cell in the periportal zone of the liver lobes in patients with CHC.

PP2-249

EXPRESSION P53, P21, P27, P16 AND KI67 IN HEPATOCELLULAR CARCINOMAS

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Background: Cell cycle control is a crucial event in the regulation of human normal tissues functions, and abnormalities of regulatory cell cycle genes are considered to contribute to the development of several human malignancies. This study investigates the expression of cell cycle proteins p53, p21, p27 and p16, and the degree of cell proliferation (Ki67 expression) in HCC and their correlation with classic pathologic tumor features and patients survival. Methods: Formalin-fixed, paraffin-embedded 4μm sections obtained from 75 HCC (hepatectomy and FNA materials), were subjected to immunohistochemistry (streptavidin-biotin peroxidase) using monoclonal and polyclonal antibodies for p53, p21, p27, p16, and Ki67. Results were expressed as % of positive cells. Mean follow up time was 29.32 months (range 1-86 months). Results: Expression of p53, p16, p21 and p27 was recorded in: 45/75(60%), 52/75(69%), 42/75(56%), and 58/75(77%) cases, respectively. The mean index for Ki67 was 22.35±±9.15. p53 and p21 were more frequently expressed in advanced stage tumors (p=0.011 and 0.0017, respectively). Statistical analysis revealed: I) direct correlation between lower disease-free survival and a) vascular invasion presence (p=0.00062), b) advanced tumor stage (p=0.00071) and c) proliferating index (Ki67+ cells) (p=0.0069). II) correlation of p53 expression with lower disease-free survival only in p21(+) tumors (p=0.013). III) direct correlations between a) p21 and p27 (r=0.3266, p=0.01933), b) p21 and p53 (r = 0.58644, p = 6.1231E-6), c) p27 and p53 (r = 0.28291, p = 0.04426). Cox regression analysis revealed that independent prognostic factors were: a) vascular invasion (CI:1.167-5.408, p=0.019), b) tumor stage (CI:0.043-0.650, p=0.01) and c) proliferating index (Ki67+ cells) (CI: 1.078-44.073, p=0.039). Conclusions: This study demonstrates that in cases of HCC, vascular invasion, tumor stage and proliferation index (Ki67 expression) are independent predictors of the outcome of the patients. p53 was associated with poor prognosis only in tumors overexpressing p21. This implies that high levels of p21 in tumor cells associated with aberrant p53 protein expression may result in tumor recurrence.

PP2-250
ANGIOGENESIS AND VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN HEPATOCELLULAR CARCINOMA

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Background: Evaluation of angiogenesis in hepatocellular carcinoma (HCC) may be crucial in the effort to develop and perform an effective antiangiogenic therapy for this tumor. This study investigates the presence of angiogenesis and vascular endothelial growth factor (VEGF) expression in HCC and correlates them with tumor pathology and patients' survival. Methods: The study included 75 HCC cases (hepatectomy and FNA materials) obtained from equal number of patients. Liver tissues were evaluated for a) degree of angiogenesis (DOA) (immunostain with anti-CD34 antibody [Biogenex, Ca, USA]), b) VEGF protein presence (immunostin with anti-VEGF antibody [DBS Pleasanton, CA, USA]). CD34 staining pattern was reported as: a) number of single complete vessels counted in 10HPF, b) presence of branching, sinusoid-like channels scored as follows:0, no sinusoid-like pattern; 1+ weakly stained discontinuous and focal vessels; 2+ weakly stained diffuse branching vessels/focal well-formed vessels; 3+ diffuse well-formed sinusoid-like vessels. Results were correlated with angiographic data, tumor pathologic features and patients' survival. Mean follow up time was 29.32 months (range 1-86 months). Results: The DOA (either the number of complete vessels or the presence of sinusoid-like channels) in HCCs was correlated with tumor size: HCCs with diameter < 2 cm) showed less DOA, compared to HCCs with a diameter >2 cm ($p=0.011$ and $p=0.0037$). In addition HCC showed higher DOA compared to non-neoplastic adjacent liver. Tumor vascularity on angiography did not correlate with DOA. VEGF protein expression was higher in solid areas of the tumors compared to acinar/pseudoglandular areas ($p=0.0012$). Cirrhotic non-neoplastic livers showed higher DOA and VEGF expression compared to non-cirrhotic livers ($p=0.0017$ and $p=0.026$, respectively). The DOA in HCC was correlated with lower disease-free survival ($r=-0.68$, $p=0.0027$ $r=-0.59$, $p=0.0034$). Cox regression analysis revealed that DOA was independent prognostic factor (CI:1.203-5.413, $p=0.015$). Conclusions: This study shows that angiogenesis in HCC may contribute in the dismal prognosis of this tumor. VEGF may be associated with the angiogenic process of the cirrhotic liver, but not with angiogenesis of HCC.

PP2-251
SOLID-PSEUDOPAPILLARY TUMOR OF THE PANCREAS

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Background: Solid-pseudopapillary tumor (SPT) of the pancreas is a clinicopathologically distinctive, yet histogenetically mysterious pancreatic neoplasm that could be misdiagnosed as adenocarcinoma, acinar cell carcinoma end endocrine tumor. A greater awareness of this unique, low-grade malignant entity gives reasons to precise histopathological diagnosis that should be associated with surgical resection. Methods: Among retrospectively reviewed pancreatic tumors diagnosed in the last 10 years 3 cases of SCT were evaluated. They comprised 1 male and 2 female patients in age 49-72 years. Clinicopathological data obtained from medical records were completed with histological examination including immunostaining. Results: Due to indolent clinical course and unremarkable laboratory investigations the tumours were discovered only by imaging procedures. The patients underwent surgical resection. Huge and encapsulated cystic tumors found in the head and tail of the pancreas were completely removed and histopathologically evaluated. The neoplastic growth patterns were composed of solid and pseudopapillary areas. The cells expressed distinct immunostaining for vimentin, NSE, CD10, CD 56; keratin, alpha1antitrypsin and glucagon in 2 cases; and fail to stain for chromogranin, synaptophysin and Ckit. After pancreatic resection with complete removal of the growths the patients recovered successfully. In one case, however, local recurrence of the tumor developed after 7 years. Partial resection was performed. Postoperative complication was acute pancreatitis that was treated conservatively. During the next 2 years the patient felt healthy and began with normal physical activity. Conclusion: SCT should be regarded as a slow growing tumor with low malignant potential and favourable prognosis after aggressive treatment such as complete growth resection. Due to infiltrative growth potential local recurrence may break out. Nevertheless, even in the presence of metastases long- term survival is expected.

PP2-252
HEPATOBILIARY PAPILLOMATOSIS WITH INTRADUCTAL PAPILLARY ADENOCARCINOMA

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Background: Biliary papillomatosis (BP) is characterised by intraductal papillary proliferation of biliary epithelial cells that could reveal various degrees of dysplasia and high risk of malignant transformation. Any part of the biliary tree can be affected including intra and extrahepatic bile ducts, gall-bladder and ampullary region. The course of the disease depends upon recurrent cholangitis followed with biliary cirrhosis or the development of biliary carcinoma. Case presentation: A 52-year-old apparently healthy man was referred to the hospital to regular health control including liver biochemistry. Slightly abnormal tests of liver functions led to abdominal ultrasonography and computed tomography. In the left liver lobe a large, space-occupying lesion was revealed, accompanied with dilatation of the intrahepatic ducts and the upper common bile duct. According to suspicion on a neoplastic growth in the liver left hemihepatectomy was performed together with cholecystectomy and resection of the hepatocholedochal duct. In the resected liver a well demarcated multilocular tumor was found, measuring 5,5 x 6 x 4 cm. It demonstrated soft and friable protrusions to the major bile duct, so as to the central cavities appearing to be dilated lumina of the intrahepatic ducts. Microscopical observation revealed multifocal papillo-villous intraductal projections of biliary epithelium. In some areas these cells represented malignant characteristics, in the others the features of benign biliary papillomatosis were evident. Besides, minimal expansile invasion was detected in the fibrous border of the growth. The histopathological diagnosis pointed out the presence of biliary carcinoma with the background of the benign lesion expressing various degrees of dysplasia, as well. After an uneventful postoperative course the patient has been disease free with serologically negative tumor marker CA 19-9 for 20 months. Conclusion: BP is a rare borderline malignant lesion involving the biliary tree. Malignant transformation may start with multicentric foci of epithelial cells with altered phenotypes.The presented case of coexisting BP and intraductal papillary carcinoma might prove the concept of gradual steps in the development of the same neoplastic entity.

PP2-253

HEPATOCELLULAR CARCINOMAS WITH MALLORY BODIES AND/OR INTRACELLULAR HYALINE BODIES: CORRELATION WITH STEATOHEPATITIC FEATURES

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Background: Mallory bodies (MBs) and intracellular hyaline bodies (IHBs) present in hepatocellular carcinoma (HCC) cells are the result of overexpression and accumulation of the stress protein sequestosome1/p62 (p62). MBs contain p62, keratins and ubiquitin as major components, whereas IHBs are characterized by their p62 and ubiquitin content. Classical MBs arise if abnormal keratins are present in addition to p62, but if p62 is present alone IHBs are formed by aggregation. Our aim was to evaluate and correlate the association of steatohepatitic features in HCCs with the presence of IHBs and MBs. Method: 126 HCCs (surgical specimens) were selected from the archives of the Department of Pathology, Medical University of Graz. All available sections were evaluated for steatohepatitic features and inclusion bodies by light microscopy. In 92 selected cases immunohistochemistry for the presence of keratin 8/18, p62 and ubiquitin was performed, using a tissue microarray, and correlated with steatohepatitic features. Statistical analysis was performed by the Fisher test. Results: Of all HCCs (n=126) 17 cases (13.5%) contained both MBs and IHBs, whereas in 27 cases only MBs (21.4%) and in 14 cases only IHBs (11.1%) were found. 68 cases (54.0%) expressed no inclusion bodies. HCCs with MBs as well as HCCs with MBs and IHBs showed significantly ($p<0.001$) more frequent infiltration by neutrophils compared to HCCs with IHBs only and HCCs without inclusion bodies. In addition, HCCs with any type of inclusion showed significantly ($p<0.001$) more cellular ballooning compared to the HCCs without inclusion bodies. Moreover, HCCs with inclusion bodies showed significantly more steatosis ($p<0.05$) versus HCCs without inclusion bodies. Conclusion: HCCs with MBs as well as HCCs with MBs and IHBs are associated with neutrophilic granulocyte inflammation, whereas HCCs with IHBs are characterized by small tumor cells with dense cytoplasm without significant inflammation. Since oxidative stress is a decisive factor in the development of steatohepatitis and since p62 as well as abnormal proteins, including keratins, are preferentially induced by oxidative stress it is reasonable to assume that oxidative stress is a major player in HCCs displaying steatohepatitic features. The eventual prognostic significance remains to be elucidated.

PP2-254

THE SIGNIFICANCE OF THE FOCAL HEMOSIDEROSIS IN CHRONIC HEPATITIS

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Background: In the past 20 years, many studies showed a correlation between the abnormal iron parameters and the chronic viral hepatitis (CVH). More recently, Di Bisceglie et al (1992) demonstrated that 35-40% of patients with CVH had had high levels of serical iron, but with normal concentration of iron in the liver. Other studies (van Thiel et al 1994, Olynyk et al 1995, Piperno et al 1996) remarked that CVH patients with a high iron concentration in liver were non-responder after Interpheron alfa therapy. Aim: Histological evaluation of hemosiderin presence and distribution using Perls' reaction on liver needle biopsy from patients with CVH and its' correlation with the response to antiviral therapy. Material and methods: Biopsies were routinely processed using paraffin embedding and HE, van Gieson staining and Perls' reaction. The CVH diagnosis relayed upon the evaluation of the necro-inflammatory degree (Ishak et al, 1994) and of the fibrotic stage (METAVIR system, 1994). Semi-quantitative evaluation of hemosiderin in Kupffer cells and/or in hepatocytes was set as mild, moderate or high. Results: From 634 patients with CVH, Perls' reaction was positive in 157 patients (24.76%). In the majority of these cases (72.61%) the hemosiderin load was mild, affecting both hepatocytes and Kupffer cells in the periportal zones of the lobules. The hemosiderin presence and intensity was correlated with the viral type (HBV and mixt polyviral rather than HCV), with the hepatitis degree and stage, but not also with the antiviral therapy. Conclusion: These preliminary data recommend Perls' reaction as a routine practice, to prove the hemosiderin accumulation. We noticed that hemosiderin accumulation was significant in chronic B viral hepatitis with high necro-inflammatory degree.

PP2-255

MATRIX METALLOPROTEINASE EXPRESSION IN NONTUMORAL PANCREAS, PANCREATIC INTRAEPITHELIAL NEOPLASIA AND DUCTAL PANCREAS CARCINOMA: ROLE IN CARCINOGENESIS AND PROGNOSIS

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AIM: The aim of the study was to investigate whether the expression of a specific matrix metalloproteinase (MMP) correlated with the carcinogenesis, metastasis and prognosis of pancreatic cancer. METHODS: Seven different MMPs, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, MMP-14, were examined in 28 pancreatic adenocarcinoma by immunohistochemistry. The expression of the MMPs were evaluated in nontumoral pancreas tissue, pancreatic intraepithelial neoplasia (PanIN) and in the areas of the pancreas carcinoma tissue undergone to Whipple procedure. RESULTS: There was expression by immunohistochemistry for MMPs 7,10,11 and 14 in all of the pancreatic cancers. But MMP-2, MMP-9 and MMP-3 expression were detected in only 29% (8/28), 86% (24/28), 57% (16/28), of the pancreas carcinomas respectively. In nontumoral pancreas tissue there were differences in the expression of the MMPs in the different components of the pancreas. Endocrine islets were stained strong with MMP-9, slight with MMP-3, MMP-10, MMP-11 and MMP-14 but not with MMP-2 and MMP-7. There was not any expression of MMP-2, MMP-9, MMP-10 and MMP-14 in the acinar cells. The pancreas ducts were stained with all of the MMPs but MMP-2 stained ducts only in the 50% (14/28) of the cases. The only MMP which is not expressed in the PanIN areas was MMP-2. In the PanIN areas the MMP staining intensities were higher than carcinoma. If there was a mucinous differantiation in an adenocarcinoma, MMP expression was decreased. In three of the cases extracellular mucin secretion was present, in those cases although the epithelium did not expresse MMP, secret was positively stained. CONCLUSION: In our study, although we could not find any prognostic significance of the MMP expression as in the previous studies supposed, we found highest overexpression in the PanIN areas and the expression of MMPs in most of the pancreatic adenocarcinomas. Those results indicate that MMP expression could have a key role in carcinogenesis and progression of the pancreas adenocarcinomas.

PP2-256
THE EXPRESSION OF MATRIX METALLOPROTEINASES IN INTRAHEPATIC CHOLANGIOCARCINOMA, HILAR (KLATSKIN TUMOR), MIDDLE AND DISTAL EXTRAHEPATIC CHOLANGIOCARCINOMA, GALL BLADDER CANCER, AMPULLARY CARCINOMA; ROLE OF MATRIXMETALLOPROTEINASES IN TUMOR PROGRESSION

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AIM: Carcinomas of the biliary tree are rare tumours of the gastrointestinal tract with a rising incidence during the recent years. Biliary neoplasms are classified into intra- and extrahepatic cholangiocarcinoma (CC) (Klatskin tumour, middle and distal extrahepatic tumours), gallbladder cancer, and ampullary carcinoma. We aimed to find out the expression profile of Matrix metalloproteinase (MMP) -2, MMP-9 and MMP-14 in the biliary neoplasms classified according to their localization. METHOD: Ten gall bladder, 8 distal bile duct carcinomas, 8 Klatskin tumors, 8 intrahepatic CCs, 10 ampullary carcinomas were included in study. The immunohistochemical expression of the MMP-2, MMP-9 and MMP-14 were detected in the nontumoral, metaplastic, dysplastic and tumoral epithelia. The tumor differentiation, angiolymphatic and perineural invasion, and the stage of the tumor were determined. RESULTS: The nontumoral epithelia of the gall bladder, intrahepatic ducts, Klatskin tumor did not express MMP-2. The expression of MMP-2 was detected in 75% (6/8) of the distal part of the biliary ducts, and 50% (5/10) of the ampullary carcinoma. The metaplastic and dysplastic epithelia were positively stained in all of the gall bladder, distal CC and ampullary tumors. In the intrahepatic CC the hepatocytes were positively stained but the infiltrative tumors were spared. Klatskin tumors also were not stained with MMP-2. The gall bladder carcinoma, distal CC and ampullary carcinomas expressed MMP-2 in 30%, 30% and 40%, of the cases respectively. MMP-9 and MMP-14 were expressed in normal, metaplastic, dysplastic epithelium and tumoral cells in all of the cases of the groups. Expression of MMPs were higher in subjects with neural invasion, but there was no correlation between MMP expression and tumor differentiaiton, angiolymphatic invasion and the stage of the tumor. CONCLUSION: When we sort the tumors of the biliary system as intrahepatic and extrahepatic CCs, MMP-2 expression was present in the extrahepatic CCs including gall bladder carcinomas. Like the intrahepatic CC, Klatskin tumors also did not express MMP-2. This can be related with its growing manner. MMP-9 and MMP-14 were present in metaplasia, dysplasia, carcinoma sequence in all of the biliary tract tumors that suggests that MMPs play an important role in carcinogenesis. The higher expresion of the MMPs with neural invasion suggests the significant role in those tumors in the invasion activity.

PP2-257
NESTIN IN NEOPLASTIC AND NORMAL ENDOCRINE PANCREAS

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Background: Pancreatic endocrine cells are multifunctional, differentiated cells originating from the differentiation of foregut endodermal precursors. Identification and transplantation of endocrine precursor cells appear to be the ultimate solution to diabetes mellitus, which requires lifelong treatment. Knowledge about specific markers that are used in the determination of precursor cells is limited. Material Methods: In this study, the expression of nestin in well-differentiated pancreatic neuroendocrine tumorswas investigated. In addition, developmental characteristics of nestin expression in the fetal and adult pancreas were examined. The study included 17 well-differentiated neuroendocrine tumors or carcinomas (PENs) diagnosed between 1988 and 2006 in the Department of Pathology of the Faculty of Medicine of Hacettepe University. In addition to tumor cases, pancreatic tissue was obtained from the autopsies of 5 fetuses (range of gestational age: 17-32 weeks) and 5 adults (age range: 42-67 years) that were performed for reasons other than cancer in the same department. Results: Findings indicated that nestin is expressed in proliferating and metabolically active cells, such as endothelium, independent of developmental and neoplastic processes, rather than endocrine precursor cells of the pancreas. Conclusion: It was concluded that nestin cannot be used as the only marker to identify pancreatic endocrine cell precursors for transplantation.

PP2-258
IMMUNOREACTIVITY FOR HEPCIDIN IN THE LIVER OF BETA THALASSEMIA PATIENTS

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BACKGROUND. Hepcidin is a 25-aminoacid peptide synthetized in the liver that acts as a systemic iron-regulatory hormone responsable for iron balance and homeostasis. In turn, hepcidin synthesis is increased by iron loading and decreased by anemia and hypoxia. Enterocytes, macrophages and kupffer cells are the most important target cells of hepcidin activity. In enterocytes it halts iron absorbtion, wihile in macrophages and kupffer cells it blocks iron relise, trapping iron within. Inhibition of the cellular iron efflux, by binding to and inducing the degradation of the iron exporter ferroportin, is considered the most important mechanism of its action. The molecular mechanisms that regulate hepcidin production are only beginning to be understood, but its dysregulation is involved in the pathogenesis of a spectrum of iron disorders. Recently, differences in hepcidin serum levels have been reported in thalassemia patients: carriers of thalassemia intermedia (TI) showed hepcidin levels remarkably lower then in patients with thalassemia major (TM). The aim of our study was to analyze the localization of hepcidin in liver of both in TM and in TI patients. METHODS. For that reason, needle liver biopsies from 5 TM and 5 TI patients were immunostained with hepcidin specific antibody (Alpha Diagnostic International, Inc). RESULTS. In all TI subjects we detected a strong immunoreactivity for hepcidin in Kupffer cells. On the contrary, in TM patients immunoreactivity for hepcidin was absent in the majority of the cases. A mild immunoreactivity was observed along the cell membrane of hepatocites in 2 out of 5 cases; no immunoreactivity for hepcidin was detected in Kupffer's cells. CONCLUSIONS. The immunohistochemical pattern for hepcidin clearly differenciates TM from TI patients and confirms at tessue level the previously reported differencies in hepcidin serum levels between TM and TI patients. Our data support the hypotesis that kupffer cells could have a major role in hemocromatosis of TI patients. Indeed the observation of the immunoreactivity for hepcidin in Kupffer cells could suggest a possible site of sequestration of hepcidin in liver macrophages, which correlates with the very low or undetecteble hepcidin serum levels in TI patients. What's more, hepcidin sequestration in Kupffer cells could give an explanation to the previously reported serum hepcidin deficiency and, eventually, excessive intestinal iron absorption.

PP2-259

THE CLINICAL SIGNIFICANCE OF PPAR-γ EXPRESSION IN PANCREATIC CARCINOMA

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Background: Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a ligand-activated nuclear transcription factor that regulates differentiation and induces cell growth arrest and apoptosis in different cancer cell types. In the present study, the expression of PPAR-γ was examined in pancreatic carcinoma histopathological specimens and was correlated with clinicopathological variables and patients' final outcome. Method: PPAR-γ expression was examined immunohistochemically on paraffin-embedded pancreatic carcinoma tissue sections obtained from 52 patients. The PPAR-γ positivity, overexpression (expression in more than the mean percentage value), intensity of staining and cellular pattern of distribution (nuclear, cytoplasmic, cytoplasmic and nuclear) were correlated with patients' gender, age and TNM stage; tumors' histological grade of differentiation, proliferative capacity (Ki-67 immunohistochemical expression); and patients' survival. Results: PPAR-γ positivity was noted in 40 out of 52 (77%) of the examined pancreatic carcinoma cases and PPAR-γ overexpression in 16 out of 40 (38%) positive cases. PPAR-γ intensity of staining was mild in 7 out of 40 (17%), moderate in 22 out of 40 (55%) and intense in 11 out of 40 (28%) positive cases. The pattern of PPAR-γ distribution in pancreatic tumor cells was nuclear in 19 out of 40 (48%), cytoplasmic in 6 out of 40 cases (15%) and cytoplasmic and nuclear in 15 out of 40 (37%) positive cases. PPAR-γ intensity of staining was statistically significantly correlated with tumors' histological grade (p=0.012). PPAR-γ overexpression and pattern of cellular distribution presented a trend of correlation with tumor size (T, p=0.078) and nodular metastases (N, p=0.060), respectively. PPAR-γ intensity of staining presented also a trend of correlation with patients' survival (p=0.061). PPAR-γ positivity, overexpression, intensity of staining and cellular pattern of distribution were not statistically significantly correlated with tumors' proliferative capacity and the other clinicopathological parameters examined. Conclusion: Our data support evidence for participation of PPAR-γ in the biological mechanisms underlying carcinogenesis in the pancreatic tissue. Further molecular and clinical studies are required to delineate and confirm the significance of this receptor in prognosis and management of pancreatic carcinoma patients.

PP2-260

EXPRESSION OF L1 AND ITS RELATED MOLECULES AS A PROGNOSTIC FACTOR IN CHOLANGIOCARCINOMA

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Background: Cholangiocarcinoma is a malignancy of the biliary tree, arising from any portion of intrahepatic or extrahepatic bile duct epithelium, and that is hardly control with curative surgical resection or conventional chemoradiation therapy. Consequently, better early diagnostic methods and new therapeutic managements for improving the survival of patients with cholangiocarcinoma are pressingly needed. L1, a novel target gene for Wnt/β-catenin-TCF signaling, transforms cells and increases cell motility, growth transformation, and tumorigenesis. In addition, cholangiocarcinoma accompanies reduced expression of E-cadherin and β-catenin and EGFR mutation. Design: In the present study, we investigated the immunohistochemical expression of specific anti-L1 monoclonal antibody, E-cadherin, β-catenin and EGFR in 143 patients with cholangiocarcinoma and estimated the correlations between the clinicopathologic characteristics and the expression of these antibodies. Result: High expression of L1 (≥20% carcinoma cells stained) was observed in 38.5% (55 of 143 cases) of cholangiocarcinoma, including 27% (10 of 37 cases) of Grade 1 tumors, 37.8% (28 of 74 cases) of Grade 2 tumors and 53.1% (17 of 32 cases) of Grade 3 tumors and strongly expressed in the invasive front of tumor cells of cholangiocarcinoma. Membranous expression of E-cadherin and β-catenin were reduced in cholangiocarcinoma (93%, 92.3% respectively). Nuclear β-catenin expression was noted in 17.5% (25 of 143 cases) cholangiocarcinoma. High expression of EGFR was observed in 63.6% of cholangiocarcinoma. There is statistically significant relationship between the expression of L1 and reduced membranous expression of E-cadherin, β-catenin or high expression of EGFR. There is also significant relationship between the expression of L1 or EGFR and histologic grading, pathologic T stage, venous/lymphatic invasion or perineural invasion in cholangiocarcinoma (p value all less than 0.05). Conclusion: The results suggest that L1 is a new factor for poor prognosis of patients and plays a crucial role in tumor invasion in cholangiocarcinoma. E-cadherin and β-catenin together with L1 might contribute to tumor progression. EGFR is also an important prognostic factor in cholangiocarcinoma.

PP2-261

PRELIMINARY STUDY REGARDING IMPORTANCE OF THE CYTOPATHOLOGICAL DIAGNOSIS IN MALIGNANT PANCREATIC TUMORS

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Background The purpose of this study consist of a preliminary assessment of the cytopathologic examination for the diagnosis of pancreatic tumors with clinical suspicion of malignancy after echografic examination. Method 38 cases with clinical suspicion of pancreatic (P) malignant (M) tumor (T) and investigated using endoscopic-ultrasound-guided fine needle aspiration cytology (EUS-FNA) were selected. Classical cytopathological examination, on minimum 2 smears stained with May-Grunwald-Giemsa (for quick cytopathological diagnosis) and minimum 2 smears stained with Papanicolaou (for better visualization of nuclear details) for each case, was performed in all 38 cases. The smears were cathegorised as: benign (B), suspicious probably M, and M. The following cytopathological parameters were assessed: smear cellularity; presence, disposition, shape and number of cells with malignant features; presence of other B cells (P ductal cells, inflammatory cells, red cells). In cases with smears with suspicion of malignancy or M ones, cellular blocks were obtained (double centrifugation with ethanol 100%, formalin fixation and processing using the classical method for wax embedding) on which we performed immunohistochemical staining (IHCst) to identify the P epithelial fenotype of the M cells (anti-AE1/AE3 cytokeratines, anti-CA19-9, anti-CK20 cytokeratine and proliferation marker Ki67), and the site of a secondary PMT (HMB-45 and S100 protein). Results The smears obtained from 13 cases were diagnosed as benign. In 9 cases the smears had inflammatory background which confirmed the

diagnosis of pseudotumoral pancreatitis (Pt) at echoendoscopic examination. In 4 cases, there were frequent foamy cells and few clusters of B canalicular cells, so the final cytodiagnosis was chronic pseudocystic Pt. In 5 cases, with suspicious probably M smears, IHCst for Ki67 was performed; 3 cases were considered B with reactive cellular changes because Ki67 labelling index was under 5%; the other 2 cases were diagnosed as M because the index was very high. In 20 cases there were clustered cells showing obvious M criteria (M smears). In 21 cases considered as M, positive IHC staining for AE1/AE3 and CA19-9 confirmed the diagnosis of P adenocarcinoma. In 1 case negative IHCst for these markers and positive IHCst for HMB45 and S100 were conclusive for metastatic PT from an ocular M melanoma. Conclusions Preliminary data suggested that cytopathologic examination (on the smears and cellular blocks) on material provided by EUS-FNA is a reliable tool for the diagnosis of PMTs.

PP2-262
CLINICAL SIGNIFICANCE OF RCAS1 EXPRESSION IN PANCREATIC ADENOCARCINOMA

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Background: The tumor associated antigen "receptor-binding cancer antigen expressed on SiSo cells" (RCAS1) inhibits the growth of cells that express its receptor, inducing apoptosis. RCAS1 has been recognized as a significant biological marker assessed in human biological fluids and cancer tissue samples. The aim of the present study was to evaluate the clinical significance of RCAS1 expression in cases of human pancreatic adenocarcinoma. Method: Immunohistochemical expression of RCAS1 was performed on paraffin-embedded pancreatic adenocarcinoma tissue sections obtained from 76 patients. The RCAS1 positivity, overexpression (expression in more than the mean percentage value), and intensity of staining were correlated with patients' gender, age and TNM stage; tumors' histopathological grade of differentiation, proliferative capacity (Ki-67 immunohistochemical expression); and patients' survival. Results: RCAS1 positivity was noted in 65 out of 76 (86%) pancreatic adenocarcinoma cases. The observed pattern of RCAS1 immunostaining was cytoplasmic and membraneous. RCAS1 overexpression was noted in 36 out of 65 (55%) of the positive cases. RCAS1 intensity of immunostaining was classified as mild in 11 (16%), moderate in 27 (42%) and intense in 27 (42%) out of 65 positive cases. RCAS1 positivity was statistically significantly correlated with tumors' histopathological grade ($p=0.026$), whereas a trend of correlation with tumor size (T, $p=0.071$) was also noted. RCAS1 intensity of immunostaining and overexpression presented a trend of correlation with tumors' histopathological grade ($p=0.061$ and 0.089, respectively). RCAS1 positivity, overexpression and intensity of immunostaining were not statistically significantly correlated with tumors' proliferative capacity and the other clinicopathological parameters examined. RCAS1 positivity, overexpression and intensity of immunostaining were not statistically significantly correlated with patients' survival. Conclusion: Our data support evidence for implication of RCAS1 in pancreatic neoplasia as correlations with clinicopathological variables in pancreatic adenocarcinoma cases have been noted. However, the prediction of survival using RCAS1 expression in pancreatic adenocarcinoma patients seems uncertain.

PP2-263
AUTOIMMUNE PANCREATITIS IN HETEROTOPIC PANCREAS MIMIC DISTAL COMMON BILE DUCT CARCINOMA: A CASE REPORT.

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CONTEXT:The incidence of pancreatic heterotopia is 0.55% to 15% among autopsy specimens. The most frequent location is upper gastrointestinal tract, specially in the duodenum, stomach and jejenum. Pancreatitis in heterotopic pancreas is rarely described, and clinical symptoms caused by this heterotopic inflammation are uncommon. CASE REPORT: We report a case of heterotopic autoimmune pancreatitis, of a 39 year old non-alcholic man who was admitted to Istanbul Education and Training Hospital Emergency Service for extensive right abdominal pain, weight loss and jaundice. ERCP and MR cholangiography demonstrated a distal common bile duct stricture suspicious of carcinoma. He underwent pancreaticoduodenectomy. Macroscopic examination revealed a nodule measuring 10x5x5 mm immediately adjacent to the ampulla of Vater in the wall of the duodenum. Histologically, it was composed of pancreatic tissue, including ducts, acini, and well-formed islets. There was dense lymphotic cell infiltrate surrounding ducts. Immunohistochemical evoluation of the lymphotic cell infiltrate showed predominance of T cells (positive for CD3 and UCHL-1) with fewer B cells (positive for CD20). CONCLUSION: In our case it was impossible to differentiate between chronic pancreatitis and pancreas carcinoma preoperatively. This is a rare entity and remains difficult to diagnose, despite advances in radiological and endoscopic imaging techniques. In the absence of unequivocal imaging and when there is a suspicion of underlying malignancy, pancreaticoduodenectomy may be the only treatment option, as in this case.

PP2-264
LEIOMYOSARCOMA OF THE LIVER IN A HIV POSITIVE CHILD. REPORT OF A CASE

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Primary hepatic leiomyosarcomas are extremely rare neoplasms. The first reported case of primary hepatic leiomyosarcoma was found in a 9 years old girl with AIDS. The leiomyosarcomas are seen with increasing frequency in immunosupressed patients like HIV-infected individuals and organ transplant recipients. Our patient was a 4 years old HIV positive girl with leiomyosarcoma in the liver . Our patient had multiple lymphadenopathy. Abdominal CT showed contrast enhancing multiple lesions some with central necrosis in the liver. Histological examination of a biopsy sample showed a tumor composed of atypical spindle cells arranged in intersecting bundles and showing immunohistochemical features of smooth muscle cells. The differential diagnosis is with Kaposi's sarcoma and gastrointestinal stromal tumor (GIST) were considered. Immunohistochemical stains showed staining with actin and desmin, while CD34 and CD117 were negative. The insidence of leiomyosarcoma is increasing unusual sites are involved in the immunosupressed population. To our knowledge, this is the youngest patient with leiomyosarcoma of the liver reported to date.

PP2-265
TO EVALUATE THE CORRELATION OF SERUM TRANSAMINASE VALUES WITH HISTOLOGICAL ACTIVITY AND FIBROSIS IN HEPATITIS

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ABSRACT: The aims of this study were to assess the predictive values of age, gender and serum aminotransferase values on the histological findings in patients with chronic hepatitis. METHODS: This retrospective study was based on data related patients with HBV, HCV infection and steatohepatitis who were seen at our pathology department from 2003 to 2007. Three hundred and ninety-eight (398) consecutive patients, including 87 cirrhotics, with chronic hepatitis were studied. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels and hepatic activity index in all liver biopsies, were assessed. Liver biopsies were graded for the degree of fibrosis and histological activity by using modified histological activity index (HAI) of Ishak K et al. Normal liver, steatohepatitis and cirrhosis were also recorded. Cirrhosis was defined by a fibrosis score 5-6 in "modified HAI" classification. RESULT: The mean age of patient population was (43,11±15,44); 226 patients (%56,8) were male. By Mann Whitney U testing and Spearman's correlation analyses, serum AST values emerged as the most important predictive variable of hepatic fibrosis (r:0,311; p<0,01). A correlation was found between AST values and histological activity (r:0,295; p<0,01). On the other hand, serum ALT values did not correlate with (r:0,094; p>0,05) the extent of hepatic fibrosis but did correlate weakly with histological activity (r:0,242; p<0,01). In cirrhotics vs noncirrhotics, there were no significant differences between mean serum ALT (120,7±21,48 vs 116,11±13,49 units/liter) , but a correlation was found serum AST (129,36±21,43 vs 82,09±10,98 units/liter) levels. There were no significant correlations between age, gender or AST/ALT ratio, with the extent of histological activity or fibrosis. CONCLUSION : Serum AST values correlate well with histological activity and with the extent of hepatic fibrosis. These finding suggest that, among other factors, serum AST values should be considered in decisions regarding the need for liver biopsy and treatment in patients with chronic hepatitis.

PP2-266
CLINICAL SIGNIFICANCE OF EPHRIN RECEPTOR A1 EXPRESSION IN PANCREATIC ADENOCARCINOMA

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Background: Ephrin receptors (Ephs) belong to the largest family of tyrosine kinase receptors that mediate bi-directional signals between adjacent cells, being clustered after ligand binding. They are divided into two sub-groups: EphA (A1–A9) and EphB (B1–B6). Ephs and their cell-presented ligands, the ephrins (ephs), have been shown to be overexpressed frequently in a variety of cancer types. In the present study, the expression of Eph A1 subtype was examined in pancreatic adenocarcinoma histopathological specimens and was correlated with clinicopathological variables and patients' final outcome. Methods: Immunohistochemical expression of Eph A1 was performed on paraffin-embedded pancreatic adenocarcinoma tissue sections obtained from 67 patients. The Eph A1 positivity, overexpression (expression in more than the mean percentage value) and intensity of immunostaining were correlated with patients' age and TNM stage, tumor histological grade of differentiation, proliferative capacity (Ki-67 immunohistochemical expression) and patients' survival. Results: Eph A1 positivity was noted in 58 out 67 (87%) of the examined pancreatic adenocarcinoma cases. Eph A1 overexpression was noted in 26 out of 58 (45%) positive cases. Eph A1 intensity of immunostaining was classified as mild in 25 (43%), moderate in 32 (55%) and intense in 1 (2%) out of 58 positive cases. Eph A1 intensity of immunostaining was statistically significantly correlated with tumor size (T, p=0.007) and disease stage (p=0.040). Additionally, a trend of correlation between Eph A1 intensity of immunostaining and tumor histological grade (p=0.055) was noted. Eph A1 overexpression presented also a trend of correlation with disease stage (p=0.056). Eph A1 positivity, overexpression and intensity of immunostaining were not statistically significantly correlated with tumors' proliferative capacity and the other clinicopathological parameters examined, as well as with patients' survival. Conclusion: According to our data, implication of Eph A1 in pancreatic tumorigenesis is suggested. Further molecular and clinical studies are required to define the significance of other Ephs and their ligands (ephs) in prognosis and management of pancreatic carcinoma patients.

PP2-267
AUTOIMMUNE PANCREATITIS

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Background: Autoimmune pancreatitis (AIP) is a recently described unique type of chronic pancreatitis(CP) that is thought to involve an autoimmune mechanism. It accounts for less than 1% of cases of CP. Recognition of this entity is clinically important because it is reversible when diagnosed. Methods:We identified only five cases with AIP among 131 patients (3.8%) with CP followed in our pancreas unit. We evaluated the clinicopathologic findings in 5 patients with AIP. Intraoperative biopsy was performed in one patient, surgical resection was performed one of the other patient, 3 of the other patients diagnosed by clinical and radiologically. Results: The 5 patients consisted of 3 women and 2 men, their ages ranged from 37 to 58 years old. Presenting manifestations in 5 patients included abdominal pain, jaundice and weight loss. None of the patients had history of alcohol abuse or other predisposing factors of chronic pancreatitis.All cases were seronegative. Association with an autoimmune disorder was seen in one case.In the first case intraoperative biopsy showed lymphoplasmacytic infiltration with periductal, interstitial fibrosis. Histological description was CP without any cytological atypia. As a concequence of the biopsy result was suggestive of AIP which was supplemented by clinical, laboratory and radiological findings. In the second case surgical resection was performed becouse of suspicion of pancreatic tumor. Macroscopic features showed diffuse firm enlargement of the pancreas without distinct mass. Histopathologic findings were intense inflammatory cell infiltration around medium-sized and large interlobular duct with a cuff-like periductal fibrosis. The periductal infiltrates were composed mainly of lymphocytes and plasma cells. In addition acute inflammatory component (granulocytic epithelial lesion) was detected. The affected ducts were narrowed by an infolding of their epithelium often giving their lumen a star-like appearance. Fibrosis of the pancreas predominantly showed a periductal. There were also fibroinflammatory areas. Venulitis was mainly present in fibrotic areas All 5 patients were responsive to steroid therapy. Conclusion: The diagnosis of AIP is based on clinical, radiological and biochemical findings. Autoimmune pancreatitis may mimic pancreatic-head carcinoma.

Excluding the diagnosis of pancreatic-head carcinoma and confirming the diagnosis of AIP may avoid unnecessary pancreaticoduodenectomy. Reliable minimally invasive methods are need to diagnosis AIP.

PP2-268
A NOVEL MODEL FOR SPATIAL RECONSTRUCTION OF ANGIOARCHITECTURE OF LIVER LOBE IN CHRONIC HEPATITIS C

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Background It is indicated that, instead of morphological variety, there is essentially a common pathway of liver cirrhosis. It is nothing but process of breakup and rebuilding of liver lobules. Stil the formal pathogenesis of liver cirrhosis is unresolved. The aim Three-dimensional reconstruction of alteration of liver lobule and its angioarhitecture in chronic hepatitis C (HHC) in order to determine the formal pathogenesis regarding the transformation from chronic hepatitis to liver cirrhosis. Material and methods Ten liver byopsies with HHC were rutinly prepared for light microscopy. A liver tissue from autopsy of a men died of sudden cardiac death was used as a control. Serial sections of parafin blocks were stained by trichrom Mallory and by immunohistochemical methods for demonstrating smooth muscle actin and CD34. The analisis was done on Leica DMR (Leica Micro-Systems, France) microscope. Histological images were captured and digitalised by Leica DC300 digital camera and were stored as JPG files (8 bit, 2088x1552 pixel images) on hard disc. The collected images were than imported into Adobe® Photoshop® CS where the collor and contrast corrections were made so that the fields that were threated for CD34 could be isolated. The bitmaps that contained only the CD34 threated fields were than imported into Autodesk® Architectural Desktop and were marked as vectorised object, giving us the abbillity to reconstruct the 3D structure. Results Computed reconstruction gave various forms of altered angioarchitecture. Portal vein was preserved in all cases. The pathological changes begin on the parenchymal portion: the second step branches of the portal vein showed an increase and decrease in number and disturbance in special arrangements. In severely damaged parts of the liver tissue normal angioarhitecture was lost. It seems that parenchymal nutrition rest on the remaining third-step portal branches. Hepatic vein system seemed to be preserved. Conclusion Distortion of portal vessels reflect changes in lobular architecture. The method for three-dimensional reconstruction succesfully helped in visuelisation the early histological signs of cirrhosis developing in the course of chronic hepatitis C.

PP2-269
SINUSOIDAL CAPILLARIZATION IN DISPLASTIC NODULES AND EARLY HEPATOCELLULAR CARCINOMA

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Background Although hepatocellular carcinoma (HCC) receives an exclusively arterial blood supply, the majority of well differentiated HCCs of indistinctly nodular type (early HCC) receive aditional portal blood. Although the pathological diagnosis of high-grade dysplastic nodule (DN) and early HCC is controversal,when face the differential diagnosis, the type of vascularisation can be helpful in processing small surgical byopsies. The aim Analisis of arterialisation and changes in sinusoidal capilarisation in displastic nodules and HCC. Material and method We immunohistochemically stained 17 displastic nodules of low grade and 9 of high grade and 7 hepatocellular carcinoma, two of them being early HHC for CD34, indicating sinusoidal capillarisation. Distribution of sinusoidal capillarization was graded semiquantitatively. Resuts Sinusoidal capillarization was common finding in HHC. The relation to displastic nodules was significantly higher (p< 0,0027). There were significant differences among moderately differentiated and early HCC. In moderately differentiated HCC, endothelial cells of the sinusoidal blood spaces are positive for CD34, whereas those in early –stage HCC are only partially positive. In our cases of early HCC with "nodulle in nodulle appearance" those findings were of crutial diagnostic value. Conclusion Distribution of sinusoidal capillarization in displastic nodules support them as premalignant lesions. Uniform distribution of CD34 in HCC of moderat degree of differentiation versus incomplete distribution in small, early-stage HCCs can be of diagnostic value.

PP2-270
GALLBLADDER ADENOCARCINOMA AND P53 AND C-ERBB2 IMMUNOHISTOCHEMICAL EXPRESSION

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Background: The gallbladder adenocarcinoma is rare, but is a carcinoma with unfavourable prognosis. The aim of this study was to evaluate the immunohistochemical expression of p53 and c-erb-B2 and their possible role in tumorigenesis in gallbladder adenocarcinomas. Method: 17 archival cases of gallbladder adenocarcinoma and one case of bile duct adenocarcinoma were reviewed. Eighteen cases of chronic cholecystitis, nine with gallstones, were also selected. The patients with adenocarcinoma were 12 female and 6 male and their age ranged 48-78 years old. Paraffin sections from the selected cases were stained immunohistochemically for p53 and c-erb-B2 Results: Thirteen cases were diagnosed as low differentiated and the rest 5 cases as moderately differentiated adenocarcinomas. The serosa was infiltrated in 11/18 cases and the adipose tissue in 7/18 cases. Four cases showed infiltration of the cystic and/or the bile duct, 4 cases had lymph node metastases, 5 cases liver metastases and 2 cases peritoneal implants. P53 overexpression was found in 8 cases (44.4%), while c-erbB2 overexpression was found in 2 cases (11.1%). None case showed co-expression of p53 and c-erbB2. Two cases of chronic cholecystitis, not related to gallstones, showed p53 overexpression. Conclusion: According to the English literature, the percentages of p53 and c-erbB2 overexpression in gallbladder adenocarcinomas are quite divergent. It seems that p53 gene mutation may play a role in tumorigenesis of gallbladder adenocarcinoma, which is possibly more significant and independent from the c-erbB-2 overexpression.

PP2-271
NEUROENDOCRINE CARCINOMA OF GALLBLADDER

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Background: To present a rare case of incidental finding of neuroendocrine carcinoma of the gallbladder and the problems that occur. Method: A female patient 73 years old underwent laparoscopic cholecystectomy due to symptoms of chronic cholecystitis. Results: Microscopic evaluation revealed a small neoplasm, 4mm in greatest diameter, located near the cystic duct. The neoplasm infiltrated the mucosa, submucosa and partially the muscular layer. Small tubular structures were observed with very mild nuclear atypia and with no obvious mitotic activity. In immunohistochemical examination the neoplastic cells were positive for keratins AE1/AE3, S100 protein (nuclear and cytoplasmic), synaptophysin, Chromogranin A and NSE. The morphological and immunohistochemical findings were

suggestive for neuroendocrine carcinoma. Conclusion: The neuroendocrine carcinoma of the gallbladder is a rare entity. The few studies published contain small numbers of cases. Therefore, there is not enough evidence to support the best choice of treatment and the possible biological behavior.

PP2-272
PSEUDOTUMOROUS TYPE OF AUTOIMMUNE PANCREATITIS

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Autoimmune pancreatitis is defined as chronic fibro-inflammatory process of the bilio-pancreatic ductal tract, dominantly involving pancreas and causing organ dysfunction. An autoimmune basis for this disease is strongly supported by laboratory findings and frequent association with rheumatoid arthritis, Sjogren's syndrome, inflammatory bowel disease and other autoimmune manifestations. This entity has prevalence between 5-6 % of all chronic pancreatitis, although it is apparently a heterogeneous disease. Besides the most common diffuse type and rarely presented systemic type with widespread extrapancreratic involvement, at least a third of cases are localized pseudotumourous forms. An excellent response to corticosteroid therapy in most cases makes Whipple's resection unnecessary. We report three cases of pseudotumourous form of autoimmune pancreatitis from the Department of Histopathology Registry during 8 years (1997-2004) in 2 male (52,67 ys average age) and 1 female patients (34 ys age) operated in Clinical centre of Serbia. In all three cases there were moderately elevated serum levels of CA19-9 and suggestive CT scan low-attenuation or isoattenuation mass in the head of the pancreas, in addition to obstructive jaundice and abdominal pain, mimicking pancreatic cancer. Detailed histopathological analysis was performed on localized well-defined tumour-like lesions measuring 4-5,5 cm in diameters, presenting firm rubbery consistency in the head of the pancreas. All cases microscopically revealed admixture of diffuse or ill-defined dense lymphoplasmacytic infiltrate surrounding interlobular ducts and irregular sclerosing fibrosis predominantly affecting interlobular septa. Immunohistochemistry revealed the predominance of T cell lymphocytic immunophenotype around and within ductal epithelium and IgG4-positive plasma cells, which confirmed the morphological diagnosis. In two cases there was additional lobular involvement of the infiltrate and in one case a prominent myofibroblastic proliferation with foci of oedema of fibrotic tissue formed inflammatory pseudotumor in the periampullar region. In conclusion, these cases represent a failure of preoperative diagnostics as it was impossible to ascertain malignancy on preoperative biopsy and there was a need for more biochemical and clinical tests leading to an autoimmune disease and reaching the correct preoperative diagnosis. However, preoperative histological confirmation of the autoimmune pancreatitis is also very difficult and could be easily misdiagnosed as other types of chronic pancreatitides.

PP2-273
EXPRESSION OF P16 PROTEIN AND CYCLIN D1 IN PERIAMPULLARY CARCINOMAS

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Background: The majority of the tumors in periampullary region are pancreatic adenocarcinoma and adenocarcinoma of ampulla of Vater's. Ductal adenocarcinomas (DAC) constitute agreater proportion of pancreatic head carcinomas. Recent studies have identified that pancreatic intraepithelial neoplasia (PanIN) plays a role in development of DAC. Also, inactivation of p16 tumor supressor gene is an important factor in carcinogenesis of pancreas adenocarcinoma. The aim of this study is to compare histopathological features of carcinoma of ampulla of Vateri (AVC) with DAC in the periampullary region and to determine diagnostic and prognostic values of p16 protein, cyclin D1 expression and PanIN lesions in tumoral and peritumoral pancreatic tissues. Materials and Methods: Tissue samples from DAC and AVC were obtained from 20 and 11 patients, respectively who had undergone pancreticoduodenectomy for periampullary carcinoma at the Departments of Pathology of Universities of Pamukkale and Cumhuriyet. The group of patients included 12 women and 19 men. Their median age was 62,32 years (range 26-85 years). The factors analyzed include tumor characteristics (diameter, margin status, lymph node status, perineural invasion, differentiation, duodenal invasion), grade of PanIN, p16 and cyclin D1 expression at tumoral and peritumoral pancreatic tissues. Results: We found no significant difference between two groups in terms of the presence of PanIN at peritumoral pancreatic tissues statistically ($p>0,05$). In DAC group, perineural invasion was more frequent than the AVC group ($p=0,01$). In all cases, the loss of p16 protein expression was correlated with perineural invasion significantly ($p=0,0001$). When desmoplasia and lymphoplasmocytic stromal infiltration were examined, it was found that desmoplastic reaction was encountered more in DAC group rather than AVC group significantly ($p=0,01$). Cyclin D1 expression was found as non significant in neither of the groups ($p>0,05$). Conclusion: These results suggest that the loss of p16 protein may be an indicator of poor prognosis of periampullary cancer related principally due to perineural invasion, regardless of histopathological type. DAC and AVC may be distinguished by the presence of desmoplastic stromal reaction. The existence of PanIN lesion seems to be not specific findings for DAC.

PP2-274
VALUE OF HEPATOCYTE PARAFFIN 1 ANTIGEN (HEP PAR 1) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN PREDICTING EARLY HCC AND DIFFERENTIATING SOME METASTATIC HEPATIC LESIONS IN PATIENTS WITH CHRONIC LIVER DISEASE

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HCC is now a rather common malignancy in Egypt, usually develops on top of liver cirrhosis secondary to chronic viral infection (B/C) which increased the risk of HCC in the Egyptian patients. Hepatocyte paraffin 1 antibody (Hep Par 1) has been reported to be a sensitive marker for HCC in paraffin sections and previous studies showed that it is 90% specific for hepatocytes and is useful for differentiating HCC from liver metastases. Our aim is to assess the expression of (Hep Par 1) and vascular endothelial growth factor (VEGF) in fine needle liver biopsy specimens from patients having hepatocellular carcinoma compared to patients having chronic viral hepatitis (B or C) and to study their clinical significance as possible markers for early prediction of malignancy in chronic viral hepatitis and cirrhotic patients. The material of this study comprised 80 patients

suffering from chronic liver disease examined during the period from 2004-2006.All patients were subjected to clinical examination, abdominal ultrasonography, serological investigations, liver needle biopsies and histopathological diagnosis. They were classified as 30 patients with HCC, 30 patients with LC and 20 patients with CH (B/C), beside 10 patients with metastatic liver disease (control group). Unstained liver sections from every case were treated with the monoclonal antibodies for both Hep par 1 and VEGF. In our study we found that more VEGF expression in "CH" group than in liver cirrhosis "LC" and HCC patients. Both LC and HCC groups exhibit moderate (51-75%) and intense expression (>75%) of VEGF, indicating that angiogenesis is higher in LC and HCC than in chronic hepatitis, so VEGF plays a role in prediction of the possibility of malignancy in CH patients. We found that most of CH and LC patients show focal expression of Hep par 1, while most of HCC patients show diffuse Hep par 1 expression (≥90%) and this was significantly higher in HCC than in CH and LC patients, so Hep Par 1 when diffuse in CH and LC patients could indicate susceptibility to turn malignant. VEGF plays a role in prediction of the possibility of malignancy in CH patients as angiogenesis was found higher in liver cirrhosis and HCC than in chronic hepatitis, while VEGF expression in liver metastases was not intense. Well differentiated HCCs with diffuse and intense Hep par 1 expression warrant earlier intervention before further progression of malignancy. Hep par 1 plays an important role in the differentiation between primary HCC and liver metastases being negatively expressed in all metastatic patients.

PP2-275
CLEAR CELL PANCREATIC ADENOCARCINOMA

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Background: Adenocarcinoma of ductal origin compromises 85-90% of all pancreatic tumors. Currently the WHO recognizes clear cell carcinoma as a "miscellanous" carcinoma with ductal phenotype and presence of mutation in the k-ras oncogene. We report a case of pancreatic ductal adenocarcinoma with predominantly clear cell morphology (> 95% clear cells). Report of the case: A 78 year-old man presented in March 2006, with right upper quadrant pain. He underwent a series of evaluations, including a computed tomographic scan of the abdomen, which revealed a mass in the tail of the pancreas. The patient reported an 8 Kg weight loss during the last three months. Distal pancreatectomy and splenectomy was performed. The specimen received for pathologic analysis was a 8X3.3X2 cm recection of the distal pancreas with the attached spleen. A vaguelly circumscribed, firm, white tumor, 3 cm in diameter was found, extended into the peripancreatic soft tissue. Microscopic examination revealed highly atypical glands composed of pleomorphic cells with abudant clear cytoplasm (> 95% clear cells). Glands were surrounded by a desmoplastic stroma. The background pancreas showed atrophy, along with a spectrum of pancreatic intraepithelial neoplasia (PanIN). Histochemically intraluminal and intacytoplasmic PAS+ and PASD+ mucin droplets were revealed. Immunohistochemically the cells were positive for CK7 and CAM 5.2 and were focally positive for CK20. These cells also expressed monoclonal carcinoembryonic antigen. Stainings for the neuroendocrine markers, synaptophysin and chromogranin were negative, as were stains for vimentin, HMB-45 and CD10. The differential diagnosis includes pancreatic neuroendocrine tumors, solid pseudopapillary tumor, PEComa, mixed ductal-endocrine carcinoma, solid serous cystadenoma and metastatic renal cell carcinoma. The possibility of a neuroendocrine tumor is excluded in this case by the absence of neuroendocrine markers in the neoplastic cells. PEComa is characterized by positivity for HMB-45, which is negative in our case. Likewise, the tubuluglandular growth pattern and mucin production combined with the absence of vimentin and CD10 expression excludes metastatic renal cell carcinoma. Conclusion: We present a unique case of poorly differrentiated clear cell ductal adenocarcinoma of the pancreas. In the proper morphologic context supported by immunohistochemistry, clear cell carcinoma can be regarded as a rare variant of ductal adenocarcinoma. The patient, twenty days after the operation died, due to postoperative hemorrhage.

PP2-276
MONONUCLEAR CELL PHENOTYPES IN NONALCOHOLIC FATTY LIVER DISEASE: COMPARISON WITH CHRONIC HEPATITIS C

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AIM: Cytokine release from inflammatory cells, lipid peroxidation, and generation of reactive oxygen species are among the factors currently thought to be important in the pathogenesis of nonalcoholic fatty liver disease (NAFLD). However, innate and adaptive immune response could also have an influence in the pathogenesis of NAFLD. The aims of this study were to evaluate the frequency and distribution of intrahepatic mononuclear cell phenotypes and to assess the relationship of these cells with the grade and stage of NAFLD. METHODS: Liver biopsies of patients with NAFLD (n=40) and chronic hepatitis- C (CH-C) patients with more than 10% steatosis in their liver biopsies (n=20) were selected for immunohistochemical staining to analyze the type and distribution of mononuclear inflammatory cells. CD4 and CD8 T-lymphocytes, CD20 (B lymphocytes), CD83 (dendritic cells), and CD56 (NK) cells were detected using monoclonal antibodies on paraffin-embedded tissue. Grading and staging of the liver biopsies of NAFLD patients were evaluated with Brunt's system. RESULTS: In both NAFLD and CH-C groups, T cells were found in portal tracts and in regions of focal necrosis. Immunohistochemistry showed mild to moderate (1+ to 2+) numbers of T cells, with CD4 predominance in NAFLD. In contrary, the percentages of CD4 and CD8 cells were approximately equal in CH-C. The number of CD8 lymphocytes in NAFLD was significantly lower than that in CH-C ($p<0.05$). B cells were only rarely present in NAFLD in contrast to CH-C. CD56-positive cells were rare in both lobule and portal areas of CH-C, but CD56 staining in biopsies of NAFLD showed prominent immunoreactivity throughout the lobules especially in perivenular regions. Dendritic cells were observed mostly in portal areas associated with CD8 lymphocytes in CH-C, but in pericentral areas in NAFLD. The magnitudes of CD4(+)T-cell and NK-cell responses were correlated with the extent of liver cell damage in NAFLD. CD56-positive cells were more common in biopsies with bridging fibrosis, compared to mild perivenular fibrosis in NAFLD patients ($p<0.05$). CONCLUSION: Our results suggest that NK cell response, and also T lymphocytes, in particular CD4 (+) T cells, are critical for the pathophysiology of NAFLD.

PP2-277
ALTERED S-ADENOSYLMETHIONINE METABOLISM IN A MALLORY BODY MOUSE MODEL

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Background: Alcoholic (ASH) and nonalcoholic steatohepatitis (NASH) are highly -prevalent diseases in developed countries and are precursors of life- threatening disorders such as cirrhosis or hepatocellular carcinoma. S-adenosylmethionine (SAMe) is a

key metabolite in hepatocytes and acting not only as the most important methyl donor, but is also involved in the regulation of hepatocyte growth, apoptosis and dedifferentiation. The steady-state level of SAMe is regulated by a network of enzymes and altered methionine metabolism contributes to the pathogenesis of ASH. The aim of our study was to identify and to measure the expression pattern of SAMe regulating enzymes in a well-established animal model that shows morphologic features of human steatohepatitis such as Mallory body formation, liver cell necrosis, apoptosis, steatosis, fibrosis and ductular reaction. Methods: Swiss albino mice were fed a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-containing diet and animals were killed after 1 week and 2.5 months of DDC-treatment, as well as after 1 month DDC-free diet (=recovery) and 3 days DDC-refeeding following 2.5 months DDC intoxication. The expression of enzymes involved in SAMe synthesis and degradation were determined by Real-time quantitative RT-PCR. Results: Compared to normal mouse liver, the enzymes MAT1A and Ahcy were downregulated after 1 week of DDC-feeding (2.3 and 2.2 fold), 2.5 months DDC-feeding (3.3 and 2.9 fold) but normalised after administration of a DDC-free diet to mice for 1 month to 2.5 months pretreated animals. In contrast, MAT2A was overexpressed 1.4 fold after 1 week of DDC administration, 1.6 fold after 2.5 months of DDC administration but normalized in the recovery phase. Refeeding mice DDC for 3 days after one month of recovery on a DDC-free diet downregulated expression of MAT1A (2.9 fold) and Ahcy (2.7 fold) but induced expression of MAT2A (2 fold). Conclusion: Our data demonstrate that in the DDC animal model, reproducing pathological features of steatohepatitis, a deregulation of SAMe-metabolizing enzymes occurs. As a switch from MAT1A to MAT2A expression and depletion of hepatic SAMe is also found in human ASH, our findings support the hypothesis that SAMe and subtle interaction of its regulating enzymes play a pathophysiological role in chronic metabolic liver disorders. The biologic significance of this altered expression pattern is unclear but dysbalance in methylation reactions may lead to changes in DNA and histone methylation, thereby influencing gene expression in the affected liver.

PP2-278

IMPACT OF TUMOR SIZE AND VASCULAR INVASION ON SURVIVAL OF PATIENTS WITH SMALL HEPATOCELLULAR CARCINOMA

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BACKGROUND: Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Liver cirrhosis is the major risk factor for this tumor. Screening for HCC in patients with cirrhosis has been recommended, with the belief that detection and treatment of early HCC improves patient survival. The aims of this study were to analyze the overall survival of small HCC in cirrhotic patients and identify independent predictors of survival, in Brazil. METHODS: Between January 1998 and December 2003, seventy-four cirrhotic patients with hepatocellular carcinoma were evaluated satisfying the following criteria: HCC of 3 cm or smaller and a maximum of three lesions. Predictors of survival were identified using the Kaplan-Meier and the Cox model. RESULTS: Mean age was 58 years-old (32-77), 71% of patients were male, 64% had hepatitis C, 60% were Child-Pugh A, mean MELD score was 11 and 79% had portal hypertension. At the time of diagnosis, 71% had one tumor, the size of the main tumor was smaller than 20 mm in 47%, mean AFP level was 131 ng/ml. Three patients had portal vein thrombosis, suggesting vascular invasion. Fifty patients (67.5%) were included in the liver transplant list, but it was performed in only four patients. Tumor resection was possible in four patients. Forty-eight (64.8%) patients received percutaneous treatment (radiofrequency ablation or percutaneous ethanol injection). Nine patients did not receive any cancer treatment. The overall survival rates were 80%, 62%, 41% and 17% at 12, 24, 36 and 60 months, respectively. The mean length of follow-up after HCC diagnosis was 23 months (median 22 months, range 1–86 months) for the entire group. During follow-up a total of 39 deaths related to liver failure or HCC progression occurred. Univariate analysis of the 74 patients showed that MELD score was greater than 11 ($p = 0.016$), Child-Pugh classification ($p = 0.007$), AFP > 100 ng/ml ($p = 0.006$), more than one lesion ($p = 0.041$), tumor diameter > 20 mm ($p = 0.009$) and presence of vascular invasion ($p < 0.0001$) were significant predictors of survival. Cox regression analysis identified vascular invasion (RR = 14.60 - IC 95% = 3.3 – 64.56 – $p < 0.001$) and tumor size > 20mm (RR = 2.14 - IC 95% = 1.07 – 4.2 – $p = 0.030$) as independent predictors of decreased survival. Treatment of HCC was related to increased overall survival. CONCLUSION: Identification of small tumors of up to 2 cm diameter is related to increase survival. Nevertheless, vascular invasion, despite the small diameter of the lesions, is a factor associated with dismal prognosis.

PP2-279

INTRANUCLEAR INCLUSIONS IN THE LIVER: A RETROSPECTIVE STUDY IN 200 LIVER BIOPSIES

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INTRODUCTION: Intranuclear inclusions in liver biopsies are common histopathological findings. Their importance and clinical significance is not clear. Although they may have morphological similarity with the intranuclear glycogen accumulation they have characteristic appearance with clear borders. MATERIAL METOD In order to examine the significance and the intensity of intranuclear inclusions we included 200 cases of liver biopsies examined in 2005 and 2004 in our department to our study (55 Chronic B hepatitis, 49 Chronic C hepatitis, 4 malignancies, 3 PBS, 89 other diagnosis). The clinical information was taken from the pathology archive files. The biopsies that contain less than 3 portal tract or inadequate liver tissue were not included. The biopsies were examined in high power field by two pathologist in order to find existence and intensity of intranuclear inclusions and intranuclear glycogen accumulation. The results were classified according to disease etiology, Grade and stage of the chronic hepatitis according to modified Scheuer system. RESULTS: Intranuclear inclusions were in different sizes with distinct borders, some of them contain homogeneous eosinophilic inclusions. We observed double inclusion in one case. Intranuclear inclusion was observed in 77 (38%) of the cases and 18 (8.5%) was intensive. In viral hepatitis inclusion were observed in 121(60.5%) of the cases, in peritumoral are intranuclear inclusion was observed in 4(25%) of the cases and none of the PBS cases contain intranuclear inclusion. There was grade and stage of 79 of the cases and intranuclear inclusion was observed in 2(50%) of Grade 0, 10(30%) of Grade 1, 18(41.86%) of Grade 2, 3, 0(0%) of Grade 4 and 3(42.85%) of Stage 0, 6(21.42%) of Stage 1, 20(51.28%) of Stage 2, 3, 1(15.66%) of Stage 4 biopsies. Intranuclear glycogen accumulation was observed in 49 (24.5%) of the cases. Intranuclear glycogen accumulation was observed in 22 (28.57%) of cases with and 27 (21.95%) of cases without intranuclear inclusion. CONCLUSION: The existence of intranuclear inclusions is more that expected in liver biopsies. Their incidence is more common in viral hepatitis than other etiological agents. Their existence is not related with inflammation and fibrosis of the liver. There is no relation between intranuclear inclusions and intranuclear glycogen accumulation.

PP2-280
THE SIGNIFICANCE OF PARENCHYMAL CHANGES AND HISTOLOGICAL EOSINOPHILIA IN PREDICTING SUBSEQUENT EPISODES OF ACUTE LIVER GRAFT REJECTION

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Background. It is suggested that centrilobular alterations may be possible indicators of subsequent episodes of acute rejection or early chronic rejection or graft loss. Aim. The purpose of this study was to identify any differences between the histopathological findings in the first rejection episode biopsies in cases with and without subsequent episodes of acute rejection. Methods. We assessed the predictive value of centrilobular necrosis, central vein endothelialitis, pericentral inflammation, hepatocyte ballooning, cholestasis, hepatocyte apoptosis, lobular inflammation, presence of portal eosinophils as well as characteristic portal tract features in the poor response to anti-rejection treatment. The findings in twenty two cases with a single acute rejection episode (Group 1) were compared with those of 23 with multiple episodes (Group 2). Only the first biopsy samples of the latter group were taken into consideration. Results. Time to the first episode of acute rejection, rejection activity index (RAI) were similar (63 vs 62 days RAI: 5.2 vs RAI: 6.1) between the 2 groups. Hepatocyte apoptosis(90% vs 82%), hepatocyte ballooning (77% vs 82%), central vein endothelialitis (59% vs 69%), were common features in both groups (group 1 and group 2 respectively). The incidence of pericentral inflammation, centrilobular necrosis and presence of portal eosinophils were significantly higher in the group 2 (78% vs 50%), (65% vs 27%) and (95% vs 68%) ($p < 0.05$). Conclusion. Patients with pericentral inflammation and centrilobular necrosis during the first episode of acute rejection may be more likely to have subsequent episodes of acute rejection.

PP2-281
CORRELATION OF CAVEOLIN-1 EXPRESSION AND PROFILERATION MARKERS IN HUMAN PANCREATIC CANCER

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Caveolin 1 (Cav-1) the structural protein of caveolae, is recognized as a key switch, functioning either as a tumor supressor or as a promoter of metastasis and tumor cell survival. This study examined the expression level of Cav-1 in different pancreatic tumors. Cav-1 and proliferation markers (Ki67, p53) were determined by immunohistochemistry in 12 pair samples (tumoral/peri-tumoral) from human pacreatic cancer. The labelling index for Ki67 was 20% in tumor tissue specimens, but around 3.5% in their matched peritumoral samples. Staining for P53 was positive in 66% of tumor samples and negative results in the peritumoral samples. A positive expression of Cav-1 was associated with positivity for proliferation markers in tumor and vascular endothelial cells (tumoral tissue). By contrast, in peritumoral samples Cav-1 expression was confined to some endothelial cells. A positive corelation between the proliferative activity and overexpression of Cav-1, in concordance with tumoral grading were noted. Moreover, Cav-1 expression in tumors was in accordance with increased level of serum tumor markers (CEA, CA19.9). Further research may settle if the clinical outcome can be predicted by measuring Cav-1 expression in pancreatic tumors.

PP2-282
PROBLEMS OF DIFFERENTIAL DIAGNOSIS IN PRIMARY AND METASTATIC NODULAR LESIONS OF LIVER: AN ANALYSIS OF A SERIES WITH 169 CASES

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Background: Metastatic tumor/primer liver tumor ratio is 40:1 in Europa-North America, 2.6:1 in Japan. Primer tumors of liver are more common than metastases in Southeast Asia. The aim of this study is to determine metastatic tumor/primer liver tumor ratio in our series and analysis of criterias that could be helpful in differential diagnosis by reviewing nodular liver lesions Method: We have reviewed and analyzed consecutive 189 liver specimens (composed of trucut , wedge or excisional biopsies of liver) with nodular lesions diagnosed at the Cerrahpasa Medical College between 2004 and 2006. Results: 169 of 189 of cases are tumoral lesions. 70(%41) of them are primary liver tumors and 88(%52) of them are metastatic tumors, although 11(%7) of tumors are tumors of unknown origin. 36 of them are hepatocellular carcinomas, 4 of them are cholangiocarcinomas, 2 of them are hepatocellular adenomas, 3 of them are focal nodular hiperplasies and 1 of them is hepatoblastoma. According to origine they are classified as follows: Adenocarcinoma (AC) of colon:27(%14), pancreatic ductal AC: 7(%4), lung AC: 4 (%2), breast: 3(1.5), ovary: 3(1.5), endometrium carcinoma: 1 (%0.5), servix carcinoma: 1 (%0.5); and testis carcinoma: 1 (%0.5), surrenal gland carcinoma: 1 (%0.5), squamous cell carcinoma: 1 (%0.5), liposarcoma: 1 (%0.5). We could not specify the origine of metastases in 21 cases and could not differentiate cholangiocarcinoma from metastatic adenocarcinoma in 11 cases. 13 case with neuroendocrine tumor features are not idendified as primary or metastatic since it is not found any primary after detailed examination in clinically. After a panel of immunhistohemistry including keratin subtypes and endocrine markers, these tumors remaine unclear. Conclusion: The most common metastatic tumor in liver is colorectal adenocarcinoma..This finding is same as in literature. The most important problem in differential diagnosis of nodular lesions in liver is in between primary neuroendocrine tumors and metastatic gastro-entero-pancreatic neuroendocrine tumors and also in between primary cholangiaocarcinoma and metastatic adenocarcinoma. There is no further technique (immunhistochemistry or molecular) that help in this differentiation, so far.

PP2-283
PROBLEMS IN GRADING AND STAGING OF NONALCOHOLIC STEATOHEPATITIS: A STUDY OF 597 CASES BY TURKISH NATIONAL WORKING GROUP OF HEPATOPANCREATOBILIARY PATHOLOGY

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BACKGROUND: Grading and staging system for nonalcoholic steatohepatitis (NASH) proposed by Brunt et al in 1999 was accepted and used widely since then. We experienced many cases of NASH with some histological features of inflammation and fibrosis different from those decribed by Brunt et al. The purpose of this study is to evaluate the histological features of NASH and to determine the differences in Turkish population. METHOD: Totally 597 cases of NASH diagnosed by 14 experienced pathologist from 12 institutions, reevaluated according to the criteria including Brunts' and certain additional features such as grade, type and distribution of steatosis; frequency and distribution of balooning, severity and predominant cell type of lobular inflammation (LI); presence and severity of portal inflammation (PI) and interface activity; presence and extent of perisinusoidal, periportal and bridging fibrosis (PSF, PPF, BF) and presence of cirrhosis. RESULTS: Steatosis was found as Grade I in 180(30,2%), II in 220(36,8%), III in197(33%) of cases. Distribution of steatosis was in zone3 in 202(33,8%), zone1 in 12(2,1%) , azonal in 383(64,1%). Excluding grade III steatosis in which zonality is difficult to determine, distribution was azonal in 47,7% of grade I, 50,5% of grade II steatosis.116(19,4%) of cases showed mixed macro/microvesicular steatosis. Grade of LI was 0 in 21 (3,5%), 1 in 356(59,6%), 2 in 173 (29%), 3 in 47 (7,9%) . LI was polymorph predominant in 70(11,7%), mononuclear predominant in 275 (46,1%), and mixed in 231(38,7%) cases. Mild PI was seen in 312(52,3%), moderate in 110(18,4%) and prominent in 13(2,2%) of cases. PSF was observed focally in 291(48,7%) and diffuse in 120(20,1%) cases; no PSF was present in 186(31,2%) cases. PPF was focal in 220(36,9%), diffuse in 109(18,3%) cases. BF was found in 85(14,2%) and cirrhosis in only 12 (2%) cases. Higher grade of steatosis was significantly correlated with the higher degrees of LI ($p<0.01$) and PSF ($p<0.05$). In 22,6% and 11,8% of cases which had no PSF had focal and diffuse periportal fibrosis, respectively. The most remarkable features were the type of lobular inflammation (46,1% mononuclear, 11,7% polymorphonuclear); location of steatosis (64,2% azonal); presence of periportal fibrosis in the cases with no perisinusoidal fibrosis (34,4%). CONCLUSION: Our results suggest that original (1999) Brunt's NASH classification needs to be revised, and the features such as azonal distribution of steatosis, microvesicular steatosis, mononuclear cell predominancy in lobular inflammation and periportal fibrosis without perisinusoidal fibrosis are worth to be emphasized as seperate parameters.

PP2-284

THE VALUE OF CYTOKERATIN 20 AND 7 IN DETERMINATION OF PRIMARY AND METASTATIC LIVER ADENOCARCINOMAS

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The liver is a very common target of metastatic tumors. At the time of initial presentation, the localization of the primary tumor is frequently unknown. The histological examination of metastatic adenocarcinomas is not sufficient for diagnosis as it may have similar appearance with different primary localizations. However, accurate identification of the site of origin of metastasis has prognostic and therapeutic advantage for patients. Immunohistochemistry has an important role in identifying the primary site. Cytokeratin (CK) 20 and CK7 expression are the most helpful procedures in differential diagnosis. Seventy-two liver specimens (resection material and core needle biopsy) were evaluated (25 cholangiocarcinoma, 28 colorectal adenocarcinoma, 7 pancreatic adenocarcinoma, 6 extrahepatic cholangiocarcinoma, 4 gastric adenocarcinoma, 1 lung adenocarcinoma, 1 breast carcinoma). Primary tumor localization could be established in all. Immunohistochemical algorithm with two monoclonal CK antibodies, CK20 and CK7 was tested on 72 tumor sections. Statistical analysis was evaluated with Fisher's exact test. All metastatic colorectal adenocarcinomas were CK20 (+) (Fisher's exact test, $p<0,001$). The CK20 (+)/CK7 (-) pattern was highly characteristic for this group (23/28, %82). All primary cholangiocarcinomas were immunoreactive with CK7 (Fisher's exact test, $p<0,001$) and the CK20 (-)/CK7 (+) pattern seemed to be specific for cholangiocarcinomas (19/25, %76). Pancreatic adenocarcinomas showed CK20 (+)/CK7 (+) pattern in 6 out of 7 but there were not enough cases for statistical analysis. In conclusion, CK20 (+)/CK7 (-) pattern is highly accurate for the determination of colorectal origin whereas, CK7 (+)/CK20 (-) pattern is characteristic for cholangiocarcinomas.

PP2-285

COMPARISON OF DIFFERENT CHRONIC HEPATITIS SCORES WITH RESPECT TO INTEROBSERVER VARIABILITY: A STUDY OF TURKISH NATIONAL WORKING GROUP OF HEPATO-PANCREATO-BILIARY PATHOLOGY

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Grading and staging of chronic hepatitis (CH) using a scoring system for the semiquantitative assessment of the severity of the histological lesions is still one of the valuable tools in the management of patients with CH. One of the criteria for the best scoring system is good interobserver variability. We carried out a blinded trial to assess the interobserver variability among five experienced hepatopathologists using the Knodell, Ishak, METAVIR systems. Forty-seven liver biopsies from 23 patients with chronic hepatitis C were scored separately by five pathologists on the slides stained with hematoxylin-eosine and routine connective tissue and iron stains. The results were analyzed using ICC (one-way random model), by using SPSS for Windows (version 13.0). P values less than 0.05 were accepted significant. Reliability was accepted good over 0.75, moderate

between 0.50- 0.75 and poor under 0.50. RESULTS: The best interobserver agreement was achieved using the fibrosis score in Ishak's system (ICC: 0.82) which was followed by METAVIR fibrosis score (ICC: 0.76). There was moderate agreement with Knodell fibrosis score (ICC: 0.71). For all scoring systems, the agreement between the five pathologists was poor for the assessment of lobular necroinflammatory activity, where as, it was moderate for interface hepatitis. For portal inflammation, which is not scored in METAVIR system, it was poor for Knodell and moderate for Ishak system. CONCLUSIONS: 1) the interobserver variability is acceptable for the assessment of stage (fibrosis) in the available scores, the most detailed (Ishak system) being the best; 2) the assessment of grade has great interobserver variability in all scoring systems.

PP2-286
COMPARATIVE EVALUATION OF HISTOLOGICAL FINDINGS IN CHRONIC HEPATITIS B AND C

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Background. It is generally agreed that liver biopsy is a very specific diagnostic tool used to assess the nature and the severity of chronic hepatitis. The morphology of the lesions produced by the hepatitis viruses B and C is not very specific and an accurate diagnosis is quite impossible without clinical and serological data. Aim. Comparative analysis of histological modifications in liver biopsy of patients with chronic hepatitis B and C. Material and Methods. We examined 324 consecutive percutaneous needle liver biopsies from patients with clinical diagnosis of chronic hepatitis B (67 cases) and C (257 cases). None of the patients in the study had serological evidence of co-existing viral hepatitis. The cases with histological liver cirrhosis were excluded. All specimens were fixed in formalin, embedded in paraffin, and cut at 5 µm. The slides were stained with haematoxylin and eosin, Masson trichrome, and Gomori reticulin. In order to asses the necroinflammatory activity and the fibrosis we evaluated the components of the Ishak modified histological activity index (HAI). The portal, periportal and lobular inflammation were graded into mild, moderate and severe. The scoring system for fibrosis included: no fibrosis (0); portal fibrosis (1); periportal fibrosis without septa (2); septa formation (3). Additional parameters like bridging necrosis (absent/present), lymph follicles formation (absent/present), cholestasis (absent/present) and steatosis (absent, mild, moderate, severe) were evaluated. Statistic analyses were performed with Winstat computer program. Differences were considered significant when P values were less than 0.05. Results. There were few significant differences between the two forms of hepatitis. Only the portal inflammation, the lymph follicle formation and the steatosis presented significantly higher values in patients with hepatitis C. Conclusion. The etiologic diagnosis of chronic viral hepatitis can not be made on histological bases.

PP2-287
SIGNIFICANCE OF HISTOLOGIC LESIONS OF PLACENTAL MEMBRANES IN DIAGNOSIS OF PLACENTAL HYPOXIA

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Introduction. Features of global chronic placental hypoxia (preplacental, uteroplacental and postplacental) and acute hypoxia (infarctions) are well recognized placenta disc lesions. Only recently the clinical and placental correlations of acute (laminar necrosis) and chronic (microscopic chorionic pseudocysts) placental membrane lesions were recognized. However, the practical significance of hypoxic chorionic disc and placental membrane lesions have never been compared. Materials and Methods. 20 clinical (maternal and fetal) and 35 gross and microscopic placental features were compared ($\chi 2$) among 168 placentae having at least one disc lesion (infarct and/or global ischemia) and at lest one membrane lesion (microscopic chorionic pseudocysts and/or laminar necrosis) (Group 1), 750 placentae with at least one hypoxic disc lesion but no membrane lesion (Group 2), 480 placentae with at lest one membrane lesion but no disc lesion (Group 3), and 3192 placentae without hypoxic villous or membrane lesions (Group 4). Results. 30% of placentae showed histological hypoxic lesions. Of those, hypoxic membrane lesions without hypoxic villous lesions were seen in 30%, and in conjunction with villous hypoxic lesions, in 45% of placentae. Therefore, recognizing placental hypoxic lesions increases the sensitivity of placental examination in diagnosing placental hypoxia by 15%. Two thirds of preeclampsia cases were in Groups 1-3. Preeclampsia, chronic hypertension, abnormal cardiotocography, induction of labour, antepartum bleeding, caesarean sections, perinatal mortality, fetal growth restriction and some placental factors (abnormal coiling/insertion of umbilical cord, decidual arteriolopathy, meconium staining, perivillous fibrin deposition, retroplacental haematoma, erythroblastosis of fetal blood) statistically significantly ($p \leq 0.05$) correlated with hypoxic placental lesions, being in general the highest in Group 1. Two thirds of placentae did not show hypoxic lesions, congenital malformations and acute chorioamnionitis being most prevalent in Group 4. Umbilical cord compromise (clinical) and fetal thrombotic vasculopathy did not show significant distribution between Groups 1-4. Conclusion. Including placental membrane hypoxic lesions in the placental microscopic diagnosis increases the sensitivity of placental examination in the retrospective diagnosis of placental hypoxia. In the overwhelming majority of placentae there are no histological features of placental hypoxia either in membranes or chorionic disc as other types of pathology are more prevalent.

PP2-288
THIN CORD SYNDROME (TCS): A RARE CAUSE OF FETAL INTRAUTERINE DEATH

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Background: Little attention has been paid to the pathologic features of the umbilical cord, which might fatally damage the fetus. We determined the association of hypercoiling (more than 1 coil per 5cm) and thinning with consecutive constriction of the umbilical vessels (thin-cord-syndrome; TCS) and intrauterine fetal death (IUFD). Methods: 303 cases of consecutive fetal autopsies over a five-year period, including spontaneous and induced abortions of the second trimester of pregnancy, were examined using a standardized protocol. Results: The mean maternal age was 28.5 years, the mean gestational age 19.1 weeks

(range: 12.6 – 24.5 weeks). Thirty-six percent of all cases were induced abortions because of congenital malformations, 8.9% resulted from legal abortions, regulated by German law. 167 cases (55.1%) were spontaneous abortion specimens. The leading cause for IUFD in the spontaneous abortion group was an amnion infection (34.7%), followed by abruptio placentae (15.6%). In 25.1%, placental dysmaturity with consecutive placental insufficiency was responsible for IUFD. Pathologies of the umbilical cord as the cause of IUFD were seen in 10.2% of the cases. Most of them (15/17) were cases with thin-cord-syndrome. In 14.4% of all spontaneous abortion specimens the cause of IUFD could not be determined by autopsy. There was an apparent difference in the frequency of thin-cord-syndrome in the spontaneous abortion group (15/167 = 9%) as compared to the non-spontaneous group (2/136 = 1.5%). Conclusions: A remarkably high percentage (17/303 = 5.6%) of all cases showed TCS. In case of spontaneous abortions, TSC was causative for intrauterine death in 9% (15/167). Careful pathologic examination of the umbilical cord is recommended to detect TCS and reduce the cases with unexplained intrauterine death.

PP2-289

IMBALANCE OF MONONUCLEAR CELL INFILTRATES IN THE PLACENTAL TISSUE FROM FETUSES AFTER SPONTANEOUS ABORTION VERSUS THERAPEUTIC TERMINATION FROM WEEK 8TH TO WEEK 12TH OF GESTATIONAL AGE

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Placental macrophages (Hofbauer cells) are located close to trophoblastic cells and fetal capillaries, which make them, perfect candidates for involvement in regulatory processes within the villous core. Their capacity of producing several cytokines and prostaglandin synthesizing enzymes, and expressing vascular endothelial growth factor, indicate possible role in the placental development and angiogenesis in order to support pregnancy. The numbers of cells expressing the various markers of the monocyte-macrophage lineage change throughout pregnancy. In the present study, we investigated the immunohistochemical expression of mononuclear infiltrations in paraffin embedded placentas, from fetuses after spontaneous abortion (8th, 10th, and 12th week of gestational age), and those after therapeutic abortion at the same time, using a panel of monoclonal antibodies for the identification of leukocytes (CD45/LCA), B-lymphocytes (CD20/L-26), T-lymphocytes (CD45RO/UCHL1), CD68 and CD14 cells. Immunologic factors in human reproductive failure are plausible mechanisms of infertility and spontaneous abortion. Approximately 25% of cases of premature ovarian failure appear to result from an autoimmune etiology. The recruitment of immune cells, particularly monocytes and T cells, neovascularization around foci of invading peritoneal lesions, and the possible development of antiendometrial autoantibodies support an immunologic basis of this disorder. Here we describe the detection, isolation and characterization of CD45RO, L26, and CD68/CD14 positive cells from human early pregnancy deciduas. These cells were found in close vicinity to endometrial glands, with preference to the basal layer of the decidua. We conclude that, 1) maternal cells, apparently CD45RO/UCHL1 positive cells, cross the maternofetal barrier and participate in spontaneous (involuntary) abortions, 2) a small proportion of maternal cells (approximately 30%), apparently CD68/CD14 positive cells also cross the maternal fetal barrier and cause growth delay and recurrent reproductive failure. Further investigation of involvement of the intercellular adhesion molecules 1 and 2, platelet endothelial cell adhesion molecule, vascular cell adhesion molecule and E-selectin in leukocyte accumulation will be needed to support the passage of maternal cells to the fetus. The results were statistically significant ($p<0.0001$, student's t-test).

PP2-290

A STROMAL MYOID CELL LINE PROVOKES THYMIC T-CELL IMMIGRATION AT THE SECOND AND THIRD GESTATIONAL TRIMESTERS

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Background: Once lymphoid precursors enter the thymus form the blood stream, they come into contact with thymic stromal cells that guide their maturation into funcionally competent T cells. Thymic myoid cells are one such cell type. They have been described as a regular constituent of the thymus of embryonic and young vertebrates and express muscle proteins including myosin, desmin, acetylcholine receptor (AChR), C-protein, MyoD, troponin T, rapsyn, and utrophin. It has been emphasized recently that the thymic myoid cells play an important role in the protection of thymocytes from apoptosis, and in the process of T-cell differentiation and maturation. The aim of the present study is to provide a quantitative estimation of thymic myoid cells and T-cell population in different stages of development. A probable interaction between these two populations could explain an additional mechanism to the active T-cell migration from the thymus that is a direct contact to a specific myoid cell line. Materials and Methods: Paraffin-embedded specimens from the thymus of forty five human embryos at the first, second and third trimester of gestation respectively, were investigated by conventional histology, and immunohistology for the presence in the stroma of the thymic medulla, of myosin in the myoid cells, and UCHL1 (pan T- cell) antigen in the medullary thymocytes. Results: Our results demonstrated a quantitative difference in the second and third trimester of development concerning the expression of myosin in the stromal myoid cells of the thymic medulla over the equivalent expression of the protein in the first trimester. Similar changes in the above periods were found concerning the population of medullary thymocytes expressing UCHL1 antigen. Conclusions: Our results indicate that: 1) Thymic myoid cells play an important role in the thymic microenvironment as they are well conserved throughout species evolution. 2) The increased population of myoid cells in the medullary area during mid and late gestational age, in comparison with first trimester, probably reflects the increased demand of the growing fetus for mature T lymphocytes. Contractions of myoid cells mediated by their cytoplasmic structural proteins, including myosin which is well preserved during development, might aid the movement of thymocytes expressing UCHL1 antigen, across or out of the gland, suggesting a potential involvement of myoid cells in the thymic function. Further studies on larger series are needed to corroborate this.

PP2-291

BER-H2 (CD30) IMMUNOHISTOCHEMICAL STAINING OF HUMAN FETAL TISSUES

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OBJECTIVE: CD30 antigen has long been considered to be restricted to the tumour cells of Hodgkin's disease and of anaplastic large cell lymphoma as well as to T and B activated lymphocytes. It is now apparent that the range of normal and neoplastic cells, which may express CD30 antigen, is much wider than was at first thought. In order to gain insight into the physiological function of CD30 antigen, we studied the distribution of its expression in the tissues of fetuses from week 8th to week 16th. MATERIALS AND METHODS: We investigated the immunohistochemical expression of CD30 antigen in paraffinembedded tissue samples representing all systems from 30 fetuses after therapeutic abortion at 8th to 10th and 12th to 16th week of gestation, respectively, using the monoclonal antibody Ber-H2. RESULTS: Our results demonstrated that CD30 is expressed early in human fetal development (8th to 10th week of gestation) in several fetal tissues derived from all three germ layers (gastrointestinal tract, special glands of the postpharyngeal foregut, urinary, musculoskeletal, reproductive, nervous, endocrine systems), with the exception of the skin and hematolymphoid system (thymus), in which the antigen is expressed later on (10th week onwards). Expression of CD30 was restricted to the hematolymphoid system in the 12-16 weeks of gestation. No expression of the marker was observed in the respiratory and cardiovascular systems during the entire period examined. CONCLUSIONS: CD30 antigen is of importance in cell development, and proliferation. It is also pathway-related to terminal differentiation in many fetal tissues and organs.

PP2-292
HISTOPATHOLOGIC EVALUATION OF PLACENTAS AND CORRELATION WITH FETAL OUTCOME

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Background: Complete pathologic evaluation of placenta provides valuable information for perinatal care. Placental examination can answer specific questions about in utero insults, improvement of the risk assessment for future pregnancies and ascertainment of newborn risk for long-term neurodevelopment sequelae. Method: We conducted a prospective study of all pregnacies at 28 to 42 weeks of gestational age, delivered at the Department of Obstetrics and Gynaecology of Eskisehir Osmangazi University Hospital from November 2004 to July 2005. All maternal and neonatal records were reviewed. In this study, 329 placentas were examined macroscopically and microscopically by two pathologists. The study groups are classified according to the existence of maternal and fetal risk factors in pregnancies. Placentas from pregnancies with risk factors (n=237) and placentas from normal pregnancies (n= 92) were included in the study. Threehundredtwentynine singleton pregnancies showed significant correlation between the weight of placenta, the weight of newborn and gestational age (P<0.001). When these study groups were compared according to their histopathologic findings, it was found that there were more placental chorioamnionitis (P<0.001), acute and chronic intervillositis (P<0.05), central infarct (P<0.05) and abnormality of villous maturation (P<0.05) in the pregnancies having risk factors and these findings were statistically significant. Histopathologic findings including chorioamnionitis (p<0.001), funisitis (P<0.05), fibromusculer sclerosis (P<0.05), excessive syncytial knots (P<0.05), peripheral and central infarction (P<0.05), perivillous fibrin deposition (P<0.05) and presence of large infarct areas in gross examination (P<0.001) were significantly associated with perinatal morbidity in newborn. Presence or absence of villitis (P<0.001), deciduitis (P<0.05), hemorrhagic endovasculitis (P<0.05), perivillous fibrin deposition (P<0.05), maternal floor infarct (P<0.05), villous edema (P<0.001), nucleated fetal red blood cells in villous vessels (P<0.001) were significantly associated with perinatal mortality in newborn. In this study, the presence of perinatal morbidity, fetal and maternal risk factors were significantly associated with the type of health insurance of pregnant women (P<0.001). Conclusion: This study suggests that our hospital is an important reference center of region for high risk pregnancies and newborns and placental pathology should be a routine component of perinatal diagnosis.

PP2-293
THE EFFECTS OF AIR POLLUTION AND SMOKING ON PLACENTAL CADMIUM, ZINC CONCENTRATION AND METALLOTHIONEIN EXPRESSION

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Background: This study is designed to determine the placental zinc (Zn) and cadmium (Cd) levels in mothers who were smokers, mothers thought to be exposed to air pollution, and non-smokers to reveal the relationship among the expression of placental metallothionein (MT) binding these metals and blood progesterone level. Methods: Placental Zn and Cd levels were measured by atomic absorption spectrometry. Presence of placental MT was determined by immunohistochemically. Placental changes were examined by light microscope after H&E and PAS staining. Immunohistochemical MT staining, of syncytiotrophoblastic and villous interstitial cells were scored as positive or negative. Results: Among the 92 mothers included in the study, 33 were smokers (group I), 29 were exposed to air pollution (group II) and 30 were living in rural areas, not exposed to air pollution and non-smokers (group III). Mean birth weight of their off-springs was 3198.62±380.01 g and mean weight of the placenta was 561.38±111.55 g. In Group I, mean placental Cd and Zn were 0.063±0.022 μg/g and 39.84±15.5 μg/g, respectively, higher than other groups. In Group II, mean placental Cd and Zn levels were higher than those of Group III. Blood progesterone levels of subjects in Group I, (121 ng/mL) was the lowest of all groups. While the mean count of villi was highest in Group III; the highest mean count of syncytial knots was in Group II. Thickening of vasculo-syncytial membrane was most prominent in Group I. Similarly, MT staining was positive and very dense in 72.7% (24/33) of cases in Group I ($p \leq 0.05$). MT staining was positive in 69.0% (29/20) and denser in Group II cases compared to 36% (11/30) in Group III ($p \leq 0.05$). Conclusion: This study showed that smoking increased Cd levels in placenta and accompanied with an increase in placental MT expression immunohistochemically. The effects of exposure to air pollution are equally harmful as smoking related effects.

PP2-294
CHORANGIOSIS: THE POTENTIAL ROLE OF SMOKING AND AIR POLLUTION

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Background: Chorangiosis is considered to be strongly associated with various feto-maternal and placental conditions including pre-eclampsia, diabetes, hypertension, major congenital anomalies, and has been found to correlate with increased fetal morbidity and mortality. In this study, our aim is to investigate the pathologic effects of maternal smoking, and air pollution on the pathogenesis of chorangiosis. Methods: We investigated 94 placentas macroscopically and microscopically over a three months period (March 2006-May 2006) at Denizli State Hospital with a hope to identify the frequency of chorangiosis and potential role of maternal smoking and air pollution. Placental changes were examined by light microscopy after H&E, and immunohistochemical evaluation of CD 34, CD 68, and muscle specific actin were used to confirm the diagnosis. Results: Among the 94 mothers included in the study, 33 were smokers (group I), 31 were thought to have exposed to air pollution (group II) and 30 were living in rural areas without air pollution and maternal smoking (group III). Chorangiosis were found in 16 % (15/94) of all placentas which they were composed of 7/15 (47 %) cases in group I, 5/15 (33 %) cases in group II and 3/15 (20 %) cases in goup III. Vascular changes were found mainly in the smoking and air pollution group. There appeared to be no correlation of these vascular changes with placental weight, parity, gestational age, major congenital anomalies, and maternal factors including diabetes and pre-eclampsia. Conclusion: We present the hypothesis that smoking and air pollution may have a contributory role in the development of chorangiosis. We suggest that chorangiosis may be just an adaptive response to maternal hypoxia and studies addressing the role of smoking and air pollution in chorangiosis may provide new insights into the pathogenesis of this condition.

PP2-295

MATERNAL VASCULOPATHY IN PREECLAMSPIA: TRIAL OF CORRELATION BETWEEN HISTOLOGICAL ABNORMALTIES IN PREECLAMPTIC PLACENTAS AND CLINICAL MANIFESTATIONS

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Background: Preeclampsia (PE) is associated with significant maternal and fetal morbidity and mortality. It is an idiopathic multisystem disorder linked to human pregnancy especially to the placental formation. Material and methods We investigated 129 preeclamptic placentas during a four year period (2003-2006) in order to identify the potential usefulness of the macroscopic and microscopic placental examination in corroboration between placental vasculopathy and clinical manifestations of the disease. This study was done at the department of pathology, Farhat Hached hospital. Only the placentas from preeclampsia complicated pregnancy was included, gestational diabetes and chronic vascular pathologies were eliminated. The diagnosis of preeclampsia was confirmed by the clinical criteria assigned on the ACOG (American College of Obstetricians and Gynecologists) classification.The placental lesions that were studied included maternal decidual vasculopathy, infraction, abruption placentae, intervillous thrombi, subchorionic thrombi, lesions of the stem villi (fibrosis, calcifications, thrombi, obliterative endarteritis) and NIFD (ischemic necrosis with fibrin deposition). Standard statistics including the student t test were used. Results Among women with PE, 16, 3% had severe disease and 70.5% were primigravid women. The gestational ages at the delivery ranged between 26 and 41 weeks of gestation. The frequencies of the placental lesions were respectively, decidual vasculopathy (17.1%), infraction (38.2%), abruption placentae (7%), intervillous thrombi (18.6%), subchorionic thrombi (5.4%), lesions of the stem villi (fibrosis (34.9%), calcifications (0.8%), thrombi (1.6%), obliterative endarteritis (10.1%) and NIFD (10.9%). These finding demonstrate that maternal vascular changes and NIFD which characterize the PE, are found in approximately (28%). The correlation study showed that the maternal vascular changes is significantly correlated with primigravid women (p=0.021) and the rate of intervillous thrombi is increased with lower gestational age (p=0.026). Conclusion A case controle study will be adequate to confirm the placental histological differences induced by the PE and a multivariate statistic analysis should be done in order to correlate the placental microscopic findings to the clinical manifestations of PE.

PP2-296

CORRELATION ANALYSIS OF THE FETO-PLACENTAL PARAMETERS IN CASES OF INTRAUTERINE FETAL GROWTH RETARDATION

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A number of studies suggest that some disproportions and restrictions in fetal and placental growth may lead to various pathologies in adults. The aim of the present research was to identify alterations in correlations between placental and fetal parameters in cases of fetal growth restriction (FGR) at 20-25 weeks of gestation (wg). Methods: 18 cases with FGR and high placenta/fetal weight index (PFI) compared with 20 controls in cases of induced abortions for socio-economic reasons at 20-25 wg. Recorded data included: weights of placenta (PW) and fetus (FW), fetus length (FL); head (Ch), chest (Cch), abdominal (Ca) circumferences, weights of fetal kidneys (KW), liver (LW) and heart (HW), as well as some indices(FPI, FW/FL, LW/PW, HW/PW, KW/PW). The correlation analysis of the morphometric data was performed. Results: FGR group had smaller parameters of FW, FL, Cch, Ch, and Ca. All FGR cases had decreased LW; KW and HW. PW was unchanged at 20-22 wg and increased at 23-25 wg, compared with controls. The negative correlation found between PW and LW/PW, FPI and LW at 20-22 wg and between PW and HW/PW at 23-25 wg. The longer gestation the more new positive strong correlations have discovered within the FGR group. Positive strong correlations discovered between FPI on the one hand and HW, KW, LW, KW/PW on the other hand. Conclusions: the study suggests that the increase in the number and the intensity of correlations between morphometric parameters might represent (a) a discrepancy between the fetal and placental growth at 20-25 wg and (b) alterations in fetal organ adaptation mechanisms, which may result in future pathologies.

PP2-297

ANALYSIS OF PERINATAL MORTALITY UNDER CONDITIONS OF THE EASTERN INDUSTRIAL REGION OF UKRAINE

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National data estimate the rapidly increasing rates of perinatal mortality in the Eastern Ukraine in the past several years. Probability of dying in perinatal period in Lugansk Region, Eastern Ukraine is higher that it can be expected in Ukraine (14, 4‰ compared with 8,3-8,7‰) The aim of the present study was to find out the main causes of the fetus and newborns death in the perinatal period in the industrial region of Ukraine. The statistical data derived from the registers of the Regional Children's Hospital of the City of Lugansk (Eastern Ukraine) for 2000-2006 were analyzed. The results have shown that 426 numbers of perinatal deaths reported in Lugansk region in 2000-2006, included 131 antenatal, 33 intranatal and 262 neonatal deaths. The death cause analysis showed that intrauterine death of the

fetus in the majority of cases resulted from hypoxia caused by acute or chronic placental insufficiency and maternal complications of pregnancy (pre-eclampsia, uterine rupture -18, 5% of cases). The developmental defects (33, 3%) and hypoxia (24, 2%) were responsible for the most cases of intranatal deaths. In 2000-2002, the infection pathology (toxoplasmosis, septicemia, pneumonia, and meningoencephalitis) was the biggest killer in neonatal period, being responsible for almost 27 deaths. The second main cause was cardio-vascular and central nervous systems pathology (25 cases). From 2003 until 2006, number of neonatal deaths from congenital defects (48 cases) exceeded the number of lethal infection pathology cases (23 cases). Conclusions The comparative analysis offered the main reasons for the high perinatal mortality rate in Eastern Ukrainian Industrial region, which include high exposure to environmental hazards, such as the Chernobyl clean-up and industrial pollution of the atmosphere. High rates of congenital defects may indicate the pathogenic impact of the long-term radiation factor on maternal health fetal development.

PP2-298
EXPRESSION FACTORS OF ANGIOGENESIS IN THE PLACENTA

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The purpose of the present research is analysis of change of an expression of the following factors and receptors in a tissue of a placenta in norm and at an eclampsia: VEGF, TSP-1, VEGF-R3, TGFγ-R1. In total 10 placentas pregnant with normal pregnancy (control) and 10 placentas pregnant with an eclampsia are investigated. A delivery carried out by caesarian sections. For carrying out of immunohistochemical reaction with antibodies to VEGF, VEGF-R3, TSP-1 and TGFγ-R1 (1:50, Novocastra) used the standard protocol. At immunohistochemical research of tissue specimens of a placenta it is marked, that expression VEGF-R3 cells of a syncytiotrophoblast of villi and decidual cells of a basal plate was lower at an eclampsia (the area of an expression 4,89±1,01%), than at normal pregnancy (11,7±0,52%, $p<0,05$). At immunohistochemical research of tissue specimens of a placenta it is marked, that expression VEGF cells of a syncytiotrophoblast and decidual cells of a basal plate was authentically lower at an eclampsia (0,58±0,13%), than at normal pregnancy (3,11±0,49%, $p<0,05$). On the contrary, expression TSP-1 was above at an eclampsia (0,61±0,05%), than at normal pregnancy (0,08±0,01%, $p<0,05$). Thus expression TSP-1 was marked in a stroma of villi and in the control is submitted by individual fields of positive reaction in the stromal elements located near to vessels of mediate villi while at an eclampsia had diffuse character and prevailed in stromal elements of terminal villi. Expression TGFγ-R1 was marked mainly in range of syncytial capillary membranes by cells of a syncytial nodes. Thus expression TGFγ-R1 was lower at an eclampsia (0,21±0,02%), than at normal pregnancy (0,96±0,25%, $p<0,05$). The low expression of VEGF and VEGF-R3 in a placenta, and a high expression of TSP-1 around of vessels of a placenta at an eclampsia reflects disorder of balance of proangiogenic and antiangiogenic factors aside the last, that can be the cause of disorder of processes of an angiogenesis in a placenta. Low expression TGFγ-R1 on endothelial cells of a placenta at an eclampsia can testify both to disorder of their function, and about compensatory reaction of these cells in reply to action of antiangiogenic factors. On the other hand, it is possible, that TGFγ-R1 doesn't accept appreciable participation in mechanisms of disorder of processes of an angiogenesis in a placenta.

PP2-299
COMPARISON OF PLACENTAL PTEN AND BETA1 INTEGRIN EXPRESSION IN EARLY SPONTANEOUS ABORTION, EARLY AND LATE NORMAL PREGNANCY

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Background: The objective of this study is to investigate the expression of PTEN and □1 integrin in placental tissues of early spontaneous abortion and first and third trimesters of normal pregnancy. Method: A total of 43 placental tissue samples were evaluated using immunohistochemistry for PTEN and □1 integrin. Group 1 included placental tissues of volunteer termination of normal pregnancy during the first trimester ((5-10 wk gestation). Group 2 included placental tissues of normal vaginal delivery at the third trimester of pregnancy (36-40 wk gestation). Group 3 included placental tissues of pregnancy termination because of spontaneous abortion during the first trimester (5-10 wk gestation). Women with serious systemic disease and anembriyonic pregnancies were not included in the study. Results: PTEN expression of villous trophoblast was decreasing as the pregnancy advanced. PTEN staining of decidual cells was significantly stronger in tissue samples from early spontaneous abortion than in tissue samples from early and late normal pregnancy ($p=0.003$, $p=0.001$, respectively). There was no significant difference between □1 integrin expression of villous trophoblast and decidual cells in three groups. Conclusion: Our findings suggest that altered patterns of PTEN expression may be associated with abortion, but it seems that □1 integrin does not contribute to this process as a signaling protein. Further evaluation is needed highlight this subject.

PP2-300
OSTEOGENESIS IMPERFECTA WITH STRAIGHT BONES

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BACKGROUND The term osteogenesis imperfecta (OI) encompasses a heterogeneous group of heritable connective tissue disorders, all of which are characterised by increased bone fragility leading to susceptibility to fracture and resultant skeletal deformity. The phenotypes range from those that are lethal in the perinatal period to those that are mild, and virtually asymptomatic. This variability has been attributed to a wide ranging array of genetic abnormalities in the genes COL1A1 and COL1A2. Of the four groups, OI type II is characterised by extreme bone fragility leading to intra-uterine or early perinatal death. Affected infants are small for gestational age (dwarfism). Here, we present a phenotypically normal fetus with radiographically diagnosed OI in whom further studies revealed a hitherto unreported mutation in the COL1A1 gene. It is possible that this new mutation is responsible for an unusual phenotype where OI is not associated with dwarfism. METHODS CLINICAL SUMMARY A primigravida presented at 20 weeks gestation. Ultrasound revealed deformity of the limbs together with dilated cerebral ventricles. Termination of pregnancy was undertaken and a severely macerated female fetus was delivered. On post mortem examination there was no evidence of dwarfism, or any other abnormality of the upper or lower limbs on external examination. In view of the ultrasound findings, however, a whole body X-ray was performed together with cytogenetic studies. RESULTS X-ray report: This revealed grossly abnormal appearances, classical of OI with multiple fractures involving all long bones and most of the ribs. The skull showed no ossification of the vault. Genetics report: Normal female karyotype. PCR

examination of genomic DNA revealed a missense substitution in exon 1 of COL1A1. This mutation is predicted to interfere with post-translational processing of the collagen pro-peptide. DISCUSSION The prenatal diagnosis of OI is usually straightforward due to dwarfism detected on ultrasound. In this case however, there was no phenotypical evidence of dwarfism, although a post-mortem X-ray showed the features typical of OI. Genetic studies detected a unique mutation in this case, hitherto not described in OI. It may possibly explain the absence of limb shortening. This case emphasises the importance of performing X-ray examination in all post-mortems on fetuses with ultrasound detected anomalies. It is possible that this case represents a sub-group of lethal type II OI. Unfortunately, loss of cell lines has rendered further detailed studies in this case impossible, but the possibility of other similar cases being detected in future exists.

PP2-301
GENERAL CHARACTERISTICS OF THE PERINATAL AUTOPSIES PERFORMED IN OUR CLINIC IN 2006
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Aim: Perinatal autopsy may reveal the reason in perinatal and neonatal deaths, may increase the accuracy of perinatal diagnostic methods, may help to predict the risk of recurrence, and may guide families in planning future pregnancies. Materials and Methods: In this study 39 cases sent to our Department of Surgical Pathology in UU School of Medicine for perinatal autopsy were evaluated. The gender, reasons of death in utero, abortion, and death after birth were noted. Special attention was paid on whether or not organ anomalies were present. Results: There was a 1.3:1 male to female ratio. There were 4 cases of death after birth, 14 cases of spontaneous death in utero, and 21 cases of medical abortion due to anomalies diagnosed after US scan and/or chromosome analysis. Central nervous system anomalies were the most frequent type of anomaly (n = 23). Conclusion: Perinatal autopsy may help diagnosing the reasons of perinatal death and thus may help prevent such deaths.

PP2-302
HERPESVIRUS INFECTION – APOPTOSIS AND ANGIOGENESIS IN HUMAN PLACENTA
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The leading part in proliferation and differentiation endothelial cells plays the vascular endothelial growth factor (VEGF). Thus proliferate go in parallel with apoptotic changes. The purpose of the present research became studying expression in various parts of human placentas VEGF-R3, p53 and Mcl-1 in conditions of the infectious defeat caused by viruses of family of a herpes (a virus of a simple herpes and a cytomegalovirus). Material and methods: 81 placentas from herpesvirus by an infection, from them with a virus of a simple herpes - 32 supervision, with a cytomegalovirus - 43 supervision, a combination of two viruses - 6 supervision is studied. In control were 20 placentas without herpesvirus infection. Immunohistochemical studying expression VEGF-R3, p53 and Mcl-1 spent on the paraffin sections prepared from samples of placentas. Results: The area expression p53 in syncytiotrophoblast and cytotrophoblast has made in the central part of a placenta 1,40±0,24% (in control - 0,45±0,03%), in peripheral - 1,58±0,55% (in control - 0,39±0,05%) ($p<0,05$). In endothelial cells of umbilical vessels the area expression p53 was 2,28±0,19% (in control - 0,05±0,01%) ($p<0,05$). The area expression Mcl-1 has made in syncytiotrophoblast and cytotrophoblast of the central departments of a placenta 1,87±0,17% (in control - 2,7±0,14%), in peripheral - 1,53±0,02% (in control - 3,7±0,6%) ($p<0,05$). However, in endothelial cells of umbilical vessels the area expression below also has made 1,28±0,31% (in control - 1,16±0,3%) ($p>0,05$). At the same time, the increase in the area expression p53 and decrease in the area expression Mcl-1 in placentas with the chronic subcompensated insufficiency in comparison with placenta with the chronic compensated insufficiency is noted. The area expression VEGF-R3 in syncytiotrophoblast and cytotrophoblast has made in the central departments of a placenta 2,31±0,43% (in control - 3,71%±0,3%), in peripheral - 4,35±0,31% (in control - 5,92%±0,46%) ($p>0,05$). In endothelial cells of umbilical vessels the area expression VEGF-R3 was 2,8±0,1% (in control - 3,1±0,4%) ($p>0,05$). Thus, herpesvirus infection in a placenta leads to formation insufficiency of placenta through strengthening expression p53.

PP2-303
MORPHOGENESIS OF ENDOMETRIUM POLYPS CANCER
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Aims: to study clinical particularities and the structure of the carcinoma arising in polyps of the uterus mucous membrane (EP). The data of the clinical observations were analyzed. Endometrium scraps and the exscised uterus bodies and appendages from 27 patients with histologically proved EP cancer were subjected to common histological and histochemical examinations, the receptors of estrogen (ER) and progesterone (PR), as well as the markers of Ki67 (MIB-1) proliferation and p53, bcl-2 àpoptosis being simultaneously clearly seen. Endometrioid adenocarcinoma (ÅÀ) was diagnosed in 20 of these patients. The patients' age averaged 51,8 years, most of whom had (75,0%) their menstrual cycle keeping. 85,0% of these patients suffered adiposity, 75,0% - had hypertension, 65,0 % - diabetes. In all 20 patients ÅÀ was accompanied with àdenomiosis, uterus leiomyoma and hyperplasia of the ovarium theca. 75,0 % of the patients demonstrated follicular cysts. In 15 cases ÅÀ in Ò1à stage was well differentiated (G1). In 5 patients with Ò1b stage EA was of grade 2 (G2). ÅÀ was found to develop in polyps with diffuse (12) or focal (8) complex atypical hyperplasia. Diffusal (7) or focal (13) simple (6) and complex (14) hyperplasia was seen in surrounding endometrium. The receptors of sexual hormones in epithelium polyps and ÅÀ were exhibited abundantly. As epithelium àtypia grew, the ER content, PR in particular, was noted to decrease. The expression of Ki67, p53 was gradually intensifying, it being best seen in the foci of a moderate differentiation. Serous (SA) adenocarcinoma (G2) of T1c, Ò2 stages was found in 6 women aged 62 – 75 - years - old with the episodes of bleeding during menopausa. The tumor was embedded in adeno-fibrous polyps accompanied by endometrium cyst atrophy. No sings of àdenomatosis and epithelium metaplasia were revealed. PR in the tumor was not revealed either. Poorly positive reaction to ER was demonstrated in some single epithelial cells, SA stroma and in the surrounding polyp. Expression Ki67 and p53 in cells was positively higher, compared to that of in the polyp surrounding epithelium and in àtrophic endometrium, with bcl-2 being reduced. In 1 patient aged 62 was found SA, moderate EA end centre endometrial intraepithelial carcinoma in adeno-fibrous polyps accompanied by the atrophic endometrium. Conclusion: Cancer formed in polyps was morphogenetically different. ÅÀ correspondent to estrogen-dependent neoplasia made up 74,1%. SA (22,2%) was considered to be estrogen-independent carcinoma.

PP2-304
MATURE PLACENTAL TERATOMA CASE REPORT
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Background: We report an an extremely rare case of mature teratoma in placenta.A search of English literature revealed less than 20 cases have been reported until now.
Case: The patient 27-year-old pregnant woman was admitted to our hospital for the delivery. Macroscopical examination of placenta revealed 10x10x6 cm. mass lesion located on fetal/maternal surface of placenta. The placenta was covered with amnion sac but lacks of umbilical cord. Histopathologic examination showed that the lesion was composed of dermal appendages, mature bone, cartilagineous tissue, smooth and striated muscle fibers. There were no immature tissues.
Conclusion: Mature placental teratoma was first reported by Morville in 1925. Pathological findings, histogenesis, differantial diagnosis with review of the literature will be discussed.

PP2-305
PERINATAL AUTOPSY EXPERIENCE OF CELAL BAYAR UNIVERSITY FACULTY OF MEDICINE:
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BACKGROUND: If perinatal autopsy was performed in appropriate conditions it is very beneficial for parents. A good knowledge about frequency of anomalies and limitations of autopsy is very important. This is a perinatal autopsy archive study of our laboratory. METHOD: From 1997 to 2007 April, 152 perinatal autopsy were documanted and classified according to fetus week, anomalies and causes of abortion. From 11 week to one month newborn autopsies were included. RESULTS: 183 fetuses or newborns were sent to our department for autopsy. 31 of 183 were excluded because these are still not reported. 20/152 in term or premature newborns died because of after-birth reasons that were not noticed in utero. Remaining 132 fetuses were aborted during pregnancy for different reasons. Of them, 62 were aborted by drugs because of detected vital anomalies and 70 were spontaneously aborted. 77 cases were male and 64 were female. Sex and week of gestation were not pointed out in perinatal autopsy report in 11 and 9 cases, respectively, as these were small and autolytic fetuses. Placentas were not send in 20 of the 70 spontaneous abortion cases. In the remaining 50, 20 cases showed placental pathological findings which might be the cause of death and 12 cases proved neither fetal nor placental anomalies. 28/70 were extremely autolytic. Five of 152 were multipl pregnancies and 10/152 had umblical cord anomalies. Fetal anomalies were detected in 73 cases. Of these, 4 fetuses reported as hydrops and 2 as cystic hygroma. CVS anomalies in 19/73, skeletal system anomalies in 17/73, CNS anomalies in 42/73, GIS anomalies in 14/73, face anomalies in 11/73, urogenital system anomalies in 18/73, gastrochisis/ omphaleocel in 5/73 and severe respiratory system anomalies in 11/73 cases were detected. Examples of syndromes and chromosome anomalies were Down Syndrome, trisomy13 and trisomy18, Meckel-Gruber Syndrome, Fraser Syndrome, Potter sequence, Dandy-Walker Syndrome, Ellis-vanCreveld Syndrome, Turner Syndrome and Di George Syndrome. CONCLUSION: Most frequent anomalies were seen in CNS and most of them were neural tube defects, followed by CVS anomalies. Although the number of perinatal autopsies in Turkey increases, systematic autopsy can not be performed properly in many centers because of lacking experienced personal, time and genetic examination means. Lack of strong correlation between pathologists and clinicians is an other problem. In spite of all these difficulties, perinatal autopsy may give important information to parents and perinatologist for next time.

PP2-306
EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), HYPOXIA INDUCIBLE FACTOR 1 ALPHA (HIF-1α), TRANSFORMING GROWTH FACTOR-β1 (TGFβ1), AND (TGFβ3) IN GESTATIONAL TROPHOBLASTIC DISEASE
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Background: The aim of this study was to invastigate the relationship between vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β1- TGF-β3) and hypoxia inducible factor 1 alpha (HIF-1α) expression in gestational trophoblastic diseases for clarifying the possible histogenesis. Methods: Twenty-one partial hydatidiform moles (PHM), 19 complete hydatidiform moles (CHM), 13 choriocarcinomas, and 20 nonhydropic spontaneous abortion (control group) were evaluated by means of immunohistochemical tecniques with antibodies to VEGF, HIF-1α, TGFβ1 and TGFβ3. Immunohistochemically, extent of positivity (0%=0, 1-24%=1, 25-49%=2, 50-74%=3, and greater than 75%=4) and intensity (no staining=0, weak staining=1, medium staining=2, and strong staining=3) were recorded. Results: The age range of the patients was 17-88 years (mean age, 27.97±9.28 years). The expressions of VEGF in spontaneous abortion and choriocarcinoma higher than in the PHM and CHM. HIF-1α was strongly expressed in the choriocarcinoma than the other subgroups. Nonhydropic spontaneous abortion (control group), showed the highest TGFβ1 expressions among the case subgroups, followed by PHM, CHM, and choriocarcinoma ($p<0.001$). The expression of TGFβ3 was seen all groups, but its level was maximally expressed in choriocarcinoma. Conclusions: Expression of these molecules and detection of correlation between some molecules showed that the pathogenesis of gestastional trophoblastic disease is a complex mechanis. Highest VEGF, HIF-1α, TGFβ3 expression in choriocarcinoma might be involved in the development of tropoblastic diseases.

PP2-307
HISTOPATHOLOGICAL EXAMINATION OF PLACENTAS WITH ANTIPHOSPHOLIPID SYNDROME
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Antiphospholipid syndrome is characterized by recurrent arterial or venous thromboembolism or pregnancy loss in association with antibodies directed against anionic phospholipids or plasma proteins bound to anionic phospholipids. METHODS: We examined sera and placenta tissue from 6 patients in reproductive age with antiphospholopid syndrome and 6 controls. RESULTS: Positivity for anti-phospholipid antibodies was found mainly against anti-prothrombin antibodies in 32%, and anti-annexin V antibodies in 43.5% women. No significant levels of aPLs were found in 6 controls. Immunohistopathology of placenta (p53) also exhibited some biological changes in the trophoblast presented by apoptotic bodies and necrotic cells in trophoblast, and very few microtrombotization in some intervillous spaces and in placenta vessels. CONCLUSION: Though routine histological examination of placentas in the setting of antiphospholipid syndrome can provide important clinical information in a minority of cases, our study shows the persistence of pathological placental findings consistent with aPLs antibodies as one of the high risk factor of repeated reproductive failure.

PP2-308

A COMPARATIVE EVALUATION OF APOPTOSIS AND GROWTH FACTORS IN FETAL KIDNEYS AND CYSTIC RENAL DISEASES

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Background: In this study, the severity and distribution of transforming growth factor-α (TGF-α), vascular endothelial growth factor (VEGF) expression and apoptosis were evaluated in adult kidney tissue, fetal kidney tissue, autosomal recessive polycystic kidney disease (ARPKD), and cystic renal dysplasia (CRD). Methods: 28 cystic renal diseases (22 CRD, and 6 ARPKD), 15 fetal kidney tissue (5 from each gestational stage), 5 adult kidney tissue samples were analysis immunohistochemically for apoptosis, TGF-α and VEGF expression. Staining of cells was scored as no staining (-), weakly staining (+), and strongly staining (+++) for TGB-α and VEGF. Apoptosis was evaluated by TUNEL method. Results: With the last stages of gestation, the staining severity of TGF-α and VEGF decreased in the proximal tubules of the fetal kidney tissues ($p<0.001$). In all the gestational stages, strongly positive staining was observed in the distal tubules. In collecting ducts and in the epithelial members of the nephrogenic zone, weakly staining was observed. In the indifferentiate mesenchyme of the nephrogenic zone and in the kidney medulla, no staining was observed. In the adult renal tissues, the proximal tubules were weakly stained with TGFα, while the distal tubules and collecting ducts were strongly stained. However, the proximal tubules and collecting ducts were mildly stained with VEGF, while the distal tubules were strongly stained. In cases with RPKD and CRD, larger cyst were faintly stained with TGF-α and VEGF, whereas smaller cysts were intensely stained. In all three trimesters of gestation, the proximal-distal tubules and collecting ducts of the fetal kidney tissues had few apoptosis development. With the last stages of gestation, the rate of apoptosis decreased in the undifferentiated mesenchyme of nephrogenic zone ($p=0.003$), while the rate of interstitial apoptosis increased ($p=0.006$). In the cases with RPBH and CRD, the rate of apoptosis in cysts of larger diameter was higher than that in the cysts of smaller diameter. Conclusions: TGFα has a mitogenic effect on the growth of fetal kidneys especially seen in proximal tubules. Parallelism between VEGF and TGFα expression suggested that these growth factors may play an important role in the renal development. Apoptosis is thought to be important for the management of cell proliferation as are the growth factors in nephron morphogenesis. Our findings indicated that in cystic renal diseases, TGFα, VEGF, and apoptosis have a role in cyst development and growth.

PP2-309

A 10 YEAR RETROSPECTIVE ANALYSIS OF FETAL NEONATAL AUTOPSIES

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AIM AND METHOD: 902 autopsies between 10 weeks of gestation-deaths in first month of life, applied in Cerrahpasa Medical Faculty between 1995-2005 were selected for the analysis. The cases were divided into the following groups: Major abnormalities involving 1 organ system, 2- 3 organ systems, external abnormalities (limbs, face, ear), syndromes, associations, metabolic diseases, infections, neoplasms, cystic hygromas, immune and non-immune fetal hydropses , placental pathologies (infections, ischemic causes, miscallenaous causes and two additional groups. One group was negative autopsies defined as autopsies with no detected macroscopical (internal-external) or histopathological abnormalities. The other group was fetal losses due to intrauterine mechanical factors or complications of prematurity. CONCLUSIONS: The largest group in number was negative autopsies (32%, 293/902). A negative autopsy is itself a diagnosis and is important for follow up of future pregnancies. The second largest group was placental pathologies, infectious subgroup (10%, 94/902). The infections seem to be an important cause of fetal losses. It rises to 15% (133/902)together with infections of fetus. The largest subgroup having 1 major abnormality was fetuses with central nervous system (CNS) abnormalities (9.5%, 86/902). Another notable finding was the frequent involvement of renal system in two major abnormalities group (84%, 16/19). Abnormalities of other organ systems were carefully looked for when renal abnormality was detected in screenings. 1/3 percent of single umbilical artery cases are accompanied by CNS abnormalities. That is why a careful search is needed for additional CNS abnormalities on single umbilical artery cases.

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Gynecopathology

PP3-1
BILATERAL SERTOLI -LEYDIG TUMOR OF THE OVARY WITH RETIFORM PATTERN

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Background: Sertoli-Leydig tumor of the ovary is rare, representing less then 1 % of all ovarian tumors. The mean age of patients is usually 25 years, and is usually unilateral. The retiform pattern is seen in young patients and malignancy depend on differentiation at histopathological examination. Method: We report a case of 35 year-old woman that consulted in emergency for an acute abdominal pain. Results: Radiological investigations showed a right ovarian tumor. The patient underwent a right ovariectomy with biopsy of the peritoneum. Histological examination concluded to a Sertoli-Leydig tumor with a retiform pattern; One year later, the patient presented with a left ovarian mass. Microscopically, the mass was also a Sertoli-Leydig tumor with a retiform pattern. Clinical outcome was favourable with no local recurrence two years after. Conclusion: Sertoli-Leydig tumor of the ovary is rare and is usually unilateral; malignancy is based on the degree of differentiation at histopathological examination.

PP3-2
PRIMITIVE NEUROECTODERMAL TUMOR OF THE CERVIX: A CASE REPORT

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Background: Primitive neuroectodermal tumor of the cervix is extremely rare in this location, raising for the pathologist differential diagnosis difficulties. Immunohistochemistry is of great value to lead to the correct diagnosis. Method: We report a case of 50 year-old menopausal Tunisian woman who presented with vaginal bleeding. Physical examination revealed a 2,5 cm cervical exophytic mass. Results: The specimen biopsy showed a round small cell malignant proliferation. Diagnosis of primitive neuroectodermal tumour of the cervix was retained after immunohistochemical results (positivity of mic-2 : CD 99). The patient underwent a radical surgical treatment followed by adjuvant chemotherapy. Clinical outcome was marked by local recurrence 18 months later and the patient died 6 months later. Conclusion: Primitive neuroectodermal tumor of the cervix is uncommon, raising diagnostic difficulties. Its prognosis is usually unfavourable.

PP3-3
OVEREXPRESSION OF LAMININ-5 GAMMA 2 CHAIN IN CLEAR CELL CARCINOMA OF THE OVARY

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Background: One of the characteristic microscopic features of ovarian clear cell carcinoma (CCC) is the densely hyaline basement membrane material expanding the stroma. The biological significance of this material, however, has remained unclear. Recent studies have shown that laminin-5 (LN-5), a major component of the epithelial basement membrane, plays a more active role in cell migration or tumor invasion. Method: Twenty-five surgically resected CCCs were examined for LN-5 expression immunohistochemically, using an antibody against LN-5 gamma 2 chain. For comparison, 5 borderline clear cell tumors, 10 serous adenocarcinomas, and 10 endometrioid adenocarcinomas were examined. Three CCC cell lines were analyzed in vitro for migration over excessive recombinant LN-5, with or without function-blocking antibody against integrin α3. Results: All of the 25 CCCs showed a focal or diffuse immunoreactivity with the LN-5 gamma 2 chain in the tumor stroma; whereas, borderline clear cell tumors, serous or endometrioid adenocarcinomas rarely showed a stromal immunoreactivity. Cytoplasmic accumulation of the LN-5 gamma 2 chain was far less common than stromal accumulation, suggesting an accelerated secretion in CCC. In vitro, CCC cell lines showed a significant increase of cell migration over excessive LN-5, and the migration was blocked by an antibody against integrin α3. Conclusion: The stromal accumulation of the LN-5 gamma 2 chain is common in ovarian CCC but rare in borderline clear cell tumors or other ovarian carcinomas. It is indicated that an interaction between CCC cells and extracellularly accumulated LN-5 is responsible for cell migration and the subsequent stromal invasion of CCC.

PP3-4
REDUCED EXPRESSION OF THE METASTASIS SUPPRESSOR KAI-1 IN UTERINE TUMORS OF EPITHELIAL AND STROMAL ORIGIN: CORRELATION WITH P53 STATUS

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Background: The ability of tumor cells to metastasize often requires additional genetic changes, which result in activation and inactivation of metastasis stimulating and metastasis suppressor genes. Recently KAI1 has emerged as a tumor-suppressor and more specifically as a metastasis suppressor. A loss of KAI-1 expression is associated with the advanced stages of many human malignancies and results in the acquisition of invasive and metastatic capabilities by tumor cells. The underlying mechanisms responsible for this down-regulation of KAI-1 expression remain to be resolved. Method: In the present study, immunohistochemistry and Western blot analysis were performed on uterine tumors, hyperplasias and normal endometrium to investigate the expression pattern and cell-type specific localization of KAI-1 and to correlate it with the expression of p53 and histological and clinical data. Result: 29 (69.04%) of 42 endometrial carcinomas showed reduced or absent KAI-1 expression, which correlated with strong expression of p53 ($P < 0.001$). 13 tumors (30.95%) of 42 demonstrated a moderate score (4-7) of Kai-1 expression. It is interesting that these carcinomas were all well-differentiated tumors of malignancy grade G1/G2, which showed low expression of p53. There were significant correlations between KAI-1 expression and histological type, tumor grading and clinical stage ($P < 0.001$). Most of the investigated sarcomas of the uterus (including 5 carcinosarcomas, 8 endometrial stromal sarcomas and 2 leiomyosarcomas) were clearly negative for KAI-1, whereas these tumors displayed a strong immunostaining for p53. Conclusion: In conclusion, our results demonstrate a strong inverse correlation between KAI-1 and p53 expression and it thus seems, that reduced KAI-1 expression possibly as a result of disregulated/mutated p53 gene could be an important step in uterine carcinogenesis.

PP3-5
COMPARION OF MICROIMMUNOFLUORECENCE, ELISA, RAPID DETECTION KIT (DIMA) AND GIMENEZ STAINING FOR DETECTION OF CHLAMYDIA INDUCED CERVICITIS

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Backgroud: Chlamydia trachomatis is one of the most prevalent causative agent of STD. It causes a variety of genital tract complications such as urethritis, cervicitis, endometritis, epididymitis, and lymphogranuloma venereum. The prevalence rate of Chlamydia cervicitis is varied in different societies. In a recent study, the rate in sexually active American women was in the range of 5-15%. The purpose of this study was to evaluate the prevalence rate of Chlamydia induced cervicitis in Iranian women, and to compare the efficacy of different detection techniques. Method: A total of 137 women with cervicitis were admitted for this study. Two endocervical swabs were obtained. One was used for the rapid Dima test, and the other swab was subjected to Gimenez staining. Blood samples were also obtained for serological tests. Results: ELISA indicated that 18 patients had positive IgG antibody levels in their blood, and 4 of them had IgM antibodies against Chlamydia trachomatis. Ten patients had significant IgG levels and 3 of them had anti-Chlamydia IgM according to the MIF test results. Dima rapid detection test was able to show positive results for only 5 patients. We were not able to detect any Chlamydia inclusion bodies with direct microscopy after Gimenez staining. Conclusion: The MIF technique which is generally considered to be the Gold Standard serological detection method, showed the prevalence rate of Chlamydia cervicitis to be 7.2%. There was no statistically significant difference in IgM titers detected by ELISA and MIF methods. But there was statistically significant different IgG titer rates between ELISA and MIF. Therefore, it is suggested that any ELISA positive IgG titer samples be rechecked and reconfirmed by MIF. Due to the lack of any Chlamydia inclusion body detection by direct microscopy, Gimenez staining is not recommended as a diagnostic tool.

PP3-6
ACCURACY AND RELIABILITY OF FROZEN SECTION DIAGNOSIS IN A SERIES OF 183 OVARIAN TUMORS

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Objective: Frozen section is an important diagnostic tool in determining the nature of ovarian masses. However, it still has some pitfalls. This retrospective study was conducted to determine the accuracy of frozen section diagnosis of ovarian masses and to discuss the type and the reasons of inaccuracies associated with this procedure. Methods: From January 2002 to April 2005, 183 ovarian specimens were examined by frozen section. The frozen section results were compared with the final diagnosis in paraffin sections. The sensitivity (Se), specificity (Sp) and predictive values of frozen section diagnosis were calculated. The 95 % confidence interval of each parameter was determined. Results: Ovarian masses accounted for 11,1 % of all specimens examined extemporaneously. The frozen section diagnosis was benign in 85,8 % of the cases, borderline in 3,8 % and malignant in 7,7 %. The frozen section diagnosis was deffered to permanent sections in 2,7 % of all cases. Overall diagnostic agreement was 96,6 % (Kappa=0,85). Six cases (3,4 %) were incorrectly diagnosed by frozen section. There was one false-positive and five false-negative cases. All of them were due to erroneous interpretation. The majority of the cases of disagreement were mucinous and borderline tumors. The Se for benign, borderline and malignant tumors were 99,3 %, 66,7 % and 87,5 % respectively. The Sp for benign tumors was 80 %, for borderline tumors 99,4 % and for malignant tumors 100 %. The positive predictive value was 96,8 % for benign tumors, 85,7 % for borderline tumors and 100 % for malignant tumors. Conclusion: Frozen section diagnosis seems to be a reliable method for the surgical management of patients with ovarian masses with a high sensitivity for malignant tumors and a low false-positive rate. However, diagnostic problems can occur in mucinous and borderline tumors wich require extensive sampling.

PP3-7
OVEREXPRESSION OF COX-2 AND HER-2/NEU IN PAGETS´S DISEAE OF THE VULVA AND THE BREAST

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Background: Paget´s disease (PD) of the breast as well as the vulva is a rare condition that accounts for about 4% of breast neoplasms and 1% of vulvar malignancies. Recurrent disease after breast and vulvar surgery might be a challenge. To evaluate therapeutically relevant molecules, tissue from mammary and vulvar PD lesions was investigated immunohistochemically. Methods: Histopathologic samples from eleven patients with mammary PD and eight patients with vulvar PD were stained with antibodies against estrogen and progesterone receptors, HER-2/neu and COX-2 followed by semiquantitative evaluation of the staining results. Results: All tested mammary lesions as well as seven out of eight vulvar PD were negative for estrogen and progesterone receptor. Strong membranous staining for HER-2/neu (Score 3) was seen in all cases. Six out of eight vulvar and ten out of eleven mammary PD showed COX-2 overexpression. Conclusions: PD of the breast and the vulva is not under hormonal control of estrogens and progesterons, therefore, anti-hormonal therapy is not indicated. The high frequency of Her-2/neu and COX-2 overexpression, however, suggests that these molecules could be therapeutically relevant in patients with PD. These results require further investigation.

PP3-8
HIGH FREQUENCY OF LYMPHOVASCULAR INVASION IN EARLY ADENOCARCINOMA OF THE UTERINE CERVIX

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Abstract Backgrounds: The prognostic factors of adenocarcinoma of the uterine cervix were said to be the stage of disease and lymph node metastasis. The incidence of lymph node metastasis was reported to be significantly higher in the patients with adenocarcinoma than in those with squamous cell carcinoma of the uterine cervix even in stage Ib. The other report said that the risk of nodal metastases is higher in the patients with cervical adenocarcinoma showing stromal invasion over 5mm in depth. These studies suggested that lymphovascular involvement increased in the early stage of adenocarcinoma of the uterus. Methods: There were eight patients who underwent radical hysterectomy and bilateral salpingo-oophorectomy in 2000-2006 at Sanikukai Hospital in Tokyo. The average age of the patients was 47.1 years (36-75 in range). One or two specimen were selected for the following examinations. Each specimen was stained with Hematoxyline-Eosine and Elastica-van-Gieson. Immunohistochemical staining was also performed for anti-D2-40. Results: Histological type of the eight patients was four

endometrioid type, two endocervical type and two adenocarcinoma in situ. Stages of eight patients with cervical adenocarcinoma showed that 4 cases of stage Ib2, two cases of stage Ib1 and two cases of adenocarcinoma in situ. Three cases of four Ib2 cases showed prominent lymphatic invasion. Two of the four Ib2 cases revealed vascular invasion. Lymph vessel invasion was found on the peripheral area of the tumor, with the background of markedly lymph vessel proliferation. On the other hand, both stages of Ib1 and adenocarcinoma in situ did not show any lymphovascular involvement. No lymph nodes metastasis was found in all of the eight cases. Conclusion: Lymphovascular invasion was frequently observed in adenocarcinoma of the uterine cervix stage Ib2 even in the condition of no lymph nodes metastasis.

PP3-9

LYMPHANGIOLEIOMYOMATOSIS IN THE FEMALE GENITAL TRACT

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Background: Lymphangioleiomyomatosis (LAM) is a rare, multisystem disorder of unknown etiology that affects exclusively young women and characterized by proliferation of abnormal smooth muscle-like cells (LAM cells). LAM is usually recognized of its pulmonary manifestations characterized by cystic destruction of the lung parenchyma due to proliferation of LAM cells. LAM cells can also involve the extrapulmonary organs including the lymphatic system. However, through analysis of female genital tract in patients with LAM has not been reported. Method: Six cases with LAM involving the female genital tract (3 surgical specimens and 3 autopsy cases) were collected from archives of the Department of Humanpathology at Juntendo University. A uterus was cut from cervix to fundus to identify the LAM lesions (average, 26.4 specimens per case; range, 2-78). Adnexa were serially cut from isthmus to fimbria at 5-mm thickness, including ovary and mesovarium (average, 16.4 specimens per case; range, 2-30). Retroperitoneal lymph nodes were obtained from 5 cases. Immunohistochemical staining was carried out using monoclonal antibodies against α-smooth muscle actin (α-SMA, dilution 1:200, Dako Cytomation, Carpinteria, CA), HMB45 (dilution 1:50, Dako Cytomation), Flt-4 (VEGFR-3,dilution 1:50,R&D Co Ltd., Minneapolis, MN), and CD10 (dilution 1 : 200, Novocastra Laboratories Ltd., New Castle, UK). Results: 1) Patients' age ranged from 25 to 83 years (mean 41.3 years) when the specimens were obtained. Two patients had tuberous sclerosis complex (TSC). 2) All cases showed LAM lesions in the adnexa and the retroperitoneal lymph nodes, while four of 5 cases did them in the uterus. 3) LAM lesions were detected in the serosa and/or subserosa of the uterus in all cases. However LAM lesions were not identified in the endometrium. 4) Proliferating patterns of LAM cells in the uterus were categorized into two types: nodular and diffuse type. All of sporadic LAM patients revealed nodular type and two TSC-LAM patients did diffuse type of uterine LAM lesions. 5) LAM cell clusters (LCCs) in the lymphatic vessels were detected in all cases. Conclusion: The anatomic distribution of LAM in the female genital tract is similar to that of metastatic cancers to the uterus, and LAM lesions are accompanied by LCCs in the lymphatic vessels in all cases. These results suggest that LAM lesions in the female genital tract may be disseminated from the extragenital tract through lymphatic vessels.

PP3-10

COX- 2, HER- 2/NEU AND HORMONE RECEPTOR ANALYSIS IN PRIMARY PAPILLARY CARCINOMA OF THE PERITONEUM

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Background: Primary peritoneal carcinomas (PPC) are rare but aggressive malignancies. Evaluating therapeutic relevant molecules PPC were investigated immunohistochemically. Methods: 26 PPC were stained with antibodies against estrogen and progesterone receptors, HER-2/neu and COX-2 with semiquantitative evaluation of the staining results. Results: Twenty one out of the 26 PPC (80.8%) were negative or showed only weak expression for estrogen receptor. One half of the tumors showed moderate or strong positivity for progesterone receptor. Only three PPC (11.5%) showed strong immunoreaction for HER-2/neu (Score 3). Nearly two thirds of the PPC represented COX-2 overexpression. Conclusions: PPCs are probably not under hormonal control of estrogens and lack HER-2/neu overexpression in the majority of cases. So, anti-hormonal or antibody therapy with herceptine is not indicated. The use of COX-2 inhibitores might be helpful in PPC.

PP3-11

ALTERATION OF CELL-CYCLE REGULATORY PROTEINS AND TUMOR BIOLOGY OF SMALL CELL NEUROENDOCRINE CARCINOMAS OF THE UTERINE CERVIX

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Background: Small cell neuroendocrine carcinomas (SmCC) of the uterine cervix are rare tumors. The knowledge regarding protein-expression of several checkpoint candidates of cell-cycle-regulation, their proliferative activity and the prognostic impact of only focal neuroendocrine differentiation is limited. So, SmCC were identified from our files and studied immunohistochemically with HPV-analysis. Methods: Immunohistochemical anlysis consisted neuroendocrine markers, p53, p16, p14. pRb-1, cyclin D1 and Ki-67. PCR-analysis, using general primers was performed for HPV-analysis. In cases with mixed tumors, the percentage of SmCC-component was calculated and correlated with survival. Results: Nine out of 677 tumors (1.3%) were classified as SmCC after Grimelius-staining and immunohistochemistry for NSE, chromogranin A, synaptophysin and CD 56. Two SmCC`s represented p53- and one case p14-positivity. Cyclin D1-staining was completely negative. All buat one SmCC were negative for pRb-1. All cases showed strong nuclear and/or cytoplasmic p16-immunostaining. Seven tumors represented HPV-positivity for high risk types. Four patients died of the tumor after a median time of 36.7 months (range 15 to 56 months), representing a 5-year-survival-rate of 56%. Even a SmCC-component of 17% was associated with fatal course in mixed cases. SmCC represented significant lower proliferation (Ki-67 labeling-index) than the non-small-cell

component in the same tumor (12.8 vs. 70.8%; p<0.001). Conclusions: The overexpression of p16 in all cases but a loss of cyclin D1 and pRb1 in the majority of cases, might indicate a widespread disruption of the Rb-pathway which could be responsible for the aggressive course of SmCC. Even a small SmCC-component in mixed carcinoma of the uterine cervix was associated with adverse outcome. Proliferative activity, determined by Ki-67 labeling index, is of no prognostic value.

PP3-12
ASSOCIATION OF P63 WITH TUMOR CELL DISSOCIATION AND JUXTATUMORAL STROMAL REMODELLING IN CERVICAL CARCINOMA

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Background: p63 (syn. KET and p51) is located at the short arm at chromosome 3 (3q27-29) with tumor suppressive and oncogenic properties. Its overexpression has been reported in carcinomas of the head and neck and the lungs. The present study evaluates p63-expression on carcinoma of the cervix uteri in correlation to tumor-stromal interaction. Methods: Paraffine embedded tumoral tissue from 140 patients with cervical carcinoma FIGO stage III and IV, treated with radiation therapy, were examined immunohistochemically. Staining results were evaluated using an immunoreactive score (staining intensity (1-3) x percentage of positive stained nuclei). The score values were compared to histologic tumor type, tumor grade, pattern of invasion and grade of juxtatumoral stromal remodelling (i.e. desmoplastic change). Results: Squamous cell carcinomas showed more often p63-expression than adenocarcinomas (p=0.0001). Poorly differentiated tumors (grade 3) represented a reduced p63 expression (p=0.001). Carcinomas with high tumor cell dissociation (characterised by spray-like pattern of invasion) and those with strong peritumoral stromal reaction were also associated with a loss of p63-expression (p<0.02 and p=0.074). There was no correlation between p63-expression and response to radiation therapy nor to overall survival. Conclusions: p63 is associated with high tumor cell dissociation and strong remodelling of juxtatumoral stroma in carcinoma of the cervix uteri. But, the mechanism how acts p63 in the context of the alteration of tumor cell adhesion and stromal remodelling is not well understood at time.

PP3-13
PATTERN OF INVASION (TUMOR CELL DISSOCIATION) AS PROGNOSTIC FACTOR IN CARCINOMA OF THE UTERINE CERVIX

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Background: Different patterns of invasion (representing different grades of tumor cell dissociation) are associated with prognostic outcome in cancer. We evaluated the prognostic value of different patterns of invasion (PI) in cervical carcinomas (CX). Methods: 611 surgically treated CX (FIGO IB to IIB) were re-evaluated histologically regarding the PI, using a three-level scoring system. Closed PI was defined as cohesive growth with well-delineated (pushing) borders. In finger-like PI the tumor grows in solid cords/trabecles. Highly dissociative growth in small groups or single cells was defined as spray-like PI. Types of PI were correlated to tumor stage, histo-morphologic factors and prognostic outcome. Results: 60% of the tumors showed a spray-like PI, 30% a finger-like PI, and only 7.4% were of the closed type. Spray-like PI showed a significant correlation with advanced stage disease, lymphovascular space involvement, poorly differentiated tumors and pelvic lymph node metastases. Spray-like PI was accompanied by a reduced 5-year overall survival when compared to the finger-like and closed PI (68.7% vs. 80.9% vs. 88.5%; p=0.0004). The prognostic impact of the PI disappeared in node-positive patients (p=0.06), but persisted in patients without pelvic lymph node disease (p=0.03). In multivariate analysis, using COX-regression model, the PI represented as independent prognostic factor. Conclusions: Spray-like PI, (i.e. highest degree of tumor cell dissociation) is associated with advanced tumor stages, increased rate of recurrency and a reduced overall survival. In separate analysis of patients with and without lymph node metastases, the impact of PI persisted only in node-negative cases as an prognostic factor.

PP3-14
SURVIVIN EXPRESSION IN THE MANAGEMENT OF ENDOMETRIAL CANCER PATIENTS

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Background: Survivin is a member of the inhibitor of apoptosis (IAP) family and is also involved in the regulation of cell division. Our objective was to study survivin expression in endometrial cancer and its correlation to clinicopathological parameters. Materials and Methods. A series of 110 cases of primary untreated endometrial carcinoma hosts were studied. Survivin immunoreactivity was assessed by immunohistochemistry using a polyclonal antibody, and evaluated semiquantitatively according to the percentage of cells demonstrating distinct nuclear or diffuse cytoplasmic staining. Correlation was made with tumor stage, grade, myometrial invasion, and histologic type. Association with disease outcome was also investigated. Results. Survivin overexpression, indicated by nuclear and cytoplasmic staining of at least 10% of the tumor cells, was found in 47 (42.7%) cases. A statistically significant association was demonstrated between survivin overexpression and FIGO stage (p=0.048), histological grade (p=0.024) and myometrial invasion (p=0.021). No significant association was found with histologic type of the tumor (p=0.164). Interestingly, patients with survivin positive tumors had significantly shorter survival rate (p=0.048). Conclusions. Survivin is a specific marker of endometrial cancer and strongly correlates with tumor stage, grade and myometrial invasion. It is also an independent prognostic indicator of poor outcome.

PP3-15

PROGNOSTIC SIGNIFICANCE OF CYTOPLASMIC EXPRESSION OF C-erb-B2 IN ENDOMETRIAL CARCINOMAS

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Overexpression of the epidermal growth factor type II receptor c-erb-B2 has been associated with resistance to chemotherapy and poor survival in several human tumors. The aim of this study was to investigate the expression of cerbB2 in endometrial cancer with attention both to membranous and cytoplasmic staining, to study its correlation to established clinicopathological parameters, and to elucidate the significance of cytoplasmic signaling. Tumor tissue from 110 patients with primary untreated endometrial carcinomas was available. C-erb-B2 reactivity was assessed by immunohistochemistry using a polyclonal antibody, and evaluated semiquantitatively according to the percentage of cells demonstrating membranous or diffuse cytoplasmic staining. Correlation was made with tumor stage, grade, myometrial invasion, and histologic type. Association with disease outcome was also investigated. C-erb-B2 overexpression, indicated by membranous and cytoplasmic staining of at least 10% of the tumor cells, was found in 47 (42.7%) cases. Cytoplasmic expression of c-erb-B2 was observed more frequently than membranous (69.09% versus 5.5%), and was correlated to established clinicopathological parameters. Synchronous cytoplasmic and membranous signaling was noticed in 7.9% of cases. A statistically significant association was demonstrated between c-erb-B2 overexpression and FIGO stage (p=0.048), histological grade (p=0.024) and myometrial invasion (p=0.021). No significant association was found with histologic type of the tumor (p=0.164). Interestingly, patients with c-erb-B2 positive tumors had significantly shorter survival rate (p=0.048). These results indicate that c-erb-B2 is a specific marker of endometrial cancer and strongly correlates with tumor stage, grade and myometrial invasion. It is also an independent prognostic indicator of poor outcome. C-erb-B2 antibody demonstrated a similar cytoplasmic and membranous staining. Cytoplasmic staining is as important as membranous staining and is a specific finding as well.

PP3-16

IMMUNOHISTOCHEMICAL EXPRESSION AND CORELATION OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) AND MIB1 IN HYPERPLASTIC AND NEOPLASTIC ENDOMETRIUM: POSSIBLE PROGNOSTIC VALUE

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Background: Endometrial neoplasia describes a spectrum of proliferative disease that constitues both a morphologic and biologic continuum beginning with mild hyperplasia and ending with poorly differentiated adenocarcinoma. The proliferative activity of a hyperplasia and tumor has long been considered to bear a relationship to its clinical course. The main goal is to examine immunohistochemical expression of proliferation indices (PCNA and MIB1) in hyperplastic endometrium and endometrial adenocarcinoma and to identify possible prognostic value one of them. Methods: We conducted a retrospective study of 60 patients who had simple (n=20), complex (n=20) endometrial hyperplasia with or without atypia and endometrioid adenocarcinoma (n=20). The archival paraffin blocks from the uterine specimens were recut and assessed for histologic reexamination and PCNA and MIB1 immunostaining. Results were statistically analyzed with respect to marker expression and localization to the stromal or glandular component. Results: The PCNA index in the all forms of hyperplasia and adenocarcinoma was higher than MIB1 index (p< 0,0001; CI: 95,1%, median difference 4,5) in both, stroma and glandular components. There were no significant differences (one-factor analysis of variance [ANOVA]) in PCNA expression in hyperplastic endometrium and endometrial adenocarcinoma (F=0.48; P= 0 62; df =2,57). The MIB1 score was significantly lower and more heterogeneous in different hyperplastic and neoplastic lesions. Statistically significant difference (Scheffe test) was found in MIB1 immunoreactivity between simplex and complex hyperplasia (p= 0,0006), and complex hyperplasia and adenocarcinoma (p<0,0001), while there is no difference between simplex hyperplasia and adenocarcinoma (p=0.84). Conclusion: PCNA has a higher but constant expression in endometrial carcinoma and in hyperplasia than MIB1. These findings support that MIB1 may play a role in endometrial carcinogenesis, and that MIB1 index seems to be a better and significant prognostic parameter in hyperplastic endometrium than PCNA.

PP3-17

A STUDY COMPARING EFFECTS OF INTRAUTERINE LEVONORGESTREL AND SYSTEMIC MEDROXYPROGESTERONE AS TREATMENT FOR ENDOMETRIAL HYPERPLASIA. ASSESSMENT BY LIGHT MICROSCOPY AND MORPHOMETRY

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Background: Endometrial carcinoma, showing increasing incidence in the Western world is generally developing through endometrial hyperplasia. Correct diagnostics and optimal treatment of endometrial hyperplasia is therefore of great importance. The female sex steroid hormone progesterone and different synthetical mimics, progestins, have shown a potent antiproliferative effect in the human endometrium. This effect has been utilized in clinical regimens in the treatment of endometrial proliferative disorders, endometrial hyperplasia as well as endometrial cancers. According to the literature no standard therapy regimen for progestine has been agreed upon in treatment of endometrial hyperplasia. To investigate if local application of the levonorgestrel impregnated intrauterine device was a better therapy for endometrial hyperplasia (EH) compared to per oral gestagen treatment, two patient groups were compared. The diagnostic evaluation was based on subjective (WHO criteria) and objective (prognostic data-based morphometric and stereological method/ D-score, predicting the risk of cancer development for each single patient) evaluation. Method: Women between 30 and 70 years with EH and D-score>0 were treated with levonorgestrel impregnated intrauterine device (n=26) and the results compared to a historic group of women treated with

per oral gestagen (n=31). In both treatment groups only patients with low risk (D-score >1) and uncertain risk (D-score 0-1) of cancer development were included. Endometrial specimens were investigated prior to treatment and after 3 months of therapy. The endometrial samples from the two groups were examined by light microscopy and objective databased morphometry to assess tissue characteristics and to evaluate nuclear size variation. Results: After 3 months all patients treated with levonorgestrel intrauterine device showed regression of hyperplasia, whereas, 14 of 31 patients in the per oral group still had persisting disease. Morphometric analysis showed reduction in nuclear size for both treatment groups, including the D-score >1 as well as the D-score 0-1 patients. However, the reduction was most obvious for the levonorgestrel intrauterine device treated patients with initial D-score of 0-1. Conclusion: The present study indicates that levonorgestrel intrauterine device is a superior alternative to per oral treatment of endometrial hyperplasia. This study showed that the hyperplasia patients with the highest malignant potential (D-score 0-1) were those taking most benefit from local high dose levonorgestrel therapy.

PP3-18

bcl-2, BAX AND APOPTOSIS IN ENDOMETRIAL HYPERPLASIA AFTER HIGH DOSE GESTAGEN THERAPY

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Objectives. The aim of the study was to investigate apoptosis as a growth regulatory mechanism of gestagen in endometrial precancers and to compare differences in the immunohistochemical expression of proteins in the apoptotic cascade after high and low dose gestagen regimens. Method. Pre- and post treatment paraffin-embedded endometrial hyperplasia specimens from women treated with levonorgestrel intrauterine device (n = 26) and women treated with 10 mg medroxyprogesterone for 10 days per cycle (n = 31) were examined for changes in the expression of Bcl-2 and BAX and the amount of apoptosis after 3 months of treatment. Expression in tissue specimens for Bcl-2 and BAX were evaluated by H-score. Apoptosis was assessed by counting apoptotic cells per 100 negative cells within ten different high power field (40X). Results. All the patients in the IUD group (n=31) but only about half of the patients in per oral group (16 of 26) responded to treatment. The glandular reduction in Bcl-2 expression was markedly greater for the IUD patients than for the patients who received oral gestagen. The decrease in BAX expression after IUD treatment was less than the reduction of Bcl-2. Decrease in glandular Bcl-2 after 3 months of treatment was coincident with a significant increase in the measurable amount of apoptotic bodies. In stromal cells increase in expression of Bcl-2 and BAX was found after gestagen treatment, the response being more extensive for the IUD group. The non- responders had no Bcl-2 expression in stroma after 3 months of therapy. The number of apoptotic bodies in endometrial glands increased significantly after 3 months of treatment with gestagen intrauterine device. No difference was observed for the per oral therapy. Conclusion. Our results indicate that proteins in the apoptotic cascade are regulated by gestagen therapy in human endometrial precancers. Expression of these proteins is shown to be dependent on type and administration form of the gestagen. Stromal Bcl-2 expression appears to be a potential biomarker which can separate responders of gestagen treatment from non-responders after oral administration.

PP3-19

P16 INK4A IMMUNOSTAINING: A USEFUL TOOL TO IDENTIFY OCCULT PREMALIGNANT LESIONS OF THE UTERINE CERVIX IN HPV-POSITIVE PATIENTS

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Background: The usefulness of p16INK4a in reducing interobserver variability; its ability to detect small foci of cervical intraepithelial neoplasia (CIN); and the close relationship between high-risk human papillomavirus (HR-HPV) infection and CIN, led us to evaluate whether p16INK4a staining could help to recognize under- or over-estimated CIN lesions, which may have clinical impact in the management of cervical disease. Method: Of 1259 women having a histological study and a simultaneous HR-HPV detection using the Hybrid Capture 2 (HC2) test, we selected all patients (n=139) testing positive for HR-HPV having a negative biopsy (group A), 26 women testing negative for HR-HPV with a biopsy of CIN 1 (group B) and 11 women testing negative for HR-HPV and having a biopsy of CIN 2-3 (group C). The following groups were randomly selected as controls: 50 cases negative for HR-HPV and with negative biopsy (group D); 50 cases positive for HR-HPV and with biopsy of CIN1 (group E) and 50 cases positive for HR-HPV and with biopsy of CIN2-3 (group F). Results: All cases from group D were either completely negative or showed only focal p16INK4a immunostaining, whereas all biopsies from group F were positive for p16, with 98% showing diffuse and strong staining. Thirty-four out of 139 biopsies (24.5%) from the group A were positive for p16INK4a. Thirty of these cases (21.6%) were reclassified as harboring a CIN (11 CIN 1, 19 CIN 2/3) after reevaluation. Both the number of cases reclassified as CIN of any grade or as CIN 2/3 were significantly higher for cases with HR-HPV load above 100 RLU ($p<0.005$). Conclusion: Our results indicate that particular attention should be paid to biopsies from patients having positive HC2. The risk of harboring small foci of CIN of any type or a CIN 2/3 is significantly higher for patients with high HR-HPV load. Immunostaining with p16INK4a should be considered as a highly desirable adjunct to the histologic evaluation of cervical biopsy specimens.

PP3-20

ATYPICAL (BIZARRE) STROMAL CELLS IN VARIOUS CONDITIONS OF THE FEMALE GENITAL TRACT: AN IMMUNOPATHOLOGIC REPORT OF 9 CASES

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INTRODUCTION: Atypical stromal cells of the female genital tract have been reported in various conditions of the vulva, vagina, cervix and endometrium, predominantly associated with polypoid lesions. Their atypicality includes them in the differential diagnosis of various conditions. Their origin and pathogenesis are unknown. MATERIAL AND RESULTS: We describe the presence of atypical stromal cells in a series of 8 cases comprising: fibroepithelial polyp of the vulva (1), fibroepithelial polyps of the vagina (3), normal cervix (1), of stromal response to a squamous carcinoma of the cervix (1 case) and endometrial polyps (2). Immunohistochemical studies were performed in order to elucidate its origin and proliferative capacity using the following antibodies:CAM 5.2, desmin, S 100 protein, actin, caldesmon, RE, RP and Ki 67. CONCLUSIONS: These studies demonstrated fibroblastic or myofibroblastic features, suggesting that they may represent a reactive or degenerative process of mesenchymal stem cells from

subepithelial stroma. Although they have a benign clinical course, awareness of the pathologic and immunohistochemical spectrum of these cells is crucial in their correct differentiation from other lesions as microinvasive carcinomas, sarcomas, blue nevi and intermediate trophoblastic lesions, which also exhibit bizarre stellate cells. The differentiation with superficial myofibroblastoma is difficult since this entity does also share this cellular component that originates from a specialized band of subepithelial mesenchyme in the lower female genital tract

PP3-21

FOLLICULAR-LIKE PATTERN IN METASTASES OF FEMALE GENITAL TRACT LEIOMYOSARCOMAS AND RHABDOMYOSARCOMAS. A PITFALL IN DIFERENTIAL DIAGNOSIS

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INTRODUCTION: We present an unusual and unreported follicular-like change in the abdominal and vaginal metastases of four cases of leiomyosarcoma (LMS) and rhabdomyosarcoma (RMS) of the female genital tract; three of them originated in the uterus and the remaining one in the vaginal wall. RESULTS: This pattern was not present in the primary tumour and consisted in a moderately atypical spindle cell population, sometimes exhibiting lobular growth, which presented many pseudofollicular spaces with an apparent cuboidal lining and basophilic contents in their lumina. Primary tumours corresponded to 3 highly differentiated leiomyosarcomas of the uterus and vagina respectively and a carcinosarcoma with rhabdomyosarcoma component in the remaining one. Immunohistochemically, the tumour cells from the primary and metastases were diffusely positive for vimentin, actin, desmin, caldesmon and myosin in the last case, which demonstrated the smooth or striated muscle identity of the tumours. The luminal contents were positive for both mucicarmine and PAS stains. CONCLUSIONS: Since similar follicular- like spaces are characteristic of tumours such as juvenile granulosa cell tumour (JGCT)and small cell carcinoma associated with hypercalcemia (SCC with HC), this phenomenon should be taken into account in their differential diagnosis which should also include the alveolar patterns of various sarcomas as well as malignant melanoma which also may conform such pseudofollicular spaces.

PP3-22

REPRODUCIBILITY OF CERVICAL HISTOLOGIC INTERPRETATIONS BETWEEN PATHOLOGISTS

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Background: The interpretive reproducibility of cervical cytology and histopathology is critical to cervical cancer prevention programs. In the medical community, histopathologic interpretations are generally considered as the reference standard upon which treatment of cervical disease is based. Therefore the aim of our study was to determine the interpathologist and intrapathologist reproducibility of histologic cervical specimen interpretations at our Department. Methods: We reviewed 60 randomly selected histologic cervical specimens, which were originally diagnosed by one of four pathologists at our Department. The original number of specimens was equally distributed between the pathologists (each pathologist 15 specimens). After review we calculated intraobserver reproducibility for each pathologist (15/15) and interobserver reproducibility, where all the pathologists had to interpret all 60 histologic specimens. We also compared cytologic and histologic interpretations if cytologic interpretations were available. Results: The average interobserver agreement was 73,8% (70% - 78,3%). The lack of reproducibility was most evident for negative and less severe interpretations. For high grade SIL lesions the average reproducibility was 91,7% (88,9%-97,2%). The average intraobserver agreement was 76,7% (60%-86,7%), and for high grade lesions 93,8%. Cyto-histologic correlation showed excellent results (100%) for H-SIL lesions and moderate for low grade lesions. Conclusion: Interpretive variability is substantial in cytology and histopathology. Our results showed excellent reproducibility of cervical histologic specimens for high grade squamous lesions and only moderate for low grade squamous lesions, which is in concordance with some previous studies on larger series.

PP3-23

OVARIAN CELLULAR FIBROMAS: A CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF TEN CASES

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BACKGROUND: Traditionally, cellular fibroblastic tumors of the ovary were classified as either cellular fibroma (CF) or fibrosarcoma. A recent study suggests that cellular fibromatous neoplasms with bland cytology and elevated mitotic counts are associated with favourable prognosis and should be diagnosed as "mitotically active cellular fibroma" (MACF) rather than fibrosarcoma. In addition to clinicopathological features, immunohistochemistry may aid in further differentiating between CF and MACF, but its role has not been analyzed so far. METHOD: We retrospectively analyzed the clinicopathological and immunohistochemical features of 10 cases of ovarian cellular fibroblastic tumors diagnosed either as CF or fibrosarcoma in the last seven years. Patient records and archival pathology specimens were reviewed and immunohistochemistry was performed using pan-cytokeratin, EMA, vimentin, inhibin-alpha, calretinin, CD10, CD99, alpha-smooth muscle actin (SMA), desmin, S-100, c-kit, estrogen (ER), progesterone receptor (PR), p53, bcl-2, and MIB-1 antibody. RESULTS: Utilizing criteria proposed by Irving et al. the tumors were reclassified as CF (0-3 MFs/10 HPFs, n=5) and MACF (>4 MFs/10 HPFs, n=5). The mean age of patients with CF and MACF was 44 and 36 years, respectively. All tumors were unilateral, and the mean tumor size of CFs was 6.0 cm and 13.3 cm for MACFs. The majority of the tumors were solid; four of them had a cystic component, while ovarian surface rupture was present in one CF and one MACF. All tumors consisted of cellular, intersecting bundles of spindle cells showing slight or moderate pleomorphism. The mean highest mitotic count was 2.3 MFs/10 HPFs for CF, and 7.6 MFs/10 HPFs for MACFs. Follow-up of 4 to 79 months (mean 38 months) was available in 9 patients and was uneventful in all cases. One patient with MACF died 15 days following the operation as a result of the intercurrent disease. Immunohistochemical analyses showed that spindle cells in majority of the tumors were immunoreactive for vimentin, alpha-SMA, inhibin-alpha, calretinin, PR, and bcl-2. All tumors were negative for pan-cytokeratin, EMA, CD10, CD99, c-kit, ER, and p53, while one CF was positive for S-100, and one MACF showed positivity for desmin. In addition, the MIB-1 labeling index (LI) in MACFs was higher (mean 14.4%, range 10-25%), than that in CF (mean 5.6%, range 3-10%). CONCLUSION: Our results confirm the immunophenotypic similarity between ovarian fibromas and cellular fibromatous neoplasms, and suggest that the use of MIB-1 LI may help in differentiating between CF and MAFC.

PP3-24
PRIMARY MELANOMA OF THE UTERINE CERVIX – CASE REPORT
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Only 5% of melanocytic malignancies in women originate in the genitalia. Primary malignant melanoma of the cervix is an extremely rare histological variety of cervical tumor, with only 54 cases reported. These lesions arise from melanocytes, which may be found within the mucosa lining the female genital tract. Primary malignant melanoma of the cervix is diagnosed by the presence of junctional melanocytic abnormality and the absence of distant metastases. We present a case of the malignant melanoma of the uterine cervix in 84-year-old postmenopausal women. The suspition of malignant melnoma was recognaised on a routine Papanicolaou-stained cervical smear. The gynecologic examination showed a cervical dark tumor, with extention into the vagina, the parametrium was free. Microscopic examination of the biopsy led to diagnosis of malignant melanoma (Fig.1). The cells were positive for S-100 (Fig.2)and HMB45 (Fig.3) in immunohistoceminal stain.The tumor was stage IIA of the International Federation of Gynecology and Obstetrics classification. Chest x-ray and abdominopelvic computed tomografy scanning were normal. Radical hysterectomy with bilateral salpingo-oophorectomy, pelvic and paraaortic lymphadenectomy, partial vaginectomy, and adjuvant radiation or chemotherapy have been advocated by some investigators. In our case, due to old age the patient received palliative radiation therapy to the pelvis, total dose 20Gy. Cervical melanoma is a rare disease of which no retrospective studies exist, only case reports.Primary malignant melanoma of the uterine cervix carries a very poor prognosis. The small number of reported cases makes it difficult to evaluate the diagnosis and treatment. Immunohistochemistry plays very important role in the diagnosis of this tumor in the early stage. This is essential since cervical melanoma is incurable with standard therapies.

PP3-25
THE IMPORTANCE OF USING THE CYTOBLOCK TECHNIQUE FOR WOMEN WITH BLEEDING SYMPTOMS
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Background: Cervical sampling was changed fundamentally when in the eighties of the past century the Cytobrush was introduced. Finally, the Ayre spatula was completely replaced by various brushes. The more immature lesions, including the preneoplastic CINs, are often located in the endocervical canal and are effectively sampled by the brushes. Our results indicate that inflammatory changes can lead to cervical bleeding, probably because inflammation is accompanied by angiogenesis. It is a well known fact that (pre)invasive cervical carcinoma is rich in newly formed small blood vessels, leading to post-coital bleeding. Our purpose was to use such cervical samples to prepare Cytoblocks and exploit the paraffin section made thereof for additional immunostaining for women with bleeding symptoms. Methods: The 261 cervical samples were processed by the Shandon Cytoblock Preparation System. The vials still containing the brush were placed in a shaker for 10 minutes to dislodge the material trapped between the bristles of the Cervex-Brush. Specimens fixed in BoonFix were concentrated by centrifugation (10 min at 3000 rpm). The Cytoblock cassette (containing the concentrated material infiltrated with agar) was removed from the Cytospin and histoprocessed in the Pathos (Milestone, Italy) applying m sections were cut and stainedμmicrowave technology. From each block several 4 with the Papanicolaou method. On the Papanicolaou-stained paraffin sections made thereof a histological diagnosis was given rendered on the minibiopsies. Results: Of the total 261 women screened, 237 had a benign diagnosis, with only 138 having a completely normal histology. In 42 cases an inflammation was suggested. The CIN cases contained minibiopsies with classical feature of cervical neoplasia which were discerned with ease in the excellent paraffin sections with optimal nuclear morphology. In the two cases of squamous cell carcinoma, many minibiopsies examined displayed squamous cells connected with extended desmosomes and hyperchromatic nuclei containing a macronucleolus. The (pre)invasive cases had many Ki-67 positive nuclei displaying an S-phase staining pattern. In the Ki-67 stained sections, the glandular architecture of the two AIS cases and the two adenocarcinoma cases was highlighted. Conclusion: In the histologic paraffin sections there were enough minibiopsies to allow concise diagnosis including evaluation of proliferation. Signs of cervical angiogenesis, including post-coital bleeding, can be a strong argument to prepare Cytoblocks from samples collected by sampling brushes.

PP3-26
FREQUENCY AND CHARACTERIZATION OF ANTERIOR PITUITARY COMPONENT IN OVARIAN MATURE CYSTIC TERATOMAS
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Background: Adenohypophyseal tissues and pituitary-type tumors, namely functioning corticotroph and lactotroph adenomas, have been rarely reported in ovarian mature cystic teratomas (OMCTs), but the frequency of this feature is, to date, unknown. The purpose of this study was to evaluate the incidence of a pituitary component in a large series of OMCTs. Methods: An archival series of 200 consecutively collected and of 4 selected OMCTs was revised. On the basis of the morphological finding of cellular nests or solid islands, suggesting an endocrine tissue, 56 cases were selected for the immunohistochemical study. Immunoreactions for Chromogranin A, GH, PRL, ACTH, βFSH, βLH, βTSH, α-subunit and thyreoglobulin were routinely performed. Results:Anterior pituitary cells were detected in 8 OMCTs including 6 cases among the consecutive series (incidence: 3%) and 2 selected cases. Anterior pituitary component was frequently observed near a struma ovarii (6 cases) in form of small groups or strands of polygonal endocrine cells encircling or penetrating into the thyroid parenchyma. In one case, an adenomatous micronodule associated with a strumal carcinoid was present . In two cases the pituitary cells were localized beneath a respiratory mucosa. The following immunoreactivities were obtained: Chromogranin A (7/8 cases), PRL (6/8 cases), GH (5/8 cases), α-subunit (4/8 cases), βFSH (2/8 cases), ACTH (1/8 cases). The adenomatous micronodule showed diffuse PRL+ e focal GH+. Conclusions: Adenohpophyseal cells are present in 3% of OMTCs. The immunoprofile of pituitary cells demonstrates PRL expression in the majority of the cases, suggesting that hyperprolactinemia could contribute to the clinical syndrome of amenorrhea and/or infertility in a subset of young patients with OMTCs

PP3-27
LYMPHOEPITHELIOMA-LIKE CARCINOMA OF THE ENDOMETRIUM: AN UNUSUAL VARIANT OF ENDOMETRIAL CARCINOMA
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Lymphoepithelioma is a distinct neoplasm with unique histologic features and a consistent etiologic association with the EB virus. The neoplasm was first identified in the nasopharynx, but since

then carcinoma identical to lymphoepithelioma and designated lymphoepithelioma-like has been reported in many other organs. While lymphoepithelioma-like carcinoma of the cervix is well established lymphoepithelioma-like carcinoma of the endometrium is extremely rare with 3 cases reported to date. A 73 year-old woman with postmenopausal bleeding was admitted to hospital. The physical examination and the laboratory evaluation were both unremarkable. A total hysterectomy with bilateral salpingo-oophorectomy was performed. The hysterectomy specimen revealed a tan polypoid tumor filling the endometrial cavity and protruding partially into the cervical canal. Microscopically, the myometrium was diffusely infiltrated by a neoplasm composed mainly of plump cells with distinct cytoplasmic borders, vesicular nuclei and prominent nucleoli. The neoplastic cells were arranged mostly in groups with a syncytial appearance surrounded by an intense inflammatory infiltrate mainly with lymphocytes and plasma cells. Immunohistochemically, the neoplastic cells revealed strong keratin and EMA as well as focal estrogen and progesterone receptors positivity. The immunostains for synaptophysine, chromogranine, a-SMA, CD10, inhibin, and CEA were totally negative. In accordance with the previously reported cases the immunohistochemical analysis for the EBV was negative. The morphologic and immunohistochemical findings supported the diagnosis of a lymphoepithelioma-like carcinoma of the endometrium. We present this case as being both interesting and extremely rare and review the related literature.

PP3-28
UTERINE LIPO-LEIOMYOMA WITH FOCI OF CARTILAGINOUS DIFFERENTIATION

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The presence of foci with cartilaginous differentiation within an otherwise typical uterine leiomyoma or lipoleiomyoma is an extremely rare phenomenon, with only 3 reported cases so far in the literature. A 47-year old woman with a history of HGSIL, submitted to hospital due to uncontrolled vaginal bleeding. A total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. In the uterine corpus two intamural tumors, with diameters of 5,5 cm and 3 cm respectively, were observed. The tumors were both well-circumscribed, whitish with a soft consistency. Microscopically, the tumors were mostly composed of intervening fascicles of smooth muscle cells. Increased cellularity, cytologic atypia, necrosis or mitotic activity were totally absent. Between the smooth muscle cells, foci composed of mature cartilaginous tissue, as well as areas composed of well-differentiated adipocytes were noticed. These areas showed strong positivity for the S-100 immunostain. The histologic and immunohistochemical findings were consistent with the diagnosis of uterine lipo-leiomyoma with foci of cartilaginous differentiation. We present the case because of its rarity and we review the few reported cases in the literature, regarding its histogenesis and the related differential diagnostic problems with the other mesenchymal neoplasms of the myometrium.

PP3-29
ENDOMETRIOSIS AND ENDOMETRIOID CARCINOMA OF THE OVARY

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BACKGROUND: We present a retrospective analisis of 22 cases of endometrioid ovarian carcinoma, reviewed to identify endometriosis and its malignant transformation. METHOD: Twenty-two patients with endometrioid ovarian cancer were included in the review. Their clinical and histological data were retrospectively reviewed. The origin of the tumor was considered endometriosis-related when the presence of malignant changes in endometriosis glands leading to endometrioid carcinoma were found. RESULTS: Endometriosis was detected in three cases (3/22=14%). One of them presented a clearly benign to malignant transformation area. In another patient, the transition zone was abrupt and present in both ovaries. In the third, a pre-menopausal woman, ovarian endometriosis with only focal endometrioid carcinoma was observed. The three of them had a clear-cell carcinoma component. The presence of a clear-cell component was significantly greater in patients with endometriosis than in patients without endometriosis. Each patient had a different clinical presentation: increase in abdominal perimeter, post-menopausal vaginal haemorrhage and hypermenorrhea. Preoperative CA 125 levels were avalaible in 15 of the patients (15/22=68%). Endometriosis was found in two of these 15 patients, both with the highest CA 125 measured levels, exceeding 1700 U/ml. In the remaining of the patients, including the third one with endometriosis, CA 125 value did not exceed 35 U/ml. CONCLUSION: Although this association is not very frequent, patients with ovarian endometriosis and high CA 125 serum level should be managed with special care, regardless of their pre-menopausal or post-menopausal status.

PP3-30
PRIMARY OVARIAN HEMANGIOENDOTHELIAL SARCOMA - REPORT OF A CASE

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A primary ovarian hemangioendothelial sarcoma, also known as ovarian hemangiosarcoma or angiosarcoma is a very rare neoplasm - fewer than 25 cases have been recorded to date. A 89 year - old woman was admitted to the hospital because of a cystic tumor mass on her left ovary. She was offered a unilateral salpingo- oophorectomy. Grossly, the ovary revealed a unilocular cystic tumor of a maximum diameter 5.5 cm, with clear fluid contend, which histologically proved to be a benign serous cystadenoma. Peripherally and within the ovarian stroma, an infiltrating neoplasm composed of vascular spaces of varying size, lined by endothelial cells with prominent atypical appearance was recognized. Extra-ovarian expansion was not observed. Positivity for the vascular markers CD34 and Factor VIII confirmed the endothelial origin of the neoplasm and supported the diagnosis of ovarian hemangioendothelial sarcoma. The immunostains for pankeratin, inhibin and calretinin were negative. The neoplasm, though rare, raises differential diagnostic considerations, involving the germ cell tumors, mostly the immature teratoma, the ovarian lymphangiosarcoma and hemangiopericytoma, as well as the metastatic spread to the ovary from primacies in the spleen or the breast. The histogenesis of the neoplasm remains uncertain. The concurrence of an epithelial neoplasm, as observed in our case, has been previously reported. We present the case as being both rare and interesting and review the related literature, regarding the differential diagnostic problems and the histogenetic considerations.

PP3-31
A CASE OF MUCINOUS MICROGLANDULAR ADENOCARCINOMA OF THE ENDOMETRIUM

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Microglandular adenocarcinoma (MGA) is a rare type of endometrium carcinoma with only thirteen cases have been reported in the literature, according to our knowledge. MGA may mimic morphologically some benign and malignant lesions of the cervix. We report the fourteenth case of a 63-year-old, para 7 gravida 7, postmenopausal Tunisian woman consulted for uterine bleeding of 3-month duration. Histopathologic evaluation of the curettage specimen made the diagnosis of mucinous MGA. The patient underwent a total hysterectomy and bilateral salpingooophorectomy with pelvic lymph node sampling. Histology showed a stage IB grade II mucinous MGA. Because MGA is an uncommon type of endometrium adenocarcinomas and has potential problems in differential diagnosis with some lesions of the cervix, we discuss the clinical, histological and immunohistochemical characteristics of MGA useful in differential diagnosis.

PP3-32
ENDOCRINE AND IMMUNE PARALLELS IN LICHEN SCLEROSUS (LS) AND VULVAR INTRAEPITHELIAL NEOPLASIA (VIN): IMMUNOMORPHOLOGICAL STUDY

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Background. Immunomorphological study of vulvar biopsy specimens taken of 19 women aged from 37 to 65 diagnosed with LS and VIN, grade 3 was undertaken (LS - 10, VIN - 9). Method. Paraffin sections were incubated with monoclonal antibodies to progesterone (PGR), estrogene (ER) receptors, CD3, CD4, CD20, CD68, HPV types 6,11,18, polyclonal antibodies to IgA, IgG, IgM ("Novocastra"). Results. HPV was found in cellular nuclei of stratified squamous epithelium (SSE) in all subjects. Volume density of ER-positive cells in LS was 1.65±0.14%, in VIN - 2.95±0.19%, while PGR-positive - 3.93±0.21% and 5.46±0.23% respectively. ER-positive and PGR-positive cells expression in SSE was mainly noted in basal layers, particularly in acanthotytic bands, where hormone sensitive cells representation was total. Volume density of various subpopulations of lymphocytes and macrophages did not change significantly aggravated: for CD4-lymphocytes it averaged 0.5±0.08%, CD8-positive – 1.05±0.16%. CD4/CD8-ratio was 0.47. The number of IgG-producing cells remained stable and averaged 39%. As VIN got more severe the number of IgA-producing cells decreased from 28% in LS to 17% in VIN3, the number of IgM-producing cells increased from 33% to 44%. Conclusion. Thus, as VIN becomes more severe some changes in local humoral immune status are observed, particularly an increased level of IgM-producing cells. Cellular immunity transformation is expressed in the decreased number of CD4-lymphocytes.The number of cells expressing receptors to sex hormones increased significantly at the site of SSE growth.

PP3-33
FIBROSIS AND SMOOTH MUSCLE METAPLASIA IN INTESTINAL ENDOMETRIOSIS

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Background: In case of endometriosis of the colon, serosa and muscularis propria are involved much more frequently than submucosa and mucosa. This finding suggest that lesions of endometriosis spread from the serosa to the mucosal layer. Itoga et al. examined the histological findings of endometriosis in the rectovaginal space, and showed that the degree of fibrosis and smooth muscle metaplasia might increase with relation of the age of ectopic endometrial tissue. This conclusion also suggested that fibrosis and smooth muscle metaplasia could represent the chronic phase of ectopic endometrial lesion. We examined the degree of fibrosis in the ectopic endometrium in each layers of the colon, for decision of which lesions early or chronic phase are. Method: Three women with endometriosis of the rectum were archived from the medical records of our hospital. All of them underwent surgical resection of the rectum. These materials were fixed by 10% formalin at least overnight. Ninety-four lesions were selected from these three specimens. These lesions were located at the subserosa (ss-lesion) (13 lesions), at the muscularis propria (mp-lesion) (78 lesions) and at the submucosa (sm-lesion) (3 lesions). No lesions were observed in the mucosa (m-lesion). Each lesion was classified into four groups in accordance with the degree of fibrosis surrounding the endometrial duct. The classification of the degree of fibrosis was, Group1: endometrial duct without stromal tissue surrounding fibrosis, Group2: endometrial duct surrounded by only endometrial stroma, Group3: endometrial duct partially surrounded by fibrous tissue, Group4: endometrial duct completely surrounded by fibrous tissue. Results: 1) Ectopic endometrial tissues were most frequently observed in the muscularis propria (sm 3, mp 78 and ss 13). 2) The lesions in the muscularis propria showed various stromal and fibrosis features (Group1 17%, Group2 32%, Group 3 22% , Group4 29%). 3) On the other hand, the lesions in the submucosa and subserosa revealed some endometrial ducts surrounding by fibrosis, completely or focally. (sm: Group3 33%, Group4 67%; ss: Group3 30.8%, Group4 69.2%). 4) Especially, in the lesions of the subserosa, smooth muscle metaplasia was found in the extensive spread of fibrous tissue. Conclusion: Ectopic endometrium in the subserosa with prominent fibrosis and smooth muscle metaplasia might represent the long-standing lesion of the enodometriosis in the colon. The first site of endometrial tissue infiltrated into the colon could be identified as the subserosa of the colon.

PP3-34
TISSUE MICROARRAY STUDY OF TUMOUR BIOMARKERS IN HPV INDUCED CERVICAL NEOPLASIA

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The aim of this study was to analyse expression patterns of tumour biomarkers Ki 67, p53, p16 (INK4A) and epidermal growth factor receptor (EGFR), in cervical carcinoma using tissue microarray technology (TMA). Methods: The expression of Ki67, p53, p16(INK4A) and EGFR was studied in series of 34 cervical cancer samples: 6 cervical intraepithelial neoplasia (CIN III), 14 squamous cell carcinomas (SCC), and 14 adenocarcinomas (ADC). TMA slides containing triplicate of 1.0 mm cores were constructed and subjected to immunohistochemical protein analysis. Results: High-risk HPV-DNA was identified in 94 % (32/34) of the cases. HPV-16 predominated in SCC and CIN III, while HPV 18 and HPV 16/18 mixed infection were detected in ADC. Ninety-one percent of samples (31/34) exhibited strong proliferative activity (+2 and +3 Ki 67 immunopositivity), 64% (22/34) exhibited high expression of p16(INK4A), 73% (25/34) demonstrated high expression of p53 and 61 % (21/34) of uterine cervix neoplasia exhibited high EGFR expression. According to the histological type, SCC and ADC demonstrated high expression of Ki67, p16(INK4A) and p53. In CIN III only high expression was detected for Ki67, while p16(INK4A) and p53 were expressed at low level. Substantial differences were shown in EGFR expression: SCC and CIN III demonstrated high level of EGFR expression, in 92 % (13/14) and 100% (6/6) samples, respectively. In eighty-five percent of

ADC's the expression of EGFR was absent (9/14) or (+1) intensity (3/14). Only two tumours demonstrated moderate/strong (+2) level of expression. Conclusion: Our study has demonstrated the reliability of tissue microarray technology in protein expression studies. Preliminary data revealed substantial differences in tumour markers expression in different histological types of cervical neoplasia. To confirm these results, larger studies are needed. Especially, EGFR expression in ADC and adenosquamous carcinoma should be investigated to elucidate the role of EGFR expression in these histological types of cervical carcinomas.

PP3-35
OVARIAN SEX CORD TUMOR WITH ANNULAR TUBULES - REPORT OF A CASE

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The sex cord tumor with annular tubules (SCTAT) was discovered as a distinctive entity when its unusual appearing microscopic pattern was encountered repetitively in a pathology consultation practice. This tumor is a distinctive ovarian neoplasm, the prominent component of which shows morphological features intermediate between those of the granulosa cell tumour and those of the Sertoli cell tumour. The aim of this paper is to report the first case of SCTAT in our Institution. Methods: We used clinico-biochemical data, macroscopic and microscopic morphology analysis, as well as immunohistochemical analysis of FFPE tissue samples. Results: A 23-year-old woman presented hirsutism and meno-metrorrhagia. Tumorous formation on the left adnexa was palpated. Ultrasound findings indicated septate cystic tumor on the left ovary. Erythrocyte Sedimentation Rate was 30/60 and CA-125 more than 40 U. (ref. 30 U.). Karyotype of the leucocytes from the peripheral blood was 46, XX. Gross examination revealed that the tumor was unilateral with smooth surface. It measured 25x22x12 cm. Cut surface exhibited multilocular cystic compartments filled with translucent gelatinous content. The compartments were separated with yellowish septa of variable width. Microscopic examination showed simple and complex annular tubules with prominent basement membrane-like material around the tubules. The lumina of tubules contained hyaline bodies. Small sheets of solid clear cells were also present, as well as calcified necrotic fields. Tumor cells were positive for cytokeratin cocktail and alpha-inhibin, they showed intermediate signal for WT1 (Wilms Tumor 1) and negative stain for cytokeratin 5/6. We can conclude that SCTAT is a very rare entity with unclear histogenesis, unpredictable prognosis and, very often, with miss out on morphology recognition.

PP3-36
BILATERAL OVARIAN WELL-DIFFERENTIATED SERTOLI-LEYDIG-CELL TUMOR(S) ASSOCIATED WITH UNILATERAL SEROUS CYSTADENOMA – A CASE REPORT

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Background: Bilateral Sertoli-Leydig-Cell Tumors of the Ovary, especially in association with unilateral Serous Cystadenoma, are exceedingly rare. We present a case of a 61 year old woman with this diagnosis, in which the Sertoli-Leydig-Cell Component shows leiomyogenic (heterologous) differentiation. On routine examination bilateral ovarian cystic tumors were detected by ultrasonography. Bilateral oophorectomy was performed, the left ovary was sent for frozen section diagnosis. Macrosopically the left ovary measured 27x16x7cm, had a weight of 1965 grams and showed multiple cysts with numerous greyish to yellowish solid nodules measuring up to 1cm. The right ovary (no frozen section diagnosis obtained) measured 4x2,5x2cm and and showed on cut surface a 2,5x1,5x0,5 cm brown to grey solid-cystic tumor. Method: The tissue was processed and stained (H&E, immunohistochemistry) according to standard protocols. To confirm the sex-cord stromal component, immunohistochemistry for α-Inhibin (Serotec, dilution 1:20) was also performed. Results: Left ovary: The frozen section revealed a serous cystadenoma/adenofibroma. The examination of the formalin-fixed paraffin-embedded tissue displayed in addition infiltrates of a well differentiated Sertoli-Leydig-Cell Tumor. The Sertoli-Leydig-Cell Tumor showed irregular infiltration of the cysts. The tumor cells lacked significant cytologic atypia and mitotic activity. Immunohistochemistry revealed intense and diffuse positivity for α-Inhibin. Within the well-differentiated sex-cord stromal tumor there were mesenchymal areas showing fascicular arrangement of spindle cell with eosinophilic, fibrillary cytoplasm. These heterologous areas were immunoreactive for smooth muscle actin. Right ovary: The tumor of the right ovary resembled a well differentiated Sertoli-Leydig-Cell Tumor. Conclusion: To the best of our knowledge, this is the first described case of a Sertoli-Leydig-Cell Tumor of the ovary occurring in a serous cystadenoma. Leiomyogenic differentiation and bilaterality are further interesting features of this particular case.

PP3-37
IS CD10 IMMUNEXPRESSION HELPFUL IN DETECTING OCCULT STROMAL CELLS IN OVARIAN ENDOMETRIOSIS?

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The diagnosis of endometriosis is based on finding glands lined by endometrioid epithelium surrounded by densely packed small fusiform cells with scanty cytoplasm and bland cytology typical of nonneoplastic endometrial stromal cells. These features may be obscured by hemorrhage, inflammation and fibrosis and diagnosis of endometriosis could be difficult. CD10 has been shown to stain some non-hematological tissues including endometrial stromal cells. In this study our aim is to detect CD10 immunreactivity in cases of presumptive ovarian endometriosis and to determine the value of CD10 in achieving a definitive diagnosis. 14 cases of ovarian biopsies diagnosed as not diagnostic but compatible with endometriosis between the years 2001-2007, were retrieved from the archives of Marmara University Department of Pathology. All slides were reevaluated and selected sections were stained with CD10 by immunohistochemistry. A case of definitive endometriosis and an endometrial tissue were used as positive controls. 2 cases with follicular cysts and inclusion cysts were used as negative controls. In 12 of the 14 cases (86%) strong CD 10 immunreactivity was seen in the stroma underlying a mainly attenuated epithelium. These areas were unnoticed in H&E sections due to fibrosis or hemorrhage. Areas that may simulate endometriosis were negative for CD 10 both in the study cases and negative controls except one. In this one case luteinized follicular cells showed weak cytoplasmic positivity for CD10. In two cases (24%) CD10 immunreactivity were negative. Overall strong positivity for CD 10 is a specific marker for endometrial stromal cells but it should remain in mind that weak staining may be seen in luteinized cells. However, such lesions are not difficult to differentiate morphologically. In conclusion, our study showed that CD 10 immunreactivity is useful and can be used in differential diagnosis of controversial cases of endometriosis.

PP3-38

HISTOPATHOLOGICAL CHANGES IN PREPUBERTAL RAT OVARIES SUBJECTED TO TORSION AND DETORSION: EFFECTS OF L-CARNITINE AND N-ACETYL CYSTEIN

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Background: We aimed to evaluate histopathological changes, to detect HIF-1α levels and to determine MDA levels in prepubertal rat ovaries, which were subjected to torsion and detorsion and treated with L-Carnitine or N-Acetyl Cystein (NAC). Methods: Forty prepubertal female Sprague-Dawley rats were used in the study. In group 1, only laparatomy was performed. In group 2, right ovaries were subjected 3 h of ischemia and removed sugically. In group 3, an ischemic period of 3 h was followed by reperfusion for 24 h. In groups 4 and 5, either L-Carnitine (100mg/kg) or NAC (20mg/kg) was infused intraperitoneally on 150th min of ischemia and ovaries were reperfused on 180th min. After 24 hours of reperfusion right ovaries were removed. Blood samples were collected before sacrification of each rat to determine serum IL-6 levels. All ovarian tissues were subjected to histopathological examination and MDA levels were determined biochemically. Immunohistochemically HIF-1α antibody was applied to all ovaries. Histopathologically a score between 0 and 9 was formed for each ovary, which was composed of sum of grades of vascular congestion, edema and paranchymal hemorrhage, each graded between 0 and 3. Immunohistochemically HIF-1α staining was scored between 0 and 9 which was the sum of separate evaluations of staining intensities of granulosa cells, theca interna cells and theca externa cells, graded between 0 and 3. Results: HIF-1α did not show any nuclear staining while there was expressive cytoplasmic staining with an unknown significance. Histopathological scores and tissue MDA levels as well as HIF-1α scores, were significantly higher in groups 2 (all $P<0.001$) and 3 ($P<0.01$, $P=0.05$ and $P<0.001$ respectively) then group 4. They were higher significantly in group 2 ($P<0.001$) then group 5. Histopathological scores and MDA levels were significantly high in group 3 when compared to group 5 ($P<0.01$ and $P<0.05$ respectively. Serum IL-6 levels were significantly high in group 2 when compared to groups 4 and 5 (both $P<0.01$). Conclusion: Histological pathologies of ovarian tissues seem to decrease after 24 hours of reperfusion of the torsioned ovaries. Tissue MDA levels and serum IL-6 levels also support those results. However, reperfused ischemic ovaries treated by both L-Carnitine and NAC, showed better recovery than the untreated group, though L-Carnitine seems to be more effective then NAC.

PP3-39

PROGNOSIS OF SQUAMOUS CELL CARCINOMA (FIGO STAGE IA2, IB AND IIA) OF THE UTERINE CERVIX PREDICTED BY MALIGNANCY GRADING SCORE EVALUATION

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BACKGROUND Most reliable prognostic factor for squamous cell carcinoma (SCC) of the uterine cervix is staging according to FIGO. Pelvic lymph node metastasis implies less favorable prognosis and such patients are usually offered adjuvant treatment. Some patients lacking metastasis will also experience relapse and would probably benefit from adjuvant treatment, but predicting such tumor behaviour has proven difficult. The malignancy grading score (MGS) system based on 8 histological parameters (structure, polymorphism, mitotic rate, mode and stage of invasion, vascular invasion, cellular response) was introduced in the seventies, but has not been taken into general use. We wanted to investigate if the MGS system and image analysis would be suitable tools for identification of high-risk metastasis-free patients. METHOD In the study 82 patients diagnosed with- and treated for early stage SCC at the University Hospital of North Norway were included. Nineteen patients had been given adjuvant treatment due to lymph node metastasis, lack of radical exciscion or high risk profile. Nine patients experienced relapse and 7 died during follow-up of 5-16 years. Information including lymph node metastasis, resection margins, depth of infiltration, tumor diameter and clinical stage according to FIGO was taken from the hospital files. On the biopsy material initially evaluated, an MGS-score was given in each case by two pathologists independently. Finally, image analysis was performed. RESULTS We found metastasis, unfree resection margins and deep infiltration to significantly predict a poor outcome. Interestingly, the MGS system proved highly significant in separating patients with high risk of relapse from those with low risk. This was true when the material was evaluated as a whole and also when node positive patients were excluded. The inter-observer reproducibility was evaluated by Cohen's Kappa to 0,9. Image analysis proved unable to predict prognosis. CONCLUSION The MGS system is a fairly simple method and can easily be implemented into routine diagnostics. Its ability to predict relapse in patients lacking metastasis at time of initial surgery is therefore especially interesting. Image analysis offers no additional information.

PP3-40

INVESTIGATION OF FASCIN PROGNOSTIC SIGNIFICANCE IN ENDOMETRIAL CARCINOMAS

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Background: Fascin is an actin cross-linking protein that has been implicated in cell motility and migration. The absence or low expression of fascin in normal epithelia is dramatically altered in many human carcinomas. The aim of this retrospective study was to investigate fascin expression in endometrial carcinoma cases and correlate with clinicopathological factors and survival data. Methods: FFPE tissue specimens from 58 patients with endometrial adenocarcinoma were arrayed (5 cores of 0.6mm) using the tissue microarray technology. Clinical, pathological and survival data were collected from all patients and correlated with the immunohistochemical data. Results: The mean age of patients was 63 years (range 35-80). The majority of the cases (96%) was of endometrioid subtype and 79% of FIGO Stage I. Fascin expression was detected in 44/56 (76%) of cases, more common in grade 1 tumors, tumors with diameter <2cm, and with superficial myometrial infiltration ($p = 0.2$, $p = 0.1$ and $p = 0.1$, respectively). The estimate overall survival (OS), cancer related survival (CRS) and disease free survival (DFS) of patients with positive fascin were not significantly higher from those with lack of fascin expression. DFS was not significantly improved when expression was correlated with different clinicopathological parameters (age, FIGO staging, grade, myometrial infiltration, tumor diameter, extrauterine disease). Conclusion: According our data, it seems that fascin expression in patients with endometrial cancer is related with favorable grade, myometrial invasion and tumor diameter. In contrast to previous study we didn't find any impact in patients' survival.

PP3-41
DOCUMENTATION OF 938 OVARIAN LESIONS (NEOPLASTIC-NONNEOPLASTIC) IN A PERIOD OF SIX YEARS
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Background : The aim of this study is to establish the relative frequency and distribution of neoplastic and nonneoplastic lesions of the ovarian materials sent to the Pathology Laboratory of Ataturk Research and Training Hospital, Izmir, Turkey during 2000-2005. Method : In the institutional database, during these 6 years, 938 ovarian lesions were evaluated according to histopathological diagnosis retrospectively. They were also subclassified according to the neoplastic nature, origin and localisation of the tumor . Results : Out of 938 ovarian lesions reviewed, 736 (78,46 %) were neoplastic and 202 (21,54 %) nonneoplastic. Of 736 neoplastic lesions, 692 (92,02 %) were primary and 44 (5,7 %) metastatic. Out of 692 primary tumors 527 (76,15 %) were epithelial, 120 (7,34 %) germ cell tumor and 45 (6,50 %) sex-cord stromal tumor. Krukenberg tumor was the most frequent metastatic tumor (40 %) noted.Out of 202 nonneoplastic lesions 80 (39 %) were endometriotic cyst. Conclusion: The data demonstrate that the profile of the studied ovarian lesions in Ataturk Research and Training Hospital quantitatively parallels most of the literature reviewed

PP3-42
SUBEROYLANILIDE HYDROXAMIC ACID, A HISTONE DEACETYLASE INHIBITOR, SELECTIVELY INDUCES GROWTH SUPPRESSION AND CELL DEATH OF ENDOMETRIAL STROMAL SARCOMA CELLS IN VITRO
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Background: Endometrial stromal sarcomas are rare uterine malignancies and molecular mechanisms involved in their pathogenesis are poorly understood. Beside surgical excision, very often followed by recurrences, other therapeutic methods like radio- and/or chemotherapy seem to be less efficient. Covalent modifications of histone proteins, in particular de/acetylation of lysine residues, play an important role in the regulation of gene transcription in normal and cancerous cells. Nevertheless, the studies about these processes in solid mesenchymal tumors are limited. In our recent work we have shown increased HDAC2 expression in ESS samples and cognate ESS-1 cell line in comparison to normal endometrial stroma cells. Methods: For in vitro experiments endometrial stromal sarcoma cells (ESS-1) and human endometrial stromal cells (HES) were grown under standard conditions. After treatment with suberoylanilide hydroxamic acid (SAHA) in different concentrations, the cells were harvested at 24, 48 and 72 hours and analyzed by flow cytometry and Western blotting. Cells were also continuously monitored and changes in cell-growth and morphology were documented by cell-observer. Results: Our study shows that SAHA, which inhibits the activity of histone deacetylases class I and II, is able to mediate the cell cycle and expression of some genes related to malignant phenotype of endometrial stromal tumors. We were able to show for the first time that SAHA caused a dose dependent decrease in viable cell number and an increase in percentage of dead endometrial stromal sarcoma cells over time. In our in vitro experiments SAHA (3μM) led to differentiation and to death of ESS-1-cells, but not of HES-cells. Exposure of HES-cells to SAHA resulted in slightly decreased cell proliferation without evidence of cell death. SAHA also increased the p21WAF1 expression and caused significant changes in the cell cycle by inhibiting the G1/S transition in ESS-1 cells. Recovery experiments indicated that these changes became irreversible when the tumor cells were treated with SAHA for longer than 24 hours. Conclusion: Taken together our study suggests that HDACs might be considered as potential drug-targets in the therapy of endometrial stromal sarcomas. For different hematologic malignancies and solid tumors SAHA has recently entered the phase II and III clinical trials. Our data suggest that SAHA might be also a promising therapeutic agent for endometrial stromal sarcoma. Further studies are needed to prove its efficacy in vivo.

PP3-43
EXPRESSION OF IL-32 AND ITS PROGNOSTIC RELEVANCE IN CERVICAL SQUAMOUS CELL CARCINOMA
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Background: IL-32 is a recently discovered cytokine that induces TNF, IL-1, IL-6, and chemokines. This study examines the expression of IL-32 and its association with clinicopathological features in cervical cancer patients. Design: The following samples were analyzed: 40 paraffin-embedded specimens of cervical squamous cell carcinoma patients treated with radiation therapy or post-operation radiation therapy. The patients were grouped into FIGO stage IB (n=16) and stage IIA-IIIB (n=24). All tissues were subjected to immunohistochemical staining for IL-32 and clinical correlation with FIGO stage and survival, and the following results were obtained. Result: IL-32 expression was observed in 55% (n=22) of all tumor tissues and was strong in the tumor invasion site. The expression was located mainly in cytoplasm as well as in some tumor cell nuclei. When IL-32 expression was investigated according to the groups with regard to the FIGO stage IB and IIA-IIIB, there was a statistically significant (Chi-square test) IL-32 expression frequency in the stage IIA-IIIB (71%) compared to stage IB (31%) (P value = 0.014). The staining intensity showed borderline significance with advanced stage (P value = 0.064). However, IL-32 expression was not correlated with survival of the patients (P value = 0.79 and 0.90 in stage IB and IIA-IIIB, respectively). Conclusion: IL-32 expression is frequently observed in cervical squamous cell carcinoma cells. Our results show that the (enhanced) expression of IL-32 by carcinoma cells is correlated with advanced stage of patients with cancer of the uterine cervix. The expression of IL-32 in itself is not associated with worse survival in these patients

PP3-44
HISTOPATHOLOGICAL STUDY AND DNA IMAGE ANALYSIS IN HYDATIDIFORM MOLE AND NON-MOLAR ABORTION: A STUDY OF 89 CASES IN TUNISIA
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Background: Complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM) are two gestational trophoblastic diseases. Commonly, CHM is genetically androgenic and diploid whereas PHM is triploid. The absence of standardized morphological criteria and the presence of atypical cases in put major problems in the histopathological recognition of these diseases. Aims: to review histological diagnosis of molar and non molar first-trimester pregnancy by two pathologists and to evaluate the correlation between the final diagnosis and some histological criteria leading to the diagnosis of mole. Then we evaluate the correlation of consensus histological diagnosis with

ploidy status for molar pregnancy. Material and methods: We proceeded on a retrospective study of 89 specimens of abortus conception, 35 CHM, 12 PHM and 42 hydropic abortions (HA). Kappa value (K) was calculated for interobserver agreement, and to compare initial and consensus diagnosis. The final histopathogical diagnosis, was compared to the results of DNA contents detected by imaging analyser (Samba 200) studying all cases of molar pregnancy. Results: In the consensus histological diagnosis the cases were reclassified into 30 CHM (initial diagnosis (ID): 27 CHM and 3 PHM), 13 PHM (ID: 7 PHM and 6 CHM) and 46 HA (ID: 42 HA, 2 CHM and 2 PHM). There was agreement among the two pathologists in 77 cases (K= 0.72, 0.52 and 0.95 respectively for CHM, PHM and HA). We objectivate a significant statistical correlation between the final diagnosis and some histological criteria leading to the diagnosis of mole: large villi ,hydropic swelling, cistern, scalped outlines, trophoblastic hyperplasia, vacuolisation of tropholastic cells, polarisation of the trophoblastic cells, atypia and trophoblastic necrosis, free trophoblastic cells, absence of vessels and fibrosis into the villous axis. Others criteria were significantly distinctive between CHM and PHM: diffuse hyperplasia of trophoblastic cells, polarisation of trophoblastic cells and the presence of trophoblatic inclusions in the core of the villi. The ploidy results showed that 56.6 % (17/30) of CHM were diploid and 53.8% (7/13) of PHM were triploid. Conclusion: Our study demonstrates that some histopathological criteria can distinguish between PHM, CHM and non molar abortion. The study of DNA content correlate to the clinical outcome is useful in some difficult cases in which these morphological criteria were not easily reproducible.

PP3-45
E-CADHERIN EXPRESSION IN ADENOMYOSIS

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Background. E-cadherin is one of the major molecules that determine epithelial cells adhesion and physiology. The aim of the study was to reveal the possible role of E-cadherin in ectopic loci formation in adenomyosis. Methods. E-cadherin determination using enzyme-linked immunosorbent assay was performed in endometrial and myometrial samples of 53 patients with adenomyosis and 24 women without adenomyosis. Immunohistochemical study with antibodies to E-cadherin was made on paraffin-embedded tissue sections of hysterectomy material from 12 women with adenomyosis and 4 women with benign lesions excepting adenomyosis. Results. E-cadherin concentration in endometrium of adenomyosis group was 15.95±7.72 ng/ml while in control group 8.98±4.09 ng/ml (p=0.007). In myometrium of adenomyosis group E-cadherin concentration was 1.76±1.92 ng/ml, in control group 0.66±0.27 ng/ml (p=0.05). Correlation between E-cadherin concentration and disease duration was revealed. In cases with anamnesis of adenomyosis less than 3 years E-cadherin concentration in endometrium was 8.77±2.24 ng/ml, in cases with duration of disease 4-7 years 15.96±4.36 ng/ml (p=0.002) and in cases with duration 8 years and more 24.88±13.53 ng/ml (p=0.03). In myometrium these values were 0.61±0.09, 1.64±0.99 (p=0.05), 4.61±2.99 ng/ml (p=0.01) respectively. Immunohistochemically strong membrane expression of E-cadherin was revealed in all glands both in eutopic and ectopic endometrium. Intensity of reaction was higher in ectopic loci than in eutopic endometrium. Conclusion. No evidences of homophilic adhesion disturbance of epithelial cells in adenomyosis were revealed. Increased E-cadherin expression according to disease duration allows to suppose compensatory intensification of adhesion in response to long-term existing of ectopic loci.

PP3-46
HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF SEX-CORD STROMAL TUMOURS OF THE OVARY

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Background: Sex cord-stromal tumours (SCST) are rare ovarian tumours, with diverse but similar microscopic patterns, a characteristic immunohistochemical (IHC) expression and a usual benign behaviour. We analysed clinical, macroscopic, microscopic and IHC parameters, in order to better classify these tumours and to predict the prognosis. Method: 12 SCST (except thecoma-fibroma group) were retrieved from our files (1989-2004), and we analysed histologic features (architectural and nuclear pattern, mitosis and presence of luteinized stromal cells) and the IHC profile (CAM 5.2, inhibin, calretinin, s-actin, EMA, CK7 and neuroendocrine markers). Results: The initial diagnosis were 9 granulosa cell tumours (8 adult – AGCT- and 1 juvenile – JGCT-), 2 Sertoli-Leydig cell tumours –SLCT- (1 well-differentiated and 1 of intermediate differentiation) and 1 unclassified SCST. The patients ranged from 16 to 75 years old (mean, 45 years), the youngest was the JGCT. The known clinical presentation was vaginal bleeding (2), pelvic pain (4) and incidental finding (1). All tumours were unilateral, solid or solid-cystic (9/12) and ranged from 0,6 to 30 cm. The tumours presented an admixed variety of patterns: macrofollicular (6/12), solid (6/12), insular (3/12), microfollicular (4/12), watery-silk (2/12), and tubular (2/12). Nuclei were grooved in 10/12, and round with nucleoli in 2. Call-Exner bodies were mainly seen in microfollicular and watered-silk patterns. No significant atypia was observed and mitotic count ranged from <1/10HPF to 12/10HPF. The tumours stained positively for calretinin (8/8), s-actin (5/8), CAM5.2 dot-like (6/8) and diffuse (2/8) and inhibin (1/8). All were negative for EMA, CK7 and neuroendocrine markers. Luteinized stromal cells were highlighted with inhibin and calretinin. We reclassified the well differentiated SLCT as Sertoli cell tumour (SCT) and the SLCT of intermediate differentiation and the unclassified SCST as AGCT. Only the SCT and one AGCT presented pelvic-abdominal recurrences 9 and 15 years later, respectively. The mitotic activity (6/10 HPF) and the poorly defined margins seemed to be the prognostic factors in the SCT. The recurrent AGCT corresponded to the largest tumour in our series. Conclusions: SCST exhibit several growth patterns. The insular, solid and tubular patterns raise the most differential diagnosis among the SCST and with other ovarian neoplasms. Nuclear grooves (hallmark of the GCT) and the IHC profile described will help in establishing the diagnosis. Some histologic features could be associated with an adverse outcome.

PP3-47
ENDOMETRIAL CARCINOMA WITH "PREVIOUS INADEQUATE SPECIMEN SAMPLING" IN ENDOMETRIAL BIOPSY

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BACKGROUND: Endometrial biopsy reported by pathologists as “inadequate specimen sampling” (“insufficient material” or “no representative sampling”) can lead to an erroneous idea of absence of malignancy. The aim of this study is to find out the overall rate of biopsies diagnosed as “inadequate sampling”, and its repercussion on the time-delay in establishing the diagnosis of endometrial cancer. METHOD: Clinical data and pathological reports from 227 patients with endometrial carcinoma, over a period of fourteen years (from 1989 to 2003) were retrospectively reviewed, and the “insufficient” or “no representative” samples proportion in endometrial biopsies, prior to histological confirmation of malignancy, was calculated. RESULTS: Eighteen out of these 227 patients (7.9%) had a previous pathological report of “inadequate sample for diagnosis”. Only two of those eighteen cases (11.1%) underwent a new biopsy in less than a month, and twelve (66.6%) had a delay in the rebiopsy of more than three months. CONCLUSIONS: Assessment of specimen adequacy is important for pathologist interpreting endometrial biopsies. Endometrial biopsies reported as “inadequate sample for diagnosis” should not be considered, for practical purposes, as a benign sample, and early rebiopsy should be recommended.

PP3-48

EXPRESSION OF SURVIVIN AND TOPOISOMERASE II ALPHA IN OVARIAN CARCINOMA: CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS AND PROGNOSIS

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Background: Ovarian carcinomas account for four percent of all female cancers and for 25% of cancers of female genital organs. The clinicopathological parameters, such as clinical stage, tumor grade, histological type may reflect the aggressiveness of ovarian tumors and clinical outcome. The additional prognostic information closely related to tumor cell biology is essential for the identification of patients with poor prognosis. Survivin is a member of the inhibitor of apoptosis protein (IAP) family that has been reported to be broadly expressed in a wide range of malignant tumors but not in most corresponding normal tissues. Topoisomerase IIα (T2a) is an enzyme with an important role in DNA topology, repair and replication. It is a cell-cycle-related protein, expressed in normal as well as neoplastic cells in the S, G2 and M phases. The aim of this study was to investigate the association of survivin and T2a expression with clinicopathological parameters and prognosis. Materials and methods: Formalin-fixed, paraffin-embedded tissue sections from 61 ovarian carcinomas were obtained from the files of the Cukurova University, Faculty of Medicine, Department of pathology. We performed primer antibodies to survivin, T2a and Ki67 by immunohistochemically. Nuclear staining for all antibodies were considered as positive reactivity, percentage of positive cells was scored on a three-tiered system with <10% as negative. and we evaluated the relationship between expressions of survivin, T2a, Ki67 and prognosis. Results: The age of patients ranged from 17 to 78 and the mean age was 52.16 ± 14.84. The 61 cases in the study comprised 35 serous carcinomas,, 12 endometrioid carcinomas, 6 mucinous carcinomas, 2 clear cell carcinoma and 6 mixed carcinoma of the ovary. Survivin expression was found in %27.9 (17 of 61) of the tumors. T2a positivity was noted in %91.8 (51 of 61) of the tumors. There was a correlation between Ki 67 and T2a expression (r=0.28, p=0.030). Survivin and T2a expression did not correlate histological type, stage or overall or disease-free survival. Conclusion: The immunohistochemical assessment of survivin and T2a does not seem to be helpful in the prognostic characterisation of ovarian carcinoma. The most important prognostic factors are stage and histological type. Further studies addressing to find the additional prognostic parameters will likely provide valuable insights into the behaviour of ovarian carcinoma.

PP3-49

IMMUNOHISTOCHEMICAL STUDY OF THE EXPRESSION CD44 IN SEROUS, BORDERLINE, MALIGN SURFACE EPITHELIAL OVARIAN TUMORS AND METASTATIC OVARIAN TUMORS

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Backround: Carcinogenesis and metastasis are a multistep process involving complex interactions between tumor cells and the environment. The main cause for the motion of the tumor cells in invasive carcinomas may be loss of completeness intercellular adherence junction. One of these adhesion receptors, CD44 binds to extracellular matrix component hyaluronic acid. In the present study, to elucidate role of CD44, we investigated a series of benign, borderline, and malignant ovarian serous neoplasms. Materials and Methods: Archives paraffin- embedded formalin-fixed blocks from benign serous tumors (n=11), benign mucinous tumors (n= 8), borderline serous tumors (n=6), borderline mucinous tumors (n=1), primer malign ovarian serous tumors (n=9) and metastatic ovarian tumors (n=12) were immunohistochemically stained for CD44. Percentage of reactive tumor cells and stromal cells with CD44 were scored. Positive staining in at least 10% of cells with strong staining considered being positive. Staining intensity was scored from 1+ to 3+. CD44 protein was preferentially expressed along the basolateral domain of plasma membrane of tumors cells. Some of tumor cells were reactive with CD44 in cytoplasm. Results: CD44 was not only detected in 1 (5, 2%) of benign tumors. Remain of the tumors were reactive with CD44 in differential percentage and localization. CD44 staining was evaluated in 4 (8, 33%), in 21 (43, 75%), in 16 (33, 33 %), in 7 (14, 58%) of all tumors as grade 0, 1, 2, 3 respectively. Statistically significant associations were found between serous and mucinous benign tumors (p=0.0001), borderline and malign serous tumors (p=0.001), malign serous tumors and metastatic carcinomas (p=0.054), and primary malign ovarian tumors and metastatic carcinomas (p=0.002) as compared CD44 staining grade. Grade 0,1,2,3 stromal staining was determined in 14 (29, 16%), in 31 (64, 58 %), in 1 (2, 08 %) and in 1 (2, 08 %) ovarian tumors respectively. But no difference was in reaction with CD44 in stromal cells statistically. In primary malign tumors, CD44 was detected more than primary benign ovarian tumors and was different statistically (p=0.0001). Conclusion: These results suggest that CD44 expression may be important in differential diagnosis between borderline and malign serous tumors, primary malign ovarian tumors and metastatic carcinomas. Also CD44 expression is a characteristic factor in stromal invasion for ovarian serous carcinomas. A large number of samples are necessary to verify prognostic significance of CD44 expression in tumor progression.

PP3-50
COMPARISON OF TWO VERSUS THREE GRADES FOR ENDOMETRIAL CARCINOMA: AN ASSESMENT OF INTEROBSERVER RELIABILITY IN ENDOMETRIAL SAMPLES AND HYSTERECTOMY SPECIMENS
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OBJECTIVE:To compare the agreement in determining the histological grade of endometrial adenocarcinomas in endometrial samples and hysterectomy specimens using the three-grade FIGO classification and the two-grade system. METHODS: Endometrial samples and subsequent hysterectomy specimens from 55 patients with endometrioid adenocarcinoma were graded according to the FIGO and the binary system (BS) proposed by Lax et al. All tumours were graded independently by four pathologists on two separate occasions. The reliability coefficient for two-facet crossed design (specimen x observer x occasion) based on generalizability theory was calculated for each grading system in endometrial samples and hysterectomy specimens. We used the generalizability coefficient (GC) as a measure of interobserver reliability and the factors which could influence it (different psychological status of the observers at different times, changes in the laboratory conditions, etc).GC was interpreted as follows: 0.00 to 0.40, poor; 0.41 to 0.60 low; 0.61 to 0.80 moderate; and 0.81 to 1.00, high. The pathologists were separated into two groups as "experienced in gynecologic pathology" and "with limited experience in gynecologic pathology". Exact agreement percentages were calculated as the percent of all cases in which the grades were identical in endometrial samples and hysterectomy specimens. The FIGO and BS were compared by using chi–square test and kappa values were calculated for each grading system. RESULTS: GCs were 0.93 for endometrial samples, and 0.83 for hysterectomy specimens for FIGO system. The BS revealed GCs of 0.89 and 0.83 for endometrial samples and hysterectomy specimens, respectively. The GC levels were higher for experienced pathologists both when using the FIGO (endometrial samples= 0.92 hysterectomy samples= 0.92), and the BS (endometrial samples=0.92 hysterectomy samples= 0.90) compared to the group with limited experience in gynecologic pathology (FIGO- 0.89- 0.49; BS- 0.68- 0.60). The agreement rates between the endometrial sampling grade and the hysterectomy grade in FIGO and BS were 247/439 (%56.2) and 361/439 (%82.2) respectively ($p<0.05$). Kappa values were 0.29 ($p<0.05$) in FIGO system and 0.38 ($p<0.05$) in BS. CONCLUSION: In this study high GC values were obtained both for the FIGO and BS. GC levels were higher for experienced pathologists in both the FIGO and BS than the group with limited experience in gynecologic pathology. The agreement rates between the endometrial sampling grade and the hysterectomy grade were higher in the BS compared to the FIGO system.

PP3-51
BABES-PAPANICOLAOU TEST METHOD TO VERIFY LSIL TREATED LESIONS
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Background: The quality verification of the treatment applied by the gynecologist is realised by periodical cytological cervico-vaginal examinations using Babeş-Papanicolaou test. This test may indicate the presence or the absence of pathologycal cells. Material and method: In the Department of Pathology from Colentina Universitary Hospital, the authors took for study a lot of 83 patients, with the age between 20-30 years, treated by the gynecologist with Podofilin or electrocautherisation for mild dysplasia and plan condyloma. The study analyzed the koilocytic cells, the intermediate squamous epithelial cells with nuclear increased size and/or mild dyscariosis, pathological changes present in mild dysplasia and plan condyloma. The smears were stained with Papanicolaou stain and the 2001-revised Bethesda System was used in order to interpret the results. Study results: From all the treated patients, 69 patients were present for a control test (63,13%), 52 of them (75,36%) being treated for plan condyloma. Among these, using the cervico-vaginal examinations, 15 cases (28,84%) have had normal aspect, 9 cases(17,30%) presented inflammatory aspects of the reparing type, 28 cases (53,84%) presented koilocytic cells. From the 17 cases (24,67%) with mild dysplasia, a number of 11 cases (64,70%) presented normal Pap smears, and 6 cases (35,30%), presented ASC-US subtype modifications. Conclusion: The study displays that, from the number of the patients examinated, almost 40% completely recovered while the other 60% remained with pathological modifications. These data prove the necessity of cytodiagnostic reevaluation after treatment.

PP3-331
UTILIZING OF KI 67 EXPRESSION AND ESTROGEN AND PROGESTERONE RECEPTORS IN DIFFERENTIAL DIAGNOSIS OF SMOOTH MUSCLE TUMORS OF THE UTERUS
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BACKGROUND: Smooth muscle tumors of the uterus are frequent and most of them are benign. Some leiomyomas may have unusual morphologic features difficult to distinguish from leiomyosarcomas. Smooth muscle tumors of uncertain malignant potential (STUMP) represent a variant of uterine leiomyomas and remain a dilemma due to their uncertain clinical behavior. MATERIAL AND METHODS: One thousand four hundreds and twenty patients operated for smooth muscle tumor of uterine origin between 2005-2007 at our hospital's department of gynecology. Seven leimyosarcoms and 7 STUMP cases were reported during this term. The average age of STUMP and leiomyosarcoma cases was 42 and 57 years respectively. The average size of STUMP and leiomyosarcoma cases was 6.8 and 12 cm respectively. Seven leiomyomas with similar age and sizes were selected to compare with STUMP cases. Number of mitosis, presence of necrosis, atypia, Ki 67 expression, presence of estrogen and progesterone receptors were evaluated in paraffin embedded tissue. CONCLUSION: There is limited experience in the clinical behaviors of problematic uterine smooth muscle tumors like STUMP. Several parameters are used to facilitate the appropriate diagnosis. Besides the criteria of the WHO classification we found that the expression of Ki 67 and the presence of estrogen and progesterone receptors in uterine leiomyomas, STUMP and leiomyosarcomas is helpful in differential diagnosis.

Uropathology

PP3-52
E-CADHERIN EXPRESSION IN TUR MATERIALS WITH LOW AND HIGH GRADE UROTHELIAL CARCINOMAS: A STUDY OF 30 CASES

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The cadherins are a family of transmemrane glycoproteins and are the primer mediators of intercellular adhesion. Cadherins are responsible for cell adhesion and transmembrane protein and expressed in all tissues. E-cadherin, the epithelial spesific cadherin mediates the selective adhesion of epithelial cells and required for the interaction and maintenance of normal epithelial integrity. Loss of E-cadherin expression leads to dissociation of cells from cohesive tissues and generates dedifferentiation and invasiveness in a variety of solid tumors, revealing the crucial role of E-cadherin as a suppressor of tumor invasion and metastasis. This study comprised with 30 transurethral resection of urinary of bladder tumor specimens. We immunostained 15 cases of low grade papillary urothelial carcinomas and 15 cases of high grade papillary urothelial carcinomas for E-cadherin using tissue arrays. We observed that E-cadherin expression in low grade tumors is more pronounced than high grade malignancias. In 5% of low grade tumors E-cadherin expression was indistinct than aspected. We think that this must be due to the increased potential of invasiveness and metastasis, contrary to their histological features. We propose to use E-cadherin as a prognostic marker in TUR materials for urothelial carcinomas.

PP3-53
RELATIONSHIP BETWEEN GLEASON SCORE AND NUCLEAR SIZE AND SHAPE FACTORS IN PROSTATIC ADENOCARCINOMA

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Background: The Gleason grading system which depends on differentiation of the glandular structures is the most common used grading system in prostatic adenocarcinoma. Recently several quantitative morphometric methods have been developed to test the objectivity of the Gleason grading system. The aim of this study is to assess the size and shape characteristics of the tumor cell nuclei, and compare the results of measurement with Gleason score of the cases by using computer-assisted analysis system. Methods: This study included 130 cases with prostatic adenocarcinoma, 100 (77%) were needle biopsy specimens and 30 (23%) were prostatectomy specimens. The ages of patients ranged 46 to 94 (Mean: 67±8.26). Each slide were reevaluated and rescored by the same pathologist without having any knowledge about the prior diagnoses. About 150 nuclei with sharply demarcated contour were included for morphometric analysis for each case. After transferring microscopic images to the computer, nuclear area, nuclear length, nuclear perimeter, roundness factor, and form ellipse were automatically measured by an image analysis program. The relationship between Gleason score and these variables were determined by correlation analysis with Spearman and Pearson correlation coefficient. Results: Among all the specimens, Gleason score were significantly correlated with mean nuclear area (r: 0.516, p=0.01), with mean nuclear length (r: 0.298, p=0.01) and with mean nuclear perimeter (r: 0.303, p=0.01). However, in the needle biopsy group Gleason score were significantly correlated with mean nuclear area (r: 0.522, p=0.01), with mean nuclear length (r: 0.398, p=0.01), with mean nuclear perimeter (r: 0.432, p=0.01) and with mean form ellipse negatively (r: -0.213, p=0.05). In the prostatectomy group Gleason score were correlated with mean nuclear area (σ: 0.619, p=0.01) and mean roundness factor negatively (σ: -0.425, p=0.05). Conclusions: As a result, nuclear size and shape factors especially mean nuclear area were found concordant with Gleason score. Nuclear size and shape assessment may support to evaluate pathologic status of the disease in the prostatic adenocarcinoma. On the other hand, the association of clinical outcome and morphometric parameters could not be evaluated because of the short observation period in this study. Therefore, more detailed studies with long period of observation are required to asses more accurate results.

PP3-54
INTRAOBSERVER AND INTEROBSERVER REPRODUCIBILITY OF THE FUHRMAN NUCLEAR GRADING FOR RENAL CELL CARCINOMA

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Background: Several systems have been proposed for grading renal cell carcinoma. Classification of renal cell carcinoma has been divided into four categories according to nuclear size and the presence of nucleoli, the most widely used grading system reported by Fuhrman. However, there are few studies in the literature assessing the intraobserver and interobserver variability of this system. The objective of the current study was to evaluate the intraobserver and interobserver reproducibility of the Fuhrman nuclear grading system. Methods: In this study, pathology slides from 46 cases of renal cell carcinoma (Conventional: 37, chromophobe: 5 and papillary: 4) were rescored according to Fuhrman nuclear grading system by two pathologists. The intraobserver variation was determined as the first pathologist's observation on two different occasions. Interobserver variation was determined by the second pathologist who did not known the result of the first. Both intraobserver and interobserver reproducibility were assessed using Cohen's kappa statistics. Results: The original Fuhrman nuclear grade was grade 1 in 4 patients (8.7%), grade 2 in 30 patients (65.2%), grade 3 in 11 patients (23.9%), and grade 4 in 1 patients (2.2%). After slides review by same pathologist, nuclear grades were reassigned as follows: grade 1 in 8 patients (17.4%), grade 2 in 23 patients (50%), grade 3 in 14 patients (30.4%), and grade 4 in 1 patients (2.2%). Intraobserver reproducibility of the Fuhrman nuclear grading system was found to be substantial (κ = 0.66). Fuhrman nuclear grading of the second pathologist was grade 1 in 11 patients (23.9%), grade 2 in 27 patients (58.7%), grade 3 in 7 patients (15.2%), and grade 4 in 1 patients (2.2%). Interobserver reproducibility of the Fuhrman nuclear grading system was found to be moderate (κ = 0.42). We found low intraobserver and interobserver agreement for the Fuhrman nuclear grade 2 and grade 3, respectively. Conclusions: Despite strong intraobserver reproducibility of the Fuhrman nuclear grading system, one should consider moderate interobserver reproducibility and low agreement for the grade 2 and grade 3. Further studies are required to confirm our results in larger series.

PP3-55

CONCORDANCE OF NUCLEAR MORPHOMETRIC ANALYSIS WITH FUHRMAN NUCLEAR GRADING IN CONVENTIONAL RENAL CELL CARCINOMA

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Background: Nuclear grade, stage and tumor size are generally used for estimating prognosis of renal cell carcinoma. Ouantitative image analysis has been applied to renal cancers for diagnostic and prognostic purposes. The aim of this study was to investigate whether there is any correlation between the results of morphometric measurement and Fuhrman nuclear grading score, pathologic stage and size of conventional (clear cell) renal cell carcinoma. Methods: Morphometric nuclear parameters such as mean area, mean roundness factor, mean form ellipse, mean length, mean breadth and mean perimeter were evaluated in hematoxylin and eosin stained slides of 37 conventional renal cell carcinoma by using computer assisted image analysis system. The relationship between Fuhrman nuclear grading score, pathologic stage, tumor size and morphometric results were determined by correlation analysis with Pearson correlation coefficient. Results: Mean nuclear area were correlated with pathologic stage (r: 0.413, p=0.05) and with Fuhrman nuclear grade (r:0.588, p=0.01). Mean nuclear length were correlated with pathologic stage (r:0.446, p=0.01) and with Fuhrman nuclear grade (r:0.580, p=0.01). Mean nuclear breadth were correlated with pathologic stage (r:0.377, p=0.05) and with Fuhrman nuclear grade (r: 0.544, p=0.01) and with tumor size (r:0.366, p=0.05). Mean nuclear perimeter were correlated with pathologic stage (r: 0.449, p=0.01) and with Fuhrman nuclear grade (r:0.593, p=0.01). Mean nuclear roundness factor were correlated with pathologic stage (r: 0.418, p=0.05) and with Fuhrman nuclear grade (r: 0.456, p=0.01). The correlation between mean nuclear form ellipse and Fuhrman nuclear grade, pathologic stage, and tumor size was not statistically significant. Conclusions: In this study, the relative concordance of morphometric results and pathologic stage, Fuhrman nuclear grade, and tumor size exhibited the importance of nuclear morphometric analysis in conventional renal cell carcinoma. Studies including larger series of cases investigating detailed nuclear morphometric analysis of conventional renal cell carcinoma are needed.

PP3-56

CHROMOPHOBE RENAL CELL CARCINOMA

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INTRODUCTION: Chromophobe renal cell carcinoma (CRCC) is an uncommon,which accounts for average 5% of all renal neoplasm. CRCC shows an intermediate prognosis between conventional cell carcinoma and oncocytoma. Oncocytoma, a benign renal tumour, is considered the aim histological differential diagnosis of CRCC. A careful anotomopathologic study, special coloration and immunostaining, allow differentiating these two entities. We report 21 cases of CRCC. The aim of our study was to evaluate the incidence, clinical presentation, anatomopathological and immunohistochemical findings, prognosis, and clinical outcome of CRCC. METHODS: Hospital records and pathological slides were reviewed for 21 patients CRCC treated at Charles Nicolle Hospital between 1999-2006. The relevant clinical and pathologic data were extracted from the clinical charts and collected into a unique database. The following parameters were noted in each patient: age, sex, symptoms at diagnosis, tumour diameter, TNM stage and grade, histological cell type, follow-up time, local recurrence, disease progression, and death. RESULTS: 21 cases of CRCC; 8 (38%) were men and 13 (62%) women. The mean patient age was 54.8 (range 31to 74 years). The tumour was located in the right kidney in 11 patients (53%) and in the left kidney in 10 (47%); no familial or bilateral disease was observed. An incidental diagnosis was founded only in two cases and accounted for 9.5% of the cases. The other patients were symptomatic. The mean tumour size was 7,9 cm (between 2,5 to 16,5cm). Grossly, it was in 95%, a mediocortical and a necrotico-hemorragic tumour. On microscope, most of the pathologic features were typical. However, in 24% we needed immunostainnig using cytokeratine 7, vimentine, CD117 and PS100. At diagnosis, 10 patients (48%) had Stage pT1a, 1 (4.7%) pT1b, 5 (24%) pT2, 1 (4.7%) pT3a, 3 (14%) pT3b, and 1 (4.7%) pT4. The lymph node status was pN0 in 19 (90.5%), and N1 in only 2 patients (9.5%). Of the 21 patients, there were no metastatic disease at diagnosis, and 1 had pulmonary metastasis. The nuclear grade was, grade 1 in 1case, grade 2 in 17 (81%), and grade 3 in 3 (14%). Radical nephrectomy was performed in 20 patients (95%). After a median follow-up of 3 years (Range 2 to 5), one local recurrence was observed. The 5-year overall survival rate for CRCC was 90%. Of the 21 patients, 3 died of unrelated causes and renal cancer, respectively. CONCLUSION: The results of our study have confirmed a general favourable outcome for CRCCs, which are predominantly low-stage and low-grade tumours. These tumours had little local aggressiveness. Although metastases at diagnosis and disease progression after nephrectomy are rare, this neoplasm should be distinguished from renal oncocytoma. PS100 A1 seems to be a powerful marker to differentiate these two entities.CONCLUSION: The results of our study have confirmed a general favourable outcome for CRCCs, which are predominantly low-stage and low-grade tumours. These tumours had little local aggressiveness. Although metastases at diagnosis and disease progression after nephrectomy are rare, this neoplasm should be distinguished from renal oncocytoma. PS100 A1 seems to be a powerful marker to differentiate these two entities.

PP3-57

RENAL ONCOCYTOMA. PATHOGICAL FINDINGS

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INTRODUCTION: We report the clinical and histopathological features of 10 cases of oncocytoma. One diagnostic problem is distinguishing oncocytoma from other renal epithelial tumours with pink cytoplasm. Oncocytomas are the most common benign epithelial tumours. It accounts about 10% of renal tumours. MATERIALS AND METHODS: Hospital records and pathological slides were reviewed for 10 patients with renal oncocytoma treated at Charles Nicolle Hospital between 1999-2006. The clinical data included age and gender. Microscopic examination was performed on all cases. RESULTS: The median age of the patients was 54,9 years (range 23-84 years). All our patients were symptomatic (10/10) flank pain (8/10) hematuria (2/10). They occur more frequently in women than in men (Female's predomination (9/10)) and have a peak incidence in the seventh decade as same as RCC. On gross examination, most of the tumours were cortical in 6 cases, and the median tumour size was 74 mm (range 30-120 mm). Oncocytomas were solitary renal tumours in the ten cases we study. Multiple and/or bilateral cases were not observed. Most of these tumours were well-circumscribed encapsulated (6/10) neoplasm, with a uniform mahogany (6/10) or red dark color (4/10). A central, white satellite scar was seen in 3 cases. Necrosis (3/10), hemorrhage (2/10). Histollogically, different architectural pattern was found with nesting (7/10), acini (4/10) and/or"tubulocystic" (4/10) architecture with a myxoid and/or hyalinized stroma (3/10). A

moderate to abundant amount of eosinophilic cytoplasm with central, small, oval–round nuclei is found in all our cases. DISCUSSION: Light microscopic differentiation of oncocytomas from RCC specifically eosinophilic variants of the chromophobe type can be very challenging. The nuclear features are an important discriminating feature; RCCs have wrinkled, raisinoid nuclear membranes, whereas oncocytomas have round nuclei with nucleoli. Additional staining techniques and immunostaining may be helpful. In fact, Hale's colloidal iron positive staining is strong and diffuse in chromophobe RCC, whereas oncocytomas exhibit focal, weak positivity. Cytokeratin 7 is often negative in oncocytoma. Conclusion: Based on our findings, oncocytomas and RCC have similar clinicopathologic features. On the other hand, the nuclear features are an important discriminating feature; RCCs have wrinkled raisinoid nuclear membranes, whereas oncocytomas have round nuclei with nucleoli, immunohistochemical stain has been helpful in some studies. Knowledge of these distinguishing histological and immunohistological features will enable to better differentiate these two pathologic entities.

PP3-58

THE EFFECT OF ORAL HIGH ALUMINIUM INTAKE ON RAT SPERMATOGENESIS

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ABSTRACT: Introduction : Aluminum is a silver-white flexible metal with a vast number of uses. It is poorly absorbed and efficiently eliminated; however, when absorption does occur aluminum is distributed mainly in bone, liver, testes, kidneys, and brain (1) . Aluminium poising causes wide range of disorders, including: a decrease in the release of neurotransmiters and inhibition of voltage dependent calcium channels. The role of calcium on GnRH release and its action is detected so, in this studying, the effect of high aluminium intake on rats spermatogenesis is investigated. Methods and Materials: The experiment performed in four groups, a control group and three experimental groups consumed 0.625, 1.25 and 2.5 mg aluminium per gram diet for 60 days. Epididymis and vas deferens were dissected cut and diluted with normal salin. In all groups weight of vas deferens , epididymis, testis and whole animal, sperm count per gram deferens and epididymis tissues were determined then, the testicular tissues fixed in formalin for study of histopathology. Results: The results have shown that in experimental groups which consoumed 1.25 and 2.5 mg aluminium per gram diet, the vas deferens, epididymis, testis and animal weight were significantly decreased. In this animals the number of sperm per gram tissues from vas deferens, epididymis were reduced. The maturation arrest is seen in seminoferous duct and it haven‘t spermatogenesis. Conclusion: This studying indicated that high aluminium in take in rat have an inhibiting effect on spermatogenesis and this effect is dose dependent.

PP3-59

COMPARISON OF HIF-1α IMMUNREACTIVITY WITH TUMOR STAGE, GRADE, ANGIOGENIC PROFILE AND PROLIFERATIVE INDEX IN BLADDER UROTHELIAL CARCINOMAS

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BACKGROUND: Hypoxia-inducible factor-1α (HIF-1α) is a critical regulatory protein of cellular response to hypoxia. In this study, we evaluated the relationship of HIF-1α immunreactivity with clinicopathologic parameters such as tumor stage and grade, as well as angiogenic profile and proliferation index. METHOD: The expression of HIF-1α was assesed in 70 primary bladder urothelial carcinoma tissue samples immunohistochemically. Vascular endothelial growth factor (VEGF) immunreactivity and microvessel density (MVD) were used to evaluate the angiogenic profile. MVD was calculated by immunohistochemical staining of endothelial cells with CD34. Proliferation index was determined by the percentage of Ki-67 nucleer staining in tumor cells. RESULTS: There was a significant relationship between HIF-1α immunreactivity and stage, as well as histological grade of the tumor ($p<0,001$). HIF-1α immunreactivity was also closely related to VEGF expression ($p<0,001$), MVD ($p=0,002$) and proliferation index ($p<0,001$). VEGF, MVD and proliferation index were found to be closely related to tumor stage and histological grade. There was no correlation between HIF-1α immunreactivity and lamina propria ($p=0,13$), muscularis propria ($p=0,009$) or vascular invasion ($p=0,1$). CONCLUSION: In this study, HIF-1α expression was found to be closely related to prognostic parameters (stage, grade, angiogenic profile and proliferation index) in urothelial carcinoma of the bladder. For this reason, it may be a useful marker to determine the prognosis and to choose the appropriate treatment modality.

PP3-60

IMMUNOHISTOCHEMICAL EXPRESSION OF P53, P16 AND P21 IN UROTHELIAL CELL CARCINOMA AND THEIR CORRELATION WITH CLINICOPATHOLOGICAL FEATURES

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Background: Transitional cell carcinoma (TCC) comprises approximately 90% of all primary tumors of the urinary bladder. Several types of mutations of cell cycle regulatory genes are commonly in those tumors. Alterations in the p53 are a predominant component in the TCC. Recently another gene – INK4A, which is located in chromosome 9p21, encodes proteins p16 and p19 which regulate the pRb and p53 pathways. P21 is both a p53-inducible and p53-indipendent cyclin-dependent kinase inhibitor that can arrest the cell by inhibiting DNA replication. The aim of this study was to determine the association of p53, p21 and p16 expression with clinocopathological features. Materials and methods: Immunohistochemical staining for p53, p16 and p21 was carried out on serial sections from (archival) specimens of 108 patients who underwent transurethral resection for TCC. Based on percentage of nuclear reactivity, p53 was considered as wild-type (0% to 10%) or altered (>10%); p21 was scored as wild-type (>10%) or altered (<10%); and p16 status was considered wild-type (1% to 50%) or altered (0% or >50%). Results: Positivity of p53 was detected in 80/108 (81,5%). P21: absence or focal positivity (<10%) of tumor cells was detected in 72/108 (66,7%) of the carcinomas. Absence of p16 was detected in 40/108 (37%) of the tumors. The p53 phenotype seems to be the strongest predictor of bladder cancer associated with bladder cancer progression. P21 expression was correlated with tumor grade and stage. P16 expression was related to recurrence and survival but not to tumor progression. The alteration of each marker was independently associated with disease progression (p[LTEQ]0,30) and disease-specific survival (p[LTEQ]0,37). Conclusion: This study suggests that p53 and p21, as well as p53 and p16, have cooperative and synergistic effects in the promotion of bladder cancer progression.

PP3-61
PROGNOSTIC VALUE OF EGFR AND HER 2 IN UROTHELIAL CARCINOMA
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Introduction: Bladder cancer is a common malignancy with highly growth patterns. HER 2 is one of the most frequently amplified oncogenes and a potential therapeutic target in bladder cancer. EGFR has been associated with genes of bladder tumor. The aim of this study was to investigate the expression of EGFR and HER 2 and examine their prognostic value in the progression of bladder cancer. Materials and methods: We examined the immunohistochemical expression of HER 2 and EGFR in formalin-fixed paraffin samples from 109 cases of urothelial carcinoma (TUR) corresponding in 77 cases of invasive (stage pT1-4, grade 1 to 3) and 32 cases of noninvasive (pTa, grade 1 to 3) carcinoma. Results: EGFR expression was stronger in invasive tumors (p<0,0001) and in high grade tumors (<0,0001). 52 of the 77 investigated invasive (67,5%) and 26 of the 32 noninvasive (81,3%) carcinomas showed overexpression of HER2 protein. Of 77 invasive carcinomas 23 (29.9%) showed 3+ positivity , 29 (37.7%) showed 2+ positivity and 25 (32,4%) were scored as negative with a score of +1 and 0. Of the 32 cases no sample was evaluated as 3+ positive, 26 (81,25%) showed a 2+ positivity and 6 (18,75%) cases were identified as 1+ positive or negative. Conclusion: The combination of HER 2 and EGFR or the correlation between these markers and histological grade will yield prognostic information that is more powerful than what histologic grade alone can provide. The staining pattern of EGFR and its widespread expression in bladder cancer make it a good target for antigrowth factor or gene therapy. The validation of over expression of HER 2 oncogene in bladder cancer may allow for the potential use of Herceptin antibody therapy.

PP3-62
CAVERNOUS HEMANGIOMA OF THE BLADDER. A CASE REPORT
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Objectives: Bladder hemangiomas are exceptional benign tumors representing 0,6% of the bladder tumors. They present as solitary unique lesions and can regress spontaneously as a result of fibrosclerosis suggesting a conservative approach wherever possible. They have a low potentional for recurrence. Asymptomatic hemangiomas do not require treatment. Case report: We report a case of a 52-year-old woman who was admitted to our hospital with the chief complaint of macroscopic haematuria, accompanied by lower abdominal pain. Cytoscopy revealed a red sessile tumor 1,2cm in diameter at the internal urethral orifice. Transurethral resection of the tumor was performed. The specimen was pathologically diagnosed as cavernous hemangioma of the urinary bladder. The typical microscopical feature was the presence of dilated and congested vascular spaces in the lamina propria of the mucosa of the urinary bladder. The patient was treated with biopsy and fulguration and she developed no recurrence 2 years after. Conclusions: Hemangioma of the urinary bladder is rare. The diagnosis is made histologically. Hemangiomas are classified into cavernous, capillary or arteriovenous types based on conventional criteria from other sites. A low incidence of recurrence is seen and the patients have a favorable outcome. Biopsy and fulguration are effective for hemangioma of the bladder when the lesion is small.

PP3-63
TERTIARY GLEASON PATTERN IN RADICAL PROSTATECTOMY SPECIMENS
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BACKGROUND: The Gleason system is one of the most widely used grading system for prostatic adenocarcinoma. The Gleason score consisting of the sum of the grades of the primary (predominant) and the secondary (second most prevalent) grades, is an important prognostic parameter for prostatic carcinoma. Although the Gleason grading system does not account for the existence of a tertiary (third most prevalent) pattern, more than two predominant Gleason patterns can be present in one prostatectomy specimen. METHOD: Between 1998 and 2003, 51 patients underwent radical prostatectomy for clinically localized prostate cancer in our hospital. Prostatectomy specimens were examined retrospectively for Gleason scores including a tertiary grade and pathological stage including margin status, seminal vesicle invasion and extraprostatic extension. RESULTS: Overall, of the 51 patients, 18 (%35.3) were found to have a tertiary pattern. In previous studies concerning the prevalence of a tertiary Gleason pattern in prostate cancer, presence of more than two patterns were reported between %16-50. Of the 18 prostatectomy specimens with a tertiary pattern in this study, 11 presented tertiary pattern 5. The Gleason score was >= 7 in 16 (%89) of the prostatectomy specimens with a tertiary pattern. All of the prostatectomy specimens (%100) with a tertiary Gleason pattern had positive margins. Extraprostatic extension was observed in 8 (%44.4) and seminal vesicle invasion was observed in 6 (%33.3) of the 18 cases with a tertiary pattern. In our study, the prevalence of a tertiary pattern was found to be consistent with previous studies published before. CONCLUSION: Prostatic carcinoma shows multiplicity and hetereogenity in many cases and it is possible that a third and even a fourth most prevalent pattern can be identified. In recent years, the prevalence of a tertiary pattern in radical prostatectomy specimens has become more important. High grade tertiary patterns are found to be associated with a more advanced pathological stage. Although further studies are needed, the presence and percentage of a tertiary, particularly less differentiated Gleason pattern can be accepted as a potential new predictive factor for prostate cancer behaviour.

PP3-64
IMMUNOHISTOCHEMICAL EXPRESSION OF RHOE IN BENIGN PROSTATE GLANDS AND PROSTATIC ADENOCARCINOMA
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BACKGROUND: RhoGTPases are key regulators of the cellular cytoskeleton, but also regulate many other processes, including cell proliferation and transformation. Accordingly, there is increasing evidence for a role of Rho proteins in human cancer, and increased expression levels of Rho-family members are found in a variety of tumours. RhoE/Rnd3 is a member of the Rnd subfamily that, in contrast to other GTPases, do not hydrolize GTP and remain always in their GTP-bound active conformation. RhoE is a ubiquitously-expressed protein antagonizing RhoA function, promoting disassembly of actin stress fibres and focal adhesions. Recently, it has been shown that RhoE can also regulate cell proliferation and transformation. RhoE overexpression inhibits cyclin D1 expression leading to

cell cycle arrest in fibroblasts and also in prostate cancer cells, in correlation with its lower expression levels in prostatic tumor samples relative to benign tissue, which have supported a tumor supressor role for RhoE in prostate cancer. METHOD: Formalin-fixed and paraffin-embedded prostatic tissue sections obtained from 12 radical prostatectomy specimens were examined by immunohistochemical staining for RhoE (labelled streptavidine-biotine method, goat anti-Human RhoE antibody, Santa Cruz Biotech. Inc.®). The selected samples included benign prostatic glands (n=12) and adenocarcinoma scoring Gleason grade (G) 3 (n=10), G4 (n=9) and G5 (n=2). The staining index (SI) scoring system was defined by staining intensity on a scale of 0 to 4 multiplied by the estimated percentage of stained cells. Benign glandular epithelium was considered as positive internal control and graded 4+. RESULTS: All samples of benign prostatic glands showed positive citoplasmic staining for RhoE. RhoE expression was decreased or absent in malignant glands compared to benign epithelia. In addition, RhoE expression in prostatic adenocarcinoma was inversely correlated to the higher Gleason-grades of tumors. SI for G3=1.31; G4=0.41; G5=0. CONCLUSIONS: RhoE expression, detected by immunohistochemical analysis in prostatectomy specimens, is lower in neoplastic glands than in neighbouring benign epithelia. And RhoE expression levels inversely correlates to Gleason grade. The loss of expression of RhoE can support its tumor supressor role in prostate cancer, and may serve as a marker of prostate cancer progression.

PP3-65
SERTOLI CELL MALIGNANT NEOPLASM IN A REDUCTIVE TESTIS. A CASE REPORT

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AIM: To present a rare malignant testicular neoplasm with location in a reductive testis, which has been treated clinically as an extratesticular tumor. MATERIALS AND METHODS: A 25-year-old male presented with a solid paratesticular mass measuring 4,5X3X1,8 cm. The intraoperative biopsy specimen, sent for a frozen section diagnosis, concerned a "paratesticular tumor" which was positive for malignancy. A tumor excision followed. The paraffin-embedded sections have been studied microscopically on hematoxylin-eosin preparations. An immunohistochemical study followed. RESULTS: The microscopic examination showed a neoplasm with a biphasic histological structure featuring tubular formations lined by high columnar, cuboidal and flat cells. Among them there were spindle cells. The neoplasm showed high pleomorphism, foci of necrosis and a high mitotic rate (30 mitoses per 20 high power fields). The immunohistochemical study showed positive for vimentin, MIC2, NSE, synaptophysin, antichymotrypsin, hCG, pancytokeratin and C-kit. The neoplasm was negative for EMA, CEA, ER, PLAP and chromogranin. CONCLUSION: A rare tumor can create diagnostic difficulties in intraoperative frozen section biopsy when is developed from testicular embryonic remnants and is mimicking a paratesticular neoplasm.

PP3-67
HISTOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF POSTRADIOTHERAPY PROSTATE NEEDLE BIOPSIES

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Introduction: Prostate needle biopsies have an important role in the follow-up of patients treated with radiotherapy, especially with regard to prognosis. However histomorphological interpretation of post-radiotherapy prostate needle biopsies is a challenging venture, mostly because practicing pathologists are not familiar with the histomorphological changes secondary to therapy. Methods: In this preliminary study, we evaluated the histomorphological changes induced by therapy in 46 of our patients treated with combined hormonal therapy and radiotherapy. All patients received the same therapy regimen and were rebiopsied after completion of radiotherapy. We used immunohistochemistry against Ki-67, vimentin, prostate specific antigen, chromogranine, p63 and high molecular weight cytokeratin in addition to H&E sections. Furthermore we compared the original diagnosis of these biopsy samples with the ones given after detailed morphological and immunohistochemical evaluation during this study. Results: The most prominent changes noted after therapy were as follows: acinar atrophy with decrease in acinus/stroms ratio (% 95.7'sinde), acinar distortion (%34.8), basal cell hyperplasia (%34.8), stromal fibrosis (%47.8), stromal edema (% 41.3) and vascular changes characterized by myointimal hyperplasia and luminal narrowing (%43.5). Nuclear enlargement (%100), nucleolar prominence (%80.4), bizzaar nuclei (%54.3) and cytoplasmic vacuolization (%93.5) was noted in the cells lining the prostate glands. Rare atypical cells in the stroma were seen to be basal cells positive for p63. Even though, originally 9 cases were reported to have residual adenocarcinoma and 3 reported as suspicious for adenocarcinoma, adenocarcinoma was confirmed in only 4 of these cases, 3 of which presented with distant metastasis during follow-up. The other cases were seen to display therapy induced changes. Most of these cases were diagnosed by practicing pathologists not experienced in prostate pathology and not familiar with the changes induced by therapy. Interestingly the morphological changes described above were not obvious in the neoplastic component of the 4 cases with residual foci of adenocarcinoma, even though they were noted in the adjacent nonneoplastic component of these cases. Conclusion: We concluded that H&E morphology is sufficient in diagnosing post-therapy needle biopsy specimens provided that the pathologist is experienced enough and familiar with the changes produced with therapy and that immunohistochemistry can aid diagnostic decision making in difficult cases.

PP3-68
MULLERIANOSIS OF THE URINARY BLADDER: A DIAGNOSTIC PITFALL IN BLADDER BIOPSY SPECIMENS

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Müllerianosis is an entitiy characterized by the presence of different Müllerian-derived tissues in the wall of this organ. The terms endocervicosis and endosalpingiosis are used when only endocervical and tubal type glands are present respectively. When endoservical, tubal and endometrial glands are found in combination in the bladder it is defined as müllerianosis. It is a rare entitity and to our knowladge up to now six cases have been reported. We present a further case in a 37- year-old woman who had 2 caesarean sections before. The patient didn't have any bladder symptoms unlike cases that have been reported earlier . She only complained from dysmenorrhea and metrorrhagia. A

bladder mass was found by pelvic ultrasonography during routine control for diabetes mellitus. Ultrasonographic findings consisted of 16x19x30 mm solid hypoechoic lesion occupying the base and posterior wall of the bladder projecting into the lumen. A complete transurethral resection , including the deep muscular layer, yielding 10 chips was performed . Cystoscopic gross appearence was different from the usual transitional cell carcinoma or adenocarcinoma, and was rather displaying a cystic form. Once the resection started, the inner surface and consistency of the tissues were mimicking the gross appearence of adenocarcinoma (of a solid tumour). Histologically there were intermediate to large-sized, irregularly shaped , haphazardly arranged, endocervical type glands in the lamina propria and in the muscularis propria, some of which were cystically dilated. In some areas rupture of the glands and inflamatory respond were seen . Siderophages also were noted in lumens and in the stroma. In focal areas there was endometrial type glands which were typically surrounded by endometrial stroma. Well differantiated adenocarcinoma has been taken into concideration. Since there was no atypia and desmoplastic reaction ,the presence of endometrial type glands and its typical stroma and the metaplastic change of superficial urothelial epithelium into the endocervical type epithelium lead us to the correct diagnosis. Although our clinicopathological findings supported metaplastic origin, still the clinical history of prior caesarean section puts doubts to this.

PP3-69

DISTRIBUTION PATTERNS OF NEURAL NETWORK ON SURFACE OF PROSTATE AND EXTENDING PATTERNS OF PERINEURAL INVASION OF PROSTATIC CANCER. A MORPHOLOGICAL STUDY USING 3-D RECONSTRUCTION METHOD

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Background: Perineural invasion would be frequently observed prostatic acinar adenocarcinoma. Although, not only distribution patterns of the invasion but also its patterns of neural network on surface of prostate were not clarified completely. So as to elucidate the mechanism of perinaural invasion, we selected several cases of prostatic cancer with extensive perineural invasion. However the distribution patterns were three-dimensional and complicated, thus we analyzed completely serial sections of prostatic cancer specimens with a computer assisted 3-D reconstruction system for this study. Materials and methods: Four surgical specimens of total prostatectomy, with cancers having extensive perineural invasion, were used. After formalin fixed and paraffin embedded, complete serial sections were mede from the specimens as 4 um thickness and H&E stained. Every four serial sections were used for strict tracing the pattern of nerve branch and carcinoma invasion, and the data were digitalized. The data were incorpotaded in a computer assisted 3-D reconstruction system and 3-D images were constructed in all its aspects for morphological analysis in detail. Result: Each number of serial sections was 170, 262, 265 and 267 sheets and their thicknesses were 510, 786, 795 and 801 um, thus searched total thickness of the prostatic tissues reached 2,892um. The 3-D images in all its aspects were apparently indicating the distribution patterns of the neural networks and extending patterns of the carcinoma, as shown in the conclusion below. Conclusion: The 3-D images showed the neural networks made complicated distribution patterns with apparent ganglions. The nerves extended narrow branch toward prostatic parenchyma. Even if tiny perinaural invasion would be showed in prostatic parenchyma, the extension of cancer could be spreading widely for outside of the prostate through the neural network. However, the invasion hardly extended from perineural space into surrounded soft tissue. The finding might explained a hypothesis that perineural invasion should not be a prognostic factor of prostatic cancer.

PP3-70

DECLINING DIAGNOSTIC VALUE OF HIGH-GRADE PIN IN PROSTATIC BIOPSIES

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Background: The detection of isolated high-grade prostatic intraepithelial neoplasia (HG-PIN) in prostatic core biopsies has long been considered an important risk factor for a concomitant adenocarcinoma. Given an estimated risk of 30%, re-biopsy within 6 months has usually been recommended. However, the diagnostic importance of HG-PIN has recently been questioned. Here, we investigate the diagnostic relevance of isolated HG-PIN in the biopsy material submitted to our institutes of pathology during the past 10 years. Methods and results: 125 patients with a diagnosis of isolated HG-PIN were identified in our databank from 1995 to 2005. All histological slides were re evaluated by the authors according to standardised criteria, and compared to the result of re-biopsies. Criteria for HG-PIN were unequivocal morphology with nuclear crowding, nuclear atypia with hyperchromasia and prominent nucleoli, as well as subepithelial retraction. 39 cases (31%) did not meet our stringent review criteria for HG-PIN and were excluded from further investigations. So far, a re-biopsy was done in 40 of the remaining 85 patients. Two of these re-biopsies (5%) showed an adenocarcinoma, and 9 (23%) showed isolated HG-PIN. 29 re-biopsies (73%) were inconspicuous. Conclusion: The diagnostic importance of isolated HG-PIN in a contemporary PSA-screened population is low, questioning the necessity of a short-term re-biopsy. Molecular markers may help to better stratify the diagnostic importance of HG-PIN in the future.

PP3-71

IMMUNOHISTOCHEMICAL STUDY OF COX-2 EXPRESSION IN PROSTATE CARCINOMA; RELATION TO APOPTOSIS AND ANGIOGENESIS

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Background: COX-2 play role in tumorigenesis of a variety of human malignancies by stimulating cell proliferation, inhibiting epithelial differentiation, inhibiting apoptosis, mediating immune suppression and increasing the production of mutagens. The objective of this study is to determine if there is relationship between COX-2, apoptosis and angiogenesis in prostate carcinomas. Material and Methods: Paraffin-embedded tissue specimens from 49 prostate adenocarcinoma were retrieved from the files of the pathology department of Cukurova University, Medical Faculty. COX-2, Bcl-2, VEGF and M30 were studied immunohistochemically. The results of staining for COX-2, Bcl-2 and VEGF were analyzed semiquantitatively by using an immunohistochemical scoring system (HSCORE) that combines the percentage of immunoreactive cells (quantity score) and an estimate of staining intensity (staining intensity score). Apoptosis was figured out by monoclonal antibody M30, which reacts with the product from the cleavage of cytokeratin 18. The number of M-30 positive cells per 1000 cells was expressed as apoptotic index. Results: The age of the patients ranged from 48 to 73 years old (average, 63.8±6.4). Twenty-one (42.8%) of the cases were Gleason score ≤ 6 and 28 (57.2%) were Gleason score ≥7. COX-

2 expression was detected in 81.6% of cases. It was significantly correlated with Bcl-2 expression (r=0.49; p<0.0001). There was no correlation between COX-2 and VEGF expression (p>0.05). Gleason score was negatively correlated with M30 (r=-0.28; p=0.04). No significant relation between Gleason score, VEGF and Bcl-2 was observed. Negative correlation between COX-2 expression and mean survival was also observed (p=0.007). Conclusion: Our study indicates that COX-2 plays role at the carcinogenesis of prostate carcinoma through inhiting apoptosis rather than promoting angiogenesis. This result may offer new insights into the treatment strategies. In addition, it may be useful for the prediction of clinical outcome.

PP3-72
PROGNOSTIC VALUE OF THE EXPRESSION OF E-CADHERIN, β CATENIN AND CD44 IN BLADDER CANCER

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Background: Loss of expression of adhesion molecules may contribute to cancer progression. Epithelial cadherin (E-cadherin) interacts with cytoskeletal proteins through the catenin complex. CD 44 is also a surface adhesion molecule. The present study was undertaken to investigate the alterations in the expression of E-cadherin, BETA catenin, and CD44 in urothelial carcinoma of the bladder, and their prognostic value. Methods: 54 patients with primary urothelial carcinoma of the bladder were included in this study. The median age was 64 (range: 29-84 years), with 49 men and 5 women. Tissue samples were obtained by radical cystectomy in patients with invasive tumours and transurethral resections in patients with superficial tumours. The histological grading was performed according to the WHO-ISUP criteria. Patients were grouped as low grade and high grade. The pathological staging was done according to the TNM classification. The tumours were grouped as superficial (Ta and T1) and invasive tumours (T2, T3, and T4). Among the 54 tumours, 24 were classified as low grade and 30 as high grade. 42 were classified as superficial tumours and 12 as invasive tumours. The proportion of immunostained cells was used as a criteria for the evaluation. Results: The staining showed a membrane pattern, except for the CD44 where staining was also found in the cytoplasm. Loss of membranous E-cadherin immunoreactivity was higher in invasive and high grade tumours, but this finding was not statistically significant. Loss of expression of BETA catenin was significantly associated with higher grade (p=0.001), but CD44 failed to show the same correlation. Both BETA catenin and CD 44 were not associated with invasiveness. Conclusion: Several studies have examined the role of E cadherin in urothelial carcinoma revealing clear associations of decreased expression, with high grade and advanced stage tumours. Our study confirms this data although not statistically significant. These findings suggest that the expression of E-cadherin and BETA catenin might be useful prognostic markers for the clinical assessment of bladder cancer. Whereas several studies have shown an altered expression pattern of CD44, some reports suggested that it has no association with clinico-pathological variables. Our findings correlated with the second group. This retrospective study with a limited number of cases does not allow definite conclusions. Large prospective clinico-pathological studies to validate the prognostic value of E-cadherin and BETA catenin immunoreactivity should be performed.

PP3-73
SHORT TERM HIGH DOSE CORTICOSTEROIDS INDUCE APOPTOSIS IN PROSTATIC EPITHELIUM

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Background: Corticosteroids (CS) induce apoptosis in various human cells. However, published data quantifying apoptosis in prostate glandular epithelium following short term high-dose CS is lacking. Design: Prostate tissue from 36 organ donors who died from head trauma and given high-dose CS for cerebral edema were evaluated. The prostate was the last organ removed of those organs consented for donation. Most of the prostate was frozen but a mid-transverse section was immediately fixed in 10% neutral buffered formalin. Apoptosis was quantitatively scored on this H E whole-mount section per 10 hpf in 4000 total epithelial cells counted. Age-approximated, non-tumor bearing autopsy prostate tissue (n=47) with no recent CS treatment served as controls. Result: The mean age for donors and controls was 32 and 39, respectively. For donors, mean apoptotic index (AI) of peripheral zone (PZ) was 1.47% compared to 0.72% in transition zone (TZ) (p< 0.05). Zonal apoptotic indices were significantly higher than the control autopsy prostate PZ and TZ epithelium; however, there was no significant difference in ejaculatory duct and seminal vesicle epithelium (table). Two donors had incidental prostatic adenocarcinoma and the tumor cells had AI of 0.1% and 0.02 % Conclusion: Short term high dose CS induces apoptosis in prostate glandular epithelium both in the PZ and TZ, an almost 50 fold increase over controls in both of these zones. However, ejaculatory duct and seminal vesicle epithelium remained unchanged in terms of AI.

PP3-74
VALUE OF HIGH GRADE T1 SUBSTAGING IN BLADDER CANCER

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BACKGROUND Since 90's there is in the literature a discussion on the value of substaging high grade T1 bladder cancer (HGT1). In our center we perform substaging according to Younes criteria since 1996 and we have demonstrate a statistically significant difference in progression for initial high grade T1b and T1c (T1b,c) urothelial carcinomas (deep invasive submucosa) vs T1a (lamina propia invasive carcinomas). From 2001 we treat T1b,c with a specific protocol that includes re-TUR after 1st BCG instilation and then a radical surgery if its shows T1c or more, or pursuit with BCG treatment if there is T1a, T1b, CIS or non-tumoral tissue. Some of the arguments against the value of substaging is that many of the so called deep invasive submucosal carcinomas could be underdiagnosed T2's. The aim of this study is to know if substaging detects real high risk neoplasias or simply reflects infrastaged (not detected T2) carcinomas. METHODS We have reviewed our reTUR performed during 2001-2006 for HGT1. We define reTUR as the procedure performed before 2 months after the initial TUR. We also reviewed 27 cases (T1b,c) looking for evidence of lymphovascular invasion with H&E. RESULTS. 53 HGT1:12T1a; 22T1b,c (9/22 -group A- followed the entire protocol and 13/22 -group B- didn't); 15T1; 4TX, with 17/53 re-TUR. 9 came from T1b,c group A, 1 fromT1b,c group B, 3 from TX , 2 from T1a and 2 from T1. Group A re-TUR (9/9) results were 1HGT1c (radical cystectomy showed pT0 pN1) ,1HGT1A , 7 non-tumoral tissue. For group B there was 1/13 re-TUR with HGT1C . For the remaining 7 re-TUR patiens we found 2HGT1 (1 from TX 1 from T1a) and 5 non-tumoral tissue (2 from TX, 1 from T1A, 2 from T1). There was no muscle infiltrating carcinomas in any of the re-TUR. 4/13 T1bc group B and 2/15

T1's patients progressed, none of T1bc group A, none of T1a and none of TX progressed. Vascular invasion was found in 6/27 cases, 2/27 were doubtful and 19/27 were without invasion . 3/6, 1/2, 2/19, progressed. CONCLUSIONS. There is no evidence to consider HGT1b,c as understaged T2. We think that deep invasive HGT1 have a high risk of progression and merits a particular treatment protocol. Deep invasive submucosa carcinomas have the capacity of lymphovascular invasion and lymph node metastases.

PP3-75

THE INCIDENCE AND CLINICOPATHOLOGICAL SIGNIFICANCE OF MULTIFOCAL PROSTATE CANCER

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Background: The presence of multiple foci of prostate cancer within the same gland is not an uncommon finding. Various studies reported incidence of multifocality in prostate cancer between 67-87%. There is an index tumor (measured by largest volume), presumed to be the driver of prognosis and one or more separate accessory tumors. Design: Ninety-four radical prostatectomies were performed between 2003 and 2006 and processed as whole specimens with all tumor foci mapped. The cases didnot have any preoperative theraphy. The tumors were further analyzed in terms of number of tumor foci. Chi square test was used for the correlations between multifocality and and patient age, preoperative PSA, extracapsular extension, surgical margin positivity, lymph node metastasis and seminal vesicle invasion. Cases operated between 2003 and 2005 had preoperative sextant core needle biopsy. For cases operated in 2006 preoperative transrectal biopsy had 12 cores. Results: As a whole, in 41.5% of cases (39 cases) prostate cancer was multifocal. For cases operated in 2006 this incidence was 67.7% The mean number of foci was 1.68. The age and the preoperative PSA value were not different in unifocal and multifocal tumors. Unifocal tumors had more frequent extracapsular extension (ECE) than the multifocal tumors.(p=0.038). Lymph node metastasis, seminal vesicle invasion and surgical margin positivity didnot have any significant difference in terms multifocality. Conclusion: In our cases, unifocal prostate tumors are more frequent than the multifocal tumors. With the standard 12 core biopsy protocol the incidence of multifocal tumors are increasing when compared to sextant biopsies. Unifocal tumors are at higher pathological stage than the multifocal tumors. This may be attributed to, assimilation of smaller volume cancers by the large volume index cancer at the time of diagnosis.

PP3-76

EFFECT OF SOY ISOFLAVONE SUPPLEMENTATION ON PROSTATE TISSUE, MARKERS OF PROLIFERATION AND APOPTOSIS AND HISTOPATHOLOGICAL ENDPOINTS OF TUMOR GRADE, VOLUME AND STAGE

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BACKGROUND Isoflavones are among the most promising potential anticarcinogenic compounds in soybeans. Epidemiological studies indicate that consumption of soybean-containing diets is associated with a lower incidence of certain human cancers like prostate cancer. METHOD Men diagnosed with localized prostate cancer who have agreed to undergo radical prostatectomy, brachytherapy or cryotherapy were enrolled in the study (21 African American, 12 White, and 1 Asian). Subjects were given soy isoflavone capsules containing 150, 300 or 600 mg genistein (PTI G-2535) or placebo capsules. The study included a four-arm, double blind, placebo-controlled parallel group dose ranging trial: arm 4 -placebo; arm 3 -150 mg; arm 2 -300 mg; arm 1 -600 mg. Both biopsy and radical prostatectomy specimens belong to this eligible patient group were examined by using H&E and immunohistochemical biomarkers. Biomarkers of cell growth, bcl-2, MIB-1, cyclinD1, CDK5, CDK6; biomarkers of cell differentiation, apoptosis, and growth control, bax, p21, p53, Cx43 and biomarkers of angiogenesis, MVD, VEGF expression in tissues were evaluated by immunohistochemistry. The results were compared with the pathological stage and tumor volume on radical prostatectomy specimens. RESULTS No statistically significant change from pre to post treatment levels in any group and no statistically significant difference between the placebo and experimental groups in terms of change from pre to post levels for Cx43, MIB-1, p21, bcl-2, bax, p53, cyclin D, CDK5, CDK6, MVD and VEGF were found. Statistical analysis revealed no significant differences between the groups except in margin positivity, which was much lower in group 1 compared to other groups. CONCLUSION Our results did not show any difference between our study groups with respect to markers of proliferation and apoptosis. This may be partly due to small sample size.

PP3-77

THE IMPACT OF UNUSUAL HISTOMORPHOLOGICAL VARIANTS ON PATIENT OUTCOME WITH HIGH GRADE MUSCLE-INVASIVE UROTHELIAL BLADDER CARCINOMA: CLINICOPATHOLOGICAL STUDY OF 63 CASES

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Our aim is to investigate the unusual histomorphological variants of high grade muscle-invasive urothelial cell carcinoma of the bladder and to assess its influence on prognosis. For this purpose we re-examine total 350 cases with urothelial bladder carcinoma. Of 350 cases 63 (18%) showed diverse morphologic features including micropapillary (10 cases), microcystic (8 cases), lymphoepithelioma-like carcinoma (3 cases), sarcomatoid carcinoma (eight cases, including pseudoangiosarcomatous type), osteoclast-rich undifferentiated carcinoma (4 cases), clear cell (5 cases), lipoid cell (5 cases), nested (3 cases), plasmacytoid/lymphoma-like variant (4 cases), rhabdoid cell (3 cases), signet ring cell (2 cases), small cell carcinoma (4 cases), inverted type (3 cases), and syncytiotrophoblastic giant cells (1 case). The extension of these non-conventional histomorphological features in tumor and the number of histological differentiation types in each tumor were examined. We found that the extension of unusual morphologic features with any proportion of conventional urothelial carcinoma had an unfavorable impact on survival time. Survival time of patients decreases significantly with increasing amount of non-conventional histological type in tumor. We also noticed that the increased number of differentiation types had an unfavorable influence on patient outcome. In conclusion we suggest that the assessment of the extension and the number of unusual histological differentiation types may be an important prognostic factor in high grade and muscle-invasive tumors.

PP3-78

THREE DIFFERENT SIMULTANEOUS TUMORS IN ONE PATIENT; CLEAR CELL TYPE RENAL CELL CARCINOMA, LOW GRADE COLLECTING DUCTUS CARCINOMA AND MICROPAPILLARY UROTHELIAL CARCINOMA

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Background: The coexistence of multiple and synchronous primary neoplasms in the genitourinary system has rarely been described in the literature. The case represented here was much more exciting since collecting ductus carcinoma has a low grade morphology,which is very rare. Method: Our case was a 57-year-old man who presented with prostatism for 2 months. Cystoscopy showed a mass in bladder, and abdominal CT revealed two masses in the middle segment and lower pole of the right kidney. With the preoperative diagnosis of high grade urothelial carcinoma, total cystoprostatectomy with urinary diversion and obturatory lymph node dissection was performed and 2 months later he underwent partial nephrectomy for the excision of renal tumors. Result: Grossly, 5x4x3 cm nodular lesion was found in the left lateral wall of the bladder. Microscopically, high grade infiltrative urothelial carcinoma with squamous differentiation and micropapillary growth pattern invaded the perivesical fatty tissue. Two metastatic lymph nodes were identified. One of the masses of the kidney had a pale gray, sponge-like cross section and it was 5.5 cm in its maximum diameter. Histology revealed a tubulocystic architecture characterized by dilated tubules lined by cuboidal to columnar cells that often have a hobnail pattern in a desmoplastic stroma. Tumor cells were positive for HMWK, vimentin, EMA and RCC. The lower pole kidney tumor was an obvious clear cell renal cell carcinoma with Fuhrman grade 2. EMA, vimentin and CD 10 immunoreactivity was detected but HMWK was negative. Conclusion: When synchronous tumors are diagnosed, standart sequential therapies may need to be reconsidered because of the possible problems concerning the effectivity of the treatment. Our patient is now being treated by a chemotherapy protocol against the metastatic carcinoma of the urinary bladder. Although we know that collecting ductus carcinoma is an agressive variant of renal carcinomas and must be treated seriously, our case was one of the very rare reported examples of “low grade” morphology with favorable prognosis. Therefore, it was not considered as our priority.

PP3-79

INVESTIGATION OF CYP 1A1 AND GST M1 GENE POLYMORPHISMS AND P53 IMMUNHISTOCHEMICAL STAINING WITH CLINICAL AND PATHOLOGIC FEATURES IN PATIENT WITH TRANSITIONAL CELL BLADDER CARCINOMA

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INTRODUCTION: Bladder cancer is the second most common malignancy of the genitourinary tract and the fourth most common type of neoplasm in men in many industrialized countries. Genetic status of the individuals and environmental interactions are thought to play an important role. Individual differences in cancer susceptibility may be mediated in part through polymorphic variability in the bioactivation and detoxification of carcinogens. The aim of this study was to identify the polymorphisms of the GST M1 and CYP 1A1 genes, making correlation between those polymorphisms and the P53 expression as well as comparing with pathological clinical parameters with the patients with transitional cell carcinoma of the bladder. RESULTS: We obtained pathologically proven transitional cell carcinoma samples from 100 (19 female, 81 male: mean age 63.69±14.42 years) bladder cancer patients who underwent transurethral resection (TUR) or radical cystectomy between 2000 and December 2005 at Istanbul University Cerrahpasa Faculty of Medicine, Department of Pathology. Healthy individuals, 101 total; 33 female, 68 male; mean age 56.46±12.86 years were chosen as the control group.DNA was extracted from formalin – fixed, paraffin –embedded blocks of the patients. We used cut-off value for P53positivity of 10% of cells or more. There is no significant difference in gene polymorphisms between the patients and control group, however, CYP 1A1 heterozygote mutant genotype was less common in patient group. GST M1 null genotype is not different between patients and conrol groups but increasing risk for perineural and vascular invasion, multifocality, involvement of the pelvic lymph nodes, metastatic spread and death from the disease. and also invasive growth pattern is greater in the patients with GST M1 null genotype. These patients show higher p53 expression as well.There is a significant difference in p53 expression between CYP 1A1 homozygote wild and both mutant genotypes. CONCLUSION: In our study, although there was no significant difference between patient and control groups for GST M1 nullgenotype we found that the patients with GST M1 null genotype had poor outcomes than the others. P53 immunoreactivity was also statistically higher in the patients who have GST M1 null genotype.

PP3-80

A RETROSPECTIVE STUDY ON BLADDER NEOPLASMS

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In Haydarpasa Numune Hospital of Pathology Laboratuary , the bladder TUR and cytology cases which have been examined between 2003 August-2007 February monts were documented. 893 TUR ve 26 urine cytology , that belongs to 505 patients, which have been sent to our laboratuary between retrospectively reviewed. The cases were between 6-91 years old. There were 802 men and 117 women. 654 of all cases were reported malign and the other 265 were benign. The 52 cases of the group , which was reported malign, were papillary urothelial neoplasia- low malignant potential, 287 low grade carcinomas and 301 high grade carcinomas. One case of all the cases in that malign group was squamous cell carcinoma, 1 case high grade malign lenfoma infiltration, 1 case embryonal rhabdomyosarcoma, 2 cases carcinosarcomas of the bladder, 2 cases adenocarcinoma infiltration, 1 case dissemine insitu carcinoma and the other 2 cases were described as amyloidosis. According to Mostofi grade, 286 of the low grade carcinomas cases , were grade II and only 1 case was grade III. 116 of the high grade carcinomas cases reported as grade II, 185 as grade III. Lamina propria invasion in 26 cases has been diagnosed low grade carcinomas group. In 147 cases of the high grade carcinomas detrusor muscle invasion defined. 40 cases reported as inistu carcinoma determined. In 162 cases squamous differantiation was reported. The 3 of the benign lesions were inverted papillom, 3 flat hyperplasia, ,1 nephrogenic adenoma, 1 papillom and 12 cases were reported papillary urothelial hyperplasia. When 180 cases described as chronic specific and nonspecific chronic cystitis, the other cases defined as normal urothelial mucosa.

PP3-81
SURVIVIN, INHIBIN AND p16INK4a EXPRESSION IN PROSTATIC ADENOCARCINOMAS

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BACKGROUND: Prostate carcinoma is one of the most commonly occurring malignancies in Western men and is a leading cause of carcinoma-related deaths. The aims of this study were to characterize and compare the expression patterns of survivin, inhibin and p16INK4a in primary prostate adenocarcinomas. METHODS: Immunohistochemical staining for survivin, inhibin and p16INK4a was carried out on archival specimens from 21 patients who underwent transurethral resection. Six of the 21 patients were low grade prostate carcinomas (final Gleason grade 2-6) and 15 were high grade prostate carcinomas (final Gleason grade 7-10). The percentage of cells that expressed survivin, inhibin and p16INK4a were classified qualitatively into 4 categories (0, +1,+2, and +3) based on intensity of staining and percentage of cells. Chi-square test and Fisher's exact test were utilized for the analysis. RESULTS: The median patient age was 63 years (40-76). p16INK4a analysis showed positive staining in 83.3 % of the low grade and 100% of the high grade carcinomas specimens. Five patients (23.8%) were positive for inhibin and 7 patients (33.3%) were positive for surviving, all of which were high grade prostate carcinomas. A statistically significant difference was found among p16INK4a, and survivin and inhibin expression. p16INK4a expression was significantly higher in prostate carcinomas (95.2%). We compared p16INK4a immunostaining scores with surviving and inhibin expressions. Regarding the inhibin and survivin immunohistochemical expression, 25% (5/20) were concomitant p16INK4a and inhibin positive, 35% (7/20) were p16INK4a and survivin positive cases. CONCLUSION: In this study, we have demonstrated p16INK4a staining might be a diagnostic aid particularly in prostate carcinomas and survivin and inhibin expression might be helpful for differentiating particularly high grade carcinomas. Increased expression of p16INK4a may be involved in the carcinogenesis of the prostate and may be a potential therapeutic target. Additional studies are needed to confirm the potential value of p16INK4a, survivin and inhibin expression in prostate adenocarcinomas.

PP3-82
EPIDEMIOLOGY OF TESTIS TUMOURS AFTER 50 YEARS OLD

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Objective : In attempt to identify causes of testicular tumors among adult after 50 years old. Materials and methods : A retrospective study was made from patients treated in the military hospital Val de Grace and hospital la Pitié Salpétrière in Paris. Clinical data and diagnosis were obtained from pathologic reports. Populations were divided according age of onset : group 1 : post pubertal patients of less than 40 years old, group 2 : patients between 40 and 49 years old, and group 3 : patients of 50 or more years old. Statistical analyses were made with Epiinfo 6.04d. Results : Between 1994 and 2006, 392 testis tumors were treated in the military hospital Val de Grace (252) and la Pitié Salpétrière (140). Mean age of patient was 35 (median 31 extreme 16-85). 281 tumors (72%) occurred in group 1, 52 tumors (13,3%) were observed in group 2 and 59 (15%) in group 3. Germ cell tumors were the most common tumors observed representing 70,9%. Sex cord stromal tumors (SCT) represented 10% and other tumors 19,1%. Regarding age of onset, germ cell tumors represented 77,23% in group 1, and 80,77% in group 2. Non-seminomatous germ cell tumors and seminomas were observed with the same frequency before 40 years old. Seminomas were the main germ cell tumor observed after 40 years old (57,69% vs 23,08% for non seminomatous germ cell tumors in group 2). In group 3, germ cell tumors represented only 32,2% cases (seminomas 23,7%, NSGT 8,5%). Sex cord stromal tumors represented 18,6% and other tumors 49,1%. Among these, lymphoma were the most common (10 cases, 17%). Tumors developed from mesothelia represented 6,8% (adenomatoid tumors 2 cases, malignant mesothelial tumor 2 cases) and spermatocytic seminomas 3,4% (2 cases). Five tumours were developed from testis adnexa (2 sarcomas and 3 benign tumors), 2 were testicular metastasis (one prostatic and one colonic cancer), 2 were benign epidermoïd cyst and 3 pseudotumoral lesion (2 orchiepididymitis and 1 amyloid pseudo tumor of the testis). Conclusion: Germ cell tumors are the most frequent tumor observed in adult testis but their incidence drop dramatically after 50 years old. At this age, sex cord stromal tumors and lymphomas are common and the use of lymphoid markers CD20 and CD79a, plus inhibin, Melan A and calretinin may be usefull in the diagnosis of a testicular tumor. Spermatocytic seminomas and adnexial tumors are very uncommon and one should consider the possibility of a metastatic origin in a tumor with atypical histologic patterns.

PP3-83
PRIMARY MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA OF THE URINARY BLADDER

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Background: Primary malignant lymphoma of the bladder is a rare tumor and account for less than 0.2% of all extranodal lymphomas. We here present a case of primary low-grade B- cell lymphoma of the MALT type of the urinary bladder. Case report: Fifty-six year old male patient presented with frequent urination, dysuria and periumbilical pain. Cystoscopic biopsies from bladder neck, right and left bladder wall, and base were taken. Histological findings revealed a dense lymphoid infiltrate. Widespread and dense mucosal lymphoepitelial lesions were present. The infiltrate mainly were composed of centrocyte-like cells and infrequent immunoblast/centroblasts. Scattered lymphoid follicles that had active residual germinal centers were seen. Immunohistochemically CD3, CD20, CD30, CD23, CD5, BCL-2, Cyclin D1, and Ki-67 were performed. CD3 was positive in 10% of the small cells, CD20 was positive in 90% of all cells, CD30 was negative, CD23 was positive at follicular dentritic cells in residual germinal centers, CD5 was positive in 10% of the small cells, BCL-2 was positive in 90% of all cells, Cyclin D1 was negative, and Ki-67 was positive in 20% of the neoplastic cells. With these histomorphological and immunuhistochemical findings, a diagnosis of extranodal marginal zone lymphoma (Malt lymphoma WHO-REAL), was given. Conclusion: Urinary bladder's lymphomas are divided into three clinical groups: 1- Primary lymphoma localized in the bladder, 2- Lymphoma presenting in the bladder as the first sign of disseminated disease (Nonlocalized lymphoma), 3- Secondary lymphoma. Our case was a primary lymphoma. Clinical and histopathological diagnosis of the MALT type lymphomas may be difficult. Immunohistochemical findings help us in recognition of these tumors in cases displaying lymphoepithelial lesions and a dense lymphocytic infiltration.

PP3-84
MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CRITERIA OF PROGNOSIS IN RENAL CELL CARCINOMA
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Aim of the study. To estimate histopathological and immunohistochemical features of renal cell carcinoma with regard to clinical course and prognosis. Material and methods. Biopsy specimens from 47 patients (aged 35-75) with renal cell carcinoma were studied histologically and immunohistochemically. Grading was performed according to Fuhrman. Immunohistochemistry was done for Ki-67, p53, bcl-2, CD34, N-cadherin and bax. Clinical records were evaluated with regard to type of surgery (resection or nephrectomy), tumor size, relapse and metastases. 13 patients showed relapsing and/or metastatic disease during 6-18 months follow-up after surgery, in 34 - the disease was local and non-progressive. Statistical analysis included p values and correlations. Results. Nearly all cases with progressive disease showed marked histological atypia. In these cases the capsule was thin or absent, the tumor had 3d -4th nuclear grade, and was characterized by low expression of N-cadherin and high proliferation rate (assessed by Ki-67 immunostaining). No clear difference was seen in bcl-2 and bax expression between the groups. A common feature associated with progressive disease was abundance of sinusoidal vessels, and invasion of tumor cells into these vessels was frequently seen. Expression of p53 gene product showed strong positive correlation with tumor nuclear grade. Conclusion Renal cell carcinomas with marked histological atypia, 3d -4th nuclear grade, having thin or no capsule, with abundance of sinusoidal vessels, low expression of N-cadherin and high proliferation rate are associated with progressive disease and unfavorable prognosis. Size of the tumor had minor influence on clinical course and prognosis in our setting.

PP3-85
p27 AND HIGH MOLECULAR WEIGHT CYTOKERATIN EXPRESSION IN PROSTATIC ATROPHY AND POST-ATROPHIC HYPERPLASIA
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Background: Longstanding chronic inflammation and atrophy of the prostatic glands have known as linked entities. Development of carcinoma in a background of chronic inflammation has shown recently in several organs. This association between inflammation and carcinoma has also been supposed as a potential mechanism regarding the prostatic carcinogenesis. Atrophy of the prostate is identified as a reduction in the volume of preexisting glands and stroma and can be divided into two major patterns, diffuse and focal. Recently, a theory was suggested as the atrophy of prostate glands and the subsequent proliferation of the glandular epithelium represent the initial lesion, which can develop into cancer. Prostatic epithelium is stained with cytokeratins and p27 immunohistochemically. p27 is a cyclin-dependent kinase inhibitor which decreases in majority of prostatic adenocarcinomas and high-grade prostatic intraepithelial hyperplasia. In this study, we evaluated the expression of p27 and high molecular weight cytokeratin (HMWC) in prostatic epithelium. Method: The study was performed in 15 prostate tissues with prostatic atrophy and post-atrophic hyperplasia. Prostate tissue samples were stained with p27 and HMWC immunohistochemically. Percentage of nuclear staining in epithelial cells with p27 was assessed semiquantitatively. T-test was used for statistical analyses. Results: All atrophic and post-atrophic hyperplastic epithelial cells were stained with HMWC discontinuedly. The average count of p27 positive cells per 1000 cells were found 67 in atrophic glands and post-atrophic hyperplastic glands, but 79 in normal prostatic glands. There were statistically significant difference between p27 values of atrophic, post-atrophic hyperplastic glands and normal prostatic glands ($p=0.001$). Conclusion: Downregulation of p27 is seen in prostatic epithelial cells of atrophy and post-atrophic hyperplastic glands. Considering the suppressor role of p27 on cell proliferation, downregulation of p27 may be a marker of carcinogenesis. Specific staining pattern of HMWC may be helpful to distinguish foci of atrophy from normal prostate glands.

PP3-86
MIXED GERM CELL – SEX CORD STROMAL TUMOR OF THE TESTIS. A CASE REPORT.
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Introduction: The mixed germ cell – sex cord stromal tumor of the testis is defined as neoplasm having neoplastic germ cells elements and neoplastic sex-cord stromal elements arranged in a diffuse pattern. Case report The case of a 45-year-old man with painless right sided testicular tumor is reported. Radiologic and laboratory investigations including serum alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) showed no abnormalities. A right radical orchidectomy was performed. A histologic examination showed biphasic tumor which was composed of two different types of cells. The majority of the tumor consisted of quite unifom predominantly spindle cells, which were immunohistochemically positive for vimentin and S-100 protein and focally for α-inhibin. The second cell type were large cells with abundant clear cytoplasm arranged in a small clusters at the periphery of the tumor. These cells showed no reactivity for immunostains mentioned above neither for cytokeratines MNF 116 and 7, AFP, HCG and PLAP. Conclusion As any rare neoplasm the mixed germ cell – sex cord stromal tumor of the testis makes a huge diagnostic problem. Clinical and biological behavior of this type of testicular neoplasm is not yet completely discovered.

PP3-87
PREDICITVE VALUE OF HISTOPATHOLOGICAL FEATURES OF HIGH GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA FOR PROSTATIC ADENOCARCINOMA
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Background: High grade prostatic intraepithelial neoplasia (HGPIN) is considered precursor of prostate carcinoma. Its detection in biopsy specimens warrants repeat biopsy for concurrent or subsequent carcinoma. Aim: In this study we aimed to determine the incidence of cancer in prostate needle biopsies after an initial diagnosis of HGPIN and ascertain whether histologic and/or clinical findings can help to predict the increased risk of finding cancer on repeat biopsy. Methods: There were 49 patients diagnosed as isolated HGPIN between January 1996 and December 2005 and had at least one repeat biopsy. Each case was reviewed and the following histopathological features were evaluated on each needle biopsy: number and

percentage of cores involved by HGPIN, number and percentage of HGPIN glands, percentage of gland involved by HGPIN, linear measurement and percentage of HGPIN glands in whole biopsy, pattern of HGPIN, feature of pattern (pure vs combined); nucleolar prominence, pleomorphism and mitotic activity in HGPIN glands. Clinical parameters including the age and preoperative PSA levels were recorded. The pathological results in repeat biopsies were divided into 3 groups: benign, HGPIN and cancer. Relationship of morphological findings of HGPIN and the clinical outcome were evaluated by chi-square and t test. Results: Prostate cancer was detected in %16.3 of the cases in repeat biopsies. Pure pattern of HGPIN and presence of pleomorphism in HGPIN were predictive to determine the cancer on repeat biopsy ($p=0.013$, $p=0.02$ respectively). The difference between groups for other morphological or clinical parameters were not significant ($p>0.05$). Conclusion:Our results indicate that presence of cellular pleomorphism and pure pattern of PIN in needle biopsies are the histological features requiring close follow up for unsampled synchronuous or metachronous prostate cancer.

PP3-88

IS THE OUTER PERIPHERAL ZONE / SUB CAPSULAR TISSUE COMPARTMENT OF THE PROSTATE MORE SUSCEPTIBLE TO CARCINOGENESIS AND EARLY NEOPLASTIC TRANSFORMATION? A TOPOGRAPHIC MORPHOLOGIC AND TISSUE MICRO ARRAY IMMUNOHISTOCHEMICAL STUDY

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Background: The approach to prostate cancer detection using needle biopsies evolved in recent years to obtain more biopsy cores and to concentrate on sampling the outer and sub capsular areas of the gland including the apical/anterior compartment. Several studies have demonstrated that this modification from the traditional “sextant” method results in better detection of particularly smaller tumors. Our objectives were to 1-document the anatomical distribution of morphological lesions characteristic of early prostatic neoplasia including high grade prostatic intraepithelial neoplasia (HGPIN) and small foci of prostatic carcinoma (PCA) in two large series of radical prostatectomy specimens and of prostate gland examined in a post mortem setting. 2- To explore whether the outer glandular epithelium is likely to express markers associated with early changes of carcinogenesis. Method: Foci of PCA and HGPIN were mapped in a series of 83 consecutive, entirely embedded glands obtained during autopsies of young men (age 22-55) and in a series of 227 radical prostatectomy specimens (RPS) harboring small tumors (total tumor volume <=1 c). The distance of PCA and HGPIN foci from the outer surface of the gland was measured. Using the hematoxilin and eosin stained section, tissue microarrays (TMAs) were constructed from benign glandular epithelium of both subcapsular and inner peripheral zone locations from both the RPS and the autopsy prostates. The TMAs were stained with cell cycle regulator/proliferative markers (p27 and Ki-67) and with Glutathione S-Transferase 1 (GSTP1), Results: Of the 235 foci of PCA found in the 83 autopsy gland, 191 (81%) were within 5 mm of the outer surface of the gland. Similarly 176 of the 223(79%) foci of HGPIN in this cohort had the same distribution. For RPS, 112 (49%) of the small volume tumors were also within 5 mm of the outer surface. GSTP 1 showed less diffuse distribution and with lower intensity in the outer versus the inner compartment of the gland. Similar pattern was found with p27 immunoreactivity while the proliferation rates were low in both compartments but slightly higher in the most outer epithelium. Conclusion: Most small volume PCA, (both sub clinical and low stage clinically detected), and most HGPIN are present in the outer subcapsular prostatic tissue. Our morphologic and TMAs findings indicate a higher vulnerability of the epithelium in the outer peripheral zone to early neoplastic transformation and are consistent with the recent biopsy sampling approaches.

PP3-89

MORPHOLOGICAL FEATURES OF KIDNEY CYSTS OF III BOSNIAK CLASSIFICATION TYPE

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Kidney cysts of III Bosniak classification type are the most complex problem in malignancy potential assessment and adequate treatment strategy selection. The aim of the research was to investigate morphological features of III type kidney cysts. 15 kidneys operated under following conditions were investigated: kidney cancer (5), multilocular cysts with possible malignant transformation (5), multicystic dysplasia of the kidney (3), benign cysts (2). There were investigated 10 males and 5 females. The median age was 54.8±2.35 years. All samples were of the following histological structure: clear cell renal cell carcinoma with cystic degeneration (4 cases), cystic renal cell carcinoma (6 cases), multicystic dysplasia of the kidney (3 cases), angiomyolipoma with hemorrhages and cystic degeneration (1 case). There was one case of synchronous multifocal tumor of the kidney: 3 angiomyolipomas were combined with cystic nephroma adult type. Cystic renal cell carcinomas are characterized by single cysts inner lining preferably of rounded or columnar cells with clear vacuolated cytoplasm, which are much in common with typical clear cell renal cell carcinoma. Similar cells were revealed in cystic cavities fluid under cytological investigation. All tumors were well-differentiated (Fuhrman nuclear grade -1) without invasive growth out of kidneys. Cystic nephroma was characterized by inner lining consists of cubical or hobnail cells monolayer with eosinophilic cytoplasm without signs of nuclear atypism and invasive growth. Thereby III type kidney cysts to Bosniak classification represent the heterogeneous group of tumorous and nonneoplastic diseases demanding preoperational and intraoperational morphologic assay to make a correct choice of type and extent of surgical treatment.

PP3-90

eNOS AND iNOS EXPRESSION IN UROTHELIAL TUMORS OF THE URINARY BLADDER

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Background: NO (nitric oxide) is one of the main factors responsible for the cytotoxic activity that macrophages exert against tumor cells. It also plays an important role in tumor growth and angiogenesis. NOS (nitric oxide synthase) has been found in various tumor types suggesting that NO may be produced in tumor tissue. The purpose of this study was to analyze the expressions of endothelial (eNOS) and inducible (iNOS) isoforms of NOS in the urothelial tumors of different grade and stage and to compare it with clinicopathological findings. Method: The medical records of 87 patients with primary urothelial carcinomas were reviewed. Grade according to WHO 2004 classification, pathological stage, invasion patterns of invasive tumors (nodular, trabecular, infiltrative, micropapillary) and stromal response types (edema and congestion, desmoplasia, fibroblastic proliferation, inflammatory response) were assessed by light microscopy. Paraffin embedded tumor sections were stained immunohistologically with eNOS and iNOS antisera and semiquantitative evaluations were performed. Association of eNOS and iNOS immunoreactivity with tumor grade, stage, invasion patterns and stromal response was examined with their

prognostic significance in predicting tumor recurrence. Statistically p values of less than 0.05 was considered statistically significant. Results: Both iNOS and eNOS expressions in tumor cells were heterogeneous, ranging from diffuse but weak expression to focal but severe staining. 73 % and 88% of tumors showed iNOS and eNOS expressions respectively. eNOS was also identified in endothelial cells. Stromal inflammatory cells showed varying degrees of iNOS expression but it was occasional. Neither eNOS nor iNOS immunoreactivity did correlate with tumor grade, stage or invasion patterns ($p>0.05$). On the other hand, tumor grade and stage were found to be statistically correlated with the invasion patterns of tumors ($p<0.05$). Conclusion: Although biological role of NO in urothelial carcinomas is still unclear, this study demonstrated that both iNOS and eNOS were being expressed in urothelial tumors. Our results were inconsistent with the literature in that we observed eNOS expression in tumor cells together with the endothelial cells of the capillaries. A clear correlation with tumor grade, stage and recurrence rate was not identified.

PP3-91
EXPRESSION OF CYCLIN D1, p16 AND Ki67 IN PROSTATE CARCINOMA

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Background: Alterations in cell cycle regulators and subsequent deregulation of the G1-S transition may cause uncontrolled cell cycle progression and may implicate in the development and progression of prostate cancer. The aim of this study was to investigate the expression of cyclin D1, p16 and Ki67 in prostate carcinoma and to correlate them with clinicopathological parameters. Materials and methods: Formalin-fixed paraffin-embedded tissue from 50 cases of radical prostatectomy was stained by immunohistochemistry for cyclin D1, p16 and Ki67. The anti-cyclin D1 (Neomarkers), the anti-p16 (NeoMarkers) and the anti-Ki67 antibody (MIB-1, Dako) monoclonal antibodies were used at dilution 1:30, 1:50 and 1:30, respectively. A step of microwave heating in a solution of sodium citrate was performed prior to incubation with the antibodies. Associations between p16 (<20% vs. [GTEQ]20%), Gleason's score (<7 vs. [GTEQ]7) and cyclin D1 (<10% vs. [GTEQ]10%), were evaluated using Fisher's exact test, while for Ki67 (median 8%, range 2%-30%) with all the above, Wilcoxon was adopted. Results: Cyclin D1 positive nuclear staining was found in 12/50 (24%) prostate carcinomas (14% of low and 86% of high grade). Benign prostate acini showed focal scattered cyclin D1 positivity of the basal and secretory epithelial cells. P16 positive nuclear and cytoplasmic staining was observed in 23/50 (31% of low and 69% of high grade). Positive staining was not found in benign prostate or hyperplastic acini. Ki67> median was found in 36/50 (40% of low and 60% of high grade). Cyclin D1 expression showed a statistically significantly correlation with higher Gleason's score ($p=0.04$) and advanced tumour's stage ($p=0.03$).There was a tendency for a significant relationship between p16 expression and Gleason's score (p-value=0.13) and a statistically significantly correlation between p16 and stage ($p=0.0003$). A trend for an inverse relationship between cyclin D1 and p16 was detected ($p=0.09$). A trend for a parallel expression between cyclin D1 and Ki67 was found. Conclusions: Cyclin D1 functions mainly in promoting cell proliferation and seems to play a role in the pathogenesis of prostate carcinoma. Increased p16 expression during tumour progression suggests that the senescence pathways are still intact in large number of prostate carcinomas.

PP3-92
FAS LIGAND EXPRESSION IN PROSTATE ADENOCARCINOMAS AND ITS RELATIONSHIP WITH HISTOPATHOLOGICAL FEATURES

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Background: Fas ligand (FasL) is a type II transmembrane tumor necrosis factor family protein, known to trigger apoptosis in cells that bear the FasL receptor, Fas. The aim of the study was to examine the expression of FasL in benign prostatic epithelium (BP) and prostate carcinoma (PC) and to investigate its relationship with histopathological features in PCs. Method: The study included 51 primary PCs (22 radical prostatectomy and 29 transurethral resection) and 28 BP specimens. All slides were re-evaluated histopathologically. In all cases Gleason grading and scoring, and in radical prostatectomy cases, extraprostatic extension, vascular and perineural invasion, high grade prostatic intraepithelial lesion (HGPIN), surgical margin status were recorded. Using tissue microarray slides, FasL examination was performed immunohistochemically. The staining was evaluated semiquantitatively as percentage of the stained cells as 1+, focal (<10%); 2+, moderate (10-50%); 3+, diffuse (>50%), and staining intensity was considered negative (0), mild (+), moderate (++), strong (+++). Results: Cytoplasmic and focal membranous FasL immunoreactivity was observed in 82.1% of BP (23/28) and 94.1% of PCs (48/51). The percentage of stained cells in PCs were classified as 10 (19.6%) focal, 10 (19.6%) moderate and 28 (54.9%) diffuse, and in BP cases, 8 (28.6%) focal, 10 (35.7%) moderate and 5 (17.9%) diffuse, respectively. The percentage of the stained cells with FasL in PCs was significantly higher compared with that in BP tissues ($p=0.003$). The PC cases with higher Gleason scores showed significantly much higher positively stained cells compared with that in lower ones ($p=0.045$). The intensity of FasL staining was also significantly different between PC and BP specimens ($p=0.005$). Mild (+) and moderate (++) staining was seen in 15 (53.6%) and 8 (28.6%) of BP specimens, respectively. Conversely, 20 (39.2%), 18 (35.3%) and 10 (19.6%) PC cases showed mild (+), moderate (++) and strong (+++) staining, respectively. In radical prostatectomy cases, the percentage of FasL staining was also significantly higher in cases with higher Gleason score and HGPIN compared with that in cases with lower Gleason score and without HGPIN ($p=0.022$, $p=0.024$, respectively). Conclusions: The increased FasL expression and its association with higher Gleason scores in prostatic adenocarcinomas suggest that FasL may be important biologic marker in the growth and progression of prostatic cancer and potential target of therapeutic intervention.

PP3-93
PATHOLOGIC CHARACTERISTICS OF PROSTATE CANCER IN AFRICAN AMERICAN AND CAUCASIAN MEN DIAGNOSED AND TREATED AT OR BELOW 50 YEARS OF AGE: RACIAL DIFFERENCES ARE MOST EVIDENT IN THE YOUNGER AGE BRACKET

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Background: Data from Wayne State University suggest that the racial discrepancies in the incidence and outcome of prostate cancer between African American (AA) and Caucasian (C), men

maybe more pronounced within the younger patient groups. The objective of this study was to compare pathological findings in both the diagnostic needle biopsy (NB) and the radical prostatectomy specimens (RPS) for the subset of men diagnosed, and the overlapping but no identical subset, who were treated by surgery at our institution. Design: For the NB subset, diagnostic serum PSA, number of cores involved by tumor and Gleason score for each involved core were recorded. For the RP subset, pathological stage, Gleason score, calculated tumor volume and extensiveness of high grade PIN were documented. Statistical analysis of the findings comparing pathological parameters between the two races for each subset was performed. Result: For the biopsy subset, 116 AA and 81 C patients were included in the study. The mean and median age for both groups were identical. Mean and median diagnostic PSA for AA were 7.4 and 5.5 ng/ml compared to 7 and 7:00 and 5.6 ng/ml for C [p< 0.05 for both values]. Forty five of the 116 (39%) AA patients, had three or more core involved by carcinoma compared to 19 of 18 (23%) C patients [p<0.05]. Nine Ten of 116 (8%) and 4 of 81 (5%) AA and C patients respectively had a biopsy Gleason score of 8 [p<0.06]. The RP cohort, included a nearly identical number of AA and C, (82 and 83 patients respectively). Organ confined (pT2), disease was found in 52 of 83 (63%) AA vs 44 of 82 (53%) C, [p<0.05]. While Gleason score distribution was similar, slight difference was found in the percentage of patients having Gleason pattern 4 or higher as their primary pattern (17% AA, 13 % C, [p= NS]). Larger tumors of 4 cc or greater were found in 31% of AA compared to 19% of C patients, [p<0.05]. Extensive HGPIN with diffuse involvement of the gland was evident in 52% vs 22% AA and C men respectively, [p<0.0005]. Conclusion: Our data suggest that the difference in pathological parameters indicative of a more aggressive prostate cancer appear to be sharper within the younger cohort of AA and C patients diagnosed with the disease. These data may indicate a potentially different pathogenesis of the disease in the younger African American population.

PP3-94
POTENTIAL DIFFERENCES IN THE CLINICOPATHOLOGICAL PROFILE OF FAMILIAL/HEREDITARY AND SPORADIC PROSTATE CANCER

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Background: There is increasing evidence implicating family history as a distinct risk factor associated with the development and possibly the aggressiveness of prostate cancer. Recent epidemiologic studies suggest a stronger familial aggregation for prostate cancer than that associated with colon or breast cancer, two malignancies with well recognized familial components. The objective of this study was to compare the clinicopathological characteristics of the patients with family history to that of the remaining radical prostatectomy patients. METHOD: Between1991 and 1997, the 1,321 patients treated by radical prostatectomy for clinically localized prostate cancer were considered for the study. The clinical history /record of our database indicated that 1074 (81.3%) of those patients were considered as having “sporadic” prostate cancer with no familial links while 247 (18.6%) met the criteria for familial/ hereditary distribution of the disease, (information self reported by patients). Of the 247, 120 were Caucasians, 114 African Americans and 13 belonged to other ethnic groups. Patients with affected first degree relative (father) or second degree relative (brother “s”) were used for this study. Results: The median preoperative PSA was 8.0, 7.9, 7.7 ng/ml in control group, father family history and brother history groups respectively. [p=NS], while the mean age at surgery was significantly younger for men with a history of a father having PCA vs the control 57 and n65 years respectively [p<0.05]. The percentages of patients with organ confined diseases were 40.4%, 47.1%) and 43.6% for patients with history of a father, a brother or no family member having PCA respectively. The corresponding figures for patient with extraprostatic extension were 18%, 16.9 % and 15 % respectively. Approximately one third 29.7% of patients with history of a father having PCA had Gleason Score 6 compared to 18.8% for patients with history of a brother having PCA and to 22.8 % for the control group. There were no differences among patients with Gleason Score 7 or higher. Median tumor volume was 3.33, 4.14 and 3.42 for patients with a history of a father, brother or no family member with PCA respectively. Conclusion A subset of patients with family history (those who have a first degree relative; father), with PCA had an earlier age of onset. The several parameters compared otherwise between the sporadic and the familial cases were not significantly different.

PP3-95
PRESERVATION OF ANTIGENICITY IN NECROTIC POST-CHEMOTHERAPY CHORIOCARCINOMA

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Introduction. Immunohistochemistry is occasionaly used to demonstrate cell lineage in spontaneously necrotic tumors, mainly carcinomas and lymphomas. Antigen preservation in necrotic post-chemotherapy germ cell tumors of the testis has not been reported. Objective. To test the immunoreactivity of necrotic metastates of testicular germ cell tumors. MATERIAL AND METHODS. Immunostaining for Cam5.2, EMA, CD10, αFP, PLAP and HCG on formalin fixed paraffin embedded tissue from resected residual metastatic masses (retroperitoneal lymph nodes: 9 specimens; lung: 6; liver: 2: mediastinal lymph nodes: 1) in patients treated with chemotherapy for germ cell tumors of the testis. Results. In the specimens from 12 patients necrotic tumor immunostaining was retained for Cam5.2, CD10 and HCG. The stained cells were mainly shadow syncytiotrophoblastic giant cells. The time from completion of chemotherapy to the resection of the residual tumors ranged from three to ten weeks. Post-chemotherapy HCG serum levels were normal in all cases (pre-chemotherapy levels were elevated in each case). PLAP, EMA, αFP and CD30 did not stain the necrotic tumor tissues. CONCLUSION. Necrotic post-chemotherapy germ cell tumors of the testis may retain immunoreactivity for Cam5.2, CD10 and HCG, thus allowing a positive diagnosis of necrotic choriocarcinoma.

PP3-96
EXPRESSION OF MUCINS AND CD10 IN ADENOCARCINOMA OF THE URINARY BLADDER

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Introduction: Mucins (MUCs) are high molecular weight membrane glicoproteins. Expression of MUC 1, 2, 5AC, and 6 genes may change during malignant transformation of epithelial

tissues according to characteristic pattern. CD10/neutral endopeptidase (NEP) is a membrane-bound zinc metallopeptidase which is expressed in urothelial neoplasms and normal urothelium. The aim of this study was to evaluate the expression of MUC1, MUC2, MUC5AC, MUC6 and CD10 in order to determine immunoprofile of the primary adenocarcinoma of the urinary bladder. Methods: Research was performed in 3 patients with vesical adenocarcinoma, at the Institute of Pathology Clinical Centre Nis. The specimens were fixed in 10% formaldehyde and embedded in paraffin. The sections were stained with HE. We detected mucin by immunohistochemical staining using the following monoclonal antibodies from Novocastra, Newcastle, UK: MUC1, MUC2, MUC5AC, MUC6 and CD10. As a control we used the normal surrounding urothelium. Results: All of the vesical adenocarcinoma displayed expression of MUC 1 (luminal membrane staining) and MUC 2 (cytoplasmic staining); MUC 6 in 2/3 cases, while MUC5AC was not detected. Expression of CD10 was present in one case. In the normal urothelium MUC1 was limited predominantly to the apical membranes of the umbrella cell layer, while CD10 was found to be expressed weakly or reaction was negative in the apical portion of the cytoplasm along the luminal surface. Conclusions: The mucin expression profile in adenocarcinoma of the urinary bladder allows the identification of MUC 1 together with intestinal mucin (MUC 2), and in certain cases MUC6. Expression of CD10 is indicative for absorptive-cell differentiation in vesical adenocarcinoma.

PP3-97

METANEPHRIC ADENOMA: A RARE BENIGN TUMOUR OF KIDNEY

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Introduction: Metanephric adenoma is a rare epithelial tumour composed of small, uniform, embrionic-appearing cells. It occurs in children and adults with female predominance. Approximately 50% of these tumours are incidental findings and they are categorized as benign metanephric tumours of kidney. Case Report: A 31-year-old female patient suffering from abdominal pain underwent ultrasonographic examination and a right renal mass was detected. Computerized tomography revealed a cystic renal mass located in the medial portion of upper pole of right kidney, which measured 3.5x3 cm in dimension. The laboratory findings, including blood counts and biochemical data, were in normal range. With the presumption of renal carcinoma an operation was planned and she underwent partial nephrectomy. Pathological examination, macroscopically, revealed a centrally cystic mass of 3.5x3x3 cm in dimention, with central haemorrhage and cut surface of grayish-tan in colour. Microscopically, a highly cellular tumour composed of tightly packed small, uniform, round tubular and acinar structures, encapsulated with thick fibrous tissue was seen. The cells were monotonous, with small uniform round to oval nuclei with delicate chromatin and inconspicious nucleoli. The cytoplasms were pale and scant. There was loose oedematous and focally hyalinized stroma. At the periphery of the tumour, there were many psammoma bodies. No mitotic figures were seen. Immunohistochemically, the tumour cells show diffuse and strong nuclear staining for WT-1, and cytoplasmic staining for vimentin and high molecular weight keratin. Also, moderate CD57 staining was detected in most tumoural cell cytoplasms. EMA, CK7 and low molecular weight keratin showed only focal positive staining. CD10 and RCC were negative. Conclusion: Metanephric adenoma is an uncommon, morphologically distinct tumour type, with characteristic histopathological features. Despite its size, it is benign and should be distinguished particularly from papillary renal cell carcinoma type 1 and Wilms' tumour. These unique features of metanephric adenoma should be pathologically and clinically recognized because of its invariably benign course.

PP3-98

CYTOKERATIN 7 AND ANTIMITOCHONDRIAL ANTIBODY EXPRESSIONS IN DIFFERENTIAL DIAGNOSES OF RENAL EPITHELIAL NEOPLASMS WITH EOSINOPHILIC CYTOPLASM

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BACKGROUND: The distinction between epithelial renal tumors with eosinophilic cytoplasm, namely, chromophobe renal cell carcinoma (ChRCC), the eosinophilic (granular) variant of clear cell (conventional) renal cell carcinoma (CRCC), and oncocytoma may remain questionable in some cases because of overlapping morphologic features. We evaluated cytokeratin 7 (CK 7) and antimitochondrial antibody (AMA) expressions to determine their potential value in distinguishing these tumors. METHOD: In this study, 22 renal tumors were included. They consisted of 11 ChRCCs, nine oncocytomas and two eosinophilic variant of CRCCs. Immunohistochemical study was performed on formalin-fixed, paraffin-embedded tumor tissue samples of these cases for CK 7 and AMA using streptavidin-biotin method. RESULTS: CK 7 staining: Seven cases of 11 ChRCCs showed strong cytoplasmic immunoreactivity with conspicuous peripheral accentuation. Among the nine oncocytomas, one case was positive, three cases were completely negative and five cases showed only focal staining in less than 5% of the tumor cell population. For two eosinophilic variant of CRCCs, only one of them was immunoreactive with CK 7. AMA staining: Five of 11 ChRCCs showed diffuse cytoplasmic coarse granular immunostaining with peripheral accentuation. Eight of nine oncocytomas demonstrated with diffuse cytoplasmic, but fine granular staining, and lastly, in one of the two eosinophilic variant of CRCCs, diffuse cytoplasmic and haphazardly distributed coarse granular immunoreactivity was observed. Combined interpretation of CK 7 and AMA stainings: Five of 11 ChRCCs showed combined CK 7 (+) and AMA (+) with diffuse coarse granular with peripheral accentuation. Eight of nine oncocytomas were CK 7 (-) and AMA (+). One of the eosinophilic variant of CRCCs was also CK 7 (-) and AMA (+) like in most of the oncocytomas, but the staining pattern of AMA was randomly distributed coarse granular, rather than fine granular which was observed in oncocytomas. CONCLUSION: When we reviewed all cases retrospectively with these immunohistochemistry results, we concluded that three of the ChRCCs were needed to be diagnosed as oncocytomas and two of them were eosinophilic variant of CRCCs. Similarly, one eosinophilic variant of CRCC was actually a ChRCC according to the CK 7 / AMA expression patterns. Lastly, only one of the nine oncocytomas was immunohistochemically consistent with the ChRCC. In conclusion, these results suggested that using CK 7 and AMA together may be a discriminative diagnostic tool in the differential diagnoses of renal epithelial tumors with eosinophilic cytoplasm, in addition to distinctive immunostaining patterns of AMA expresion

PP3-99

ONCOCYTIC PAPILLARY RENAL CELL CARCINOMA

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Introduction: Papillary renal cell carcinoma (RCC) is subclassified in type 1 displaying cells with scanty pale, cytoplasm arranged in a single layer and in type 2 showing

pseudostratified cells with eosinophilic cytoplasm. However, the existence of more variants of papillary RCC may be inferred by the recognition of few cases with different morphological features. Materials and methods: We report a series of 3 oncocytic renal papillary tumors with the aim of determining their clinicopathologic features. Results: Two patients were male and 1 female (median age, 66 years). All our patients were symptomatic (3/3) flank pain (1/3), hematuria (2/3). All of them were treated by radical nephrectomy. On macroscopic exam, Tumors (median size, 5,5 cm) were intrarenal , unifocal and well limited, with no extrarenal extension. Histologically, they consisted of thin, nonfibrotic papillae lined by a single layer of oncocytic cells, with finely granular eosinophilic cytoplasm and round regular nucleus exhibiting central nucleolus (Fuhrman grade 2). All cases were positive keratin 7, vimentin, racemase and ckit positivity in one case. Then immunohistochemical profiles and the pathologic features argued against the diagnosis of oncocytoma and suggested our cases to be part of the papillary renal cell carcinoma group. Discussion: Papillary architecture is not a feature of renal oncocytoma. In contrast, papillary renal cell carcinomas are defined by their papillary or tubulopapillary architecture. Papillary renal tumors with oncocytic cells might be a distinct variant in the papillary renal cell carcinoma group. All These results suggest that adult papillary renal tumors with oncocytic cells might be a distinct variant in the papillary renal cell carcinoma group. This distinction is of importance because oncocytomas are benign tumors with an indolent course, whereas type 2 papillary carcinomas are malignant tumors associated with a poor outcome. In conclusion, we describe an oncocytic variant of papillary RCC with distinct clinicopathologic features from type 1 and type 2.

PP3-100
EXPRESSION OF Ki-67, p53 AND E-CADHERIN IN RAT UROTHELIAL CARCINOGENESIS INDUCED BY N-BUTYL-N-(4-HYDROXYBUTYL) NITROSAMINE

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Background: N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) induced urothelial carcinogenesis is a useful model for studying urothelial tumours. The present study investigated the expression of Ki-67, p53 and E-cadherin in chemically induced rat urothelial lesions. Material and Methods: Female Fisher 344 rats (n=40) were given 0.05% BBN in their drinking water for 10 and 20 weeks and then euthanized. Twenty animals were used as negative control. Results: At the 10th week 100% of rats exposed to BBN exhibited simple hyperplasia, 40% nodular hyperplasia, 90% dysplasia, 20% papiloma, 10% of papillary neoplasm of low-malignant potential, and 20% squamous metaplasia; after 20 weeks 30% of rats had developed simple hyperplasia, 80% nodular hyperplasia, 100% dysplasia, 40% papilloma, 50% papillary neoplasm of low-malignant potential, 70% low-grade carcinoma, 20% high grade carcinoma, 10% invasive carcinoma, and 60% squamous metaplasia. Ki-67, p53 and E-cadherin were analysed immunohistochemically in paraffin embedded tissue. Low- and high-grade papillary carcinoma showed labelling and apoptotic index greater than other lesions ($p<0.05$). Simple hyperplasia and squamous metaplasia showed a similar E-cadherin pattern when compared with normal urothelium, with their expression confined to the cell membrane. Nodular hyperplasias, dysplasias and papillomas exhibited weak E-cadherin expression. All papillary neoplasm of low-malignant potential, low-and high-grade carcinoma revealed an increase in cytoplasm reactivity and cell discontinuous membrane positivity. E-cadherin immunoreactivity correlated significantly with p53 index ($p<0.05$) and Ki-67 ($p<0.05$) antigen index. Conclusion: We concluded from our data that immunohistochemical Ki-67, p53, E-cadherin staining may provide additional information on rat urothelial carcinogenesis.

PP3-101
MALACOPLAKIA OF THE GENITOURINARY TRACT: A CLINICOPATHOLOGICAL STUDY OF 5 CASES

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Introduction: Malacoplakia (MPK) is a rare granulomatous inflammatory disease that predominantly affects the urinary tract (60%) with a predilection for the bladder while renal parenchymal involvement is less common, occurring in 16% of cases. Testicular and prostatic MPK are exceedingly rare. This benign lesion is histologically characterized by histiocytes containing distinct basophilic inclusions called “Michaellis-Gutmann bodies” which are believed to result from abnormal macrophage function. Patients & Methods: Between January 1999 and February 2007, 5 cases of genitourinary tract MPK involving the prostate (n = 2), the bladder (n = 1), the kidney (n=1) and the testis (n=1) were diagnosed at the pathology department of La Rabta Hospital. Medical records and microscopic slides of each patient were retrospectively reviewed. Results: There were 3 male and 2 female patients aged between 50 and 80 years (mean age = 65,40 years). The chief complaints were fever (n=3), dysuria (n=3), nocturia (n=2), bladder outlet obstructive symptoms (n=2), lumbar pain (n =1) and testicular swelling (n =1). One patient underwent right nephrectomy for a cystic renal mass associated with pyelonephritis. Radical orchidectomy was performed in another patient. A prostatic (n=2) and vesical (n=1) biopsy under ultrasonic guidance was realized in the remainder of cases. Histological examination of the surgical and biopsy specimen showed a granulomatous inflammatory process characterized by the presence within histiocytes (von Hansermann‘s cells) and also extracellularly in the stroma, of concentrically layered, target or ring-like structures that reacted positively to periodic Schiff reagent. The final pathological diagnosis was malacoplakia. During the follow-up period that ranged between 6 months and 3 years, all patients were well and alive.

PP3-102
DIAGNOSTIC AND PROGNOSTIC VALUES OF c-KIT, Ki–67, MATRIX METALLOPROTEINASE-1 (MMP-1) EXPRESSIONS IN GERM CELL TUMORS OF TESTIS

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Germ cell tumors constitude 90-95% of all the testis tumors. Testicular germ cell tumors may be either in pure or in mixed form. Pure germ cell tumors are divided into two groups as seminomatous (seminoma and spermatocytic seminoma) and non-seminomatous (embryonal carcinoma, yolk sac tumor, trophoblastic tumor and teratoma) tumors. Mixed germ cell tumors include mainly embryonal carcinoma-teratoma, teratoma-seminoma, choriocarcinoma and different combinations of the others. H&E stained slides of 49 cases found in the archieves of Cumhuriyet University Faculty of Medicine Pathology Department which were diagnosed as germ cell tumor were reexamined according to the criterias of WHO defined in 2004. Slides obtained from paraffine blocks were stained with PLAP,

C-kit, Ki-67 and MMP-1 immunohistochemically. When pure and mixed germ cell tumors were reexamined distribution of the tumors were found as 30 cases of seminoma, 25 cases of embryonal carcinoma, 13 cases of yolk sac tumor, 14 cases of teratoma and two cases of spermatocytic seminoma. Statistical analysis of the study was done among the tumors with different histopathological types which also include intratubular germ cell neoplasia. In respect to PLAP and C-kit expression there is a significant difference betweeen seminoma and non-seminomatous tumors which have strong and intermediate membranous staining property ($p<0.05$). The highest Ki-67 proliferation index was determined in spermatocytic seminoma (0.63±0.01) and embryonal carcinoma (0.50±0.12). The difference between these two tumors and the others having low proliferation index such as seminoma, yolk-sac tumor, and teratoma was also significant ($p<0.05$). As a result, strong and intermediate membranous staining with PLAP and C-kit is a reliable marker in differential diagnosis between seminoma and non-seminomatous tumors. PLAP expression suggested the diagnosis of IGCN more than C-kit. When we considered the Ki-67 staining property, spermatocytic seminoma and embryonal carcinoma had higher proliferation indexes than the other germ cell tumors. Increase in Ki-67 index with an increase in primary tumor stage showed that those markers are important in prognostic aspect.

PP3-103
IMMUNOCHEMICAL FRACTAL ANALYSIS OF NEOVASCULAR NETWORK IN PROSTATE CANCER

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BACKGROUND: Fractal analysis is being applicated in the field of urological pathology with increasing frequency. In the present study, fractal analysis was performed in order to be investigated whether prostate cancer neovascular network fractal dimension (FD) differs from that of normal prostate vasculature. METHOD: Archival material from 34 cases of prostate cancer as well as 10 normal or hyperplastic prostates were examined. From each case two representative CD34 immunohistochemically stained fields (x200) were randomly selected and digitised as high quality JPEG files. Using especially designed image analysis applications the images were automatically processed to threshold the vascular section, by the use of a modified and extended clastering method for colour images based on an algorythm proposed by Otsu. The solid areas of the binary images were then converted to an outline of single pixels, and finally the fractal dimension was estimated using a box - counting algorythm implemented in our application. RESULTS: The normal prostate vasculature presented a mean FD of 1,959. The corresponding value of prostate cancer neovascular network was 1,3297. Both values were statistically different from the topological dimension (= 1,000). The difference of the two values was statistically significant (all $p<0,05$). CONCLUSION: Our findings suggest that by using the proposed methodology the vascular network of prostate cancer presents fractal charateristics. Thus, FD seems to be an important morphometric parameter possibly valuable for the future development of an unbiased and reproducible image analysis system for the study of prostate cancer pathology.

PP3-104
HYBRID RENAL CELL CARCINOMA WITH EOSINOPHILIC CHROMOPHOBE AND UNDIFFERENTIATED FEATURES DEMONSTRATING A THIRD FEATURE WITHIN IPSILATERAL ADRENAL METASTASES

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Background: Hybrid renal cell carcinoma (RCC), containing tumor cells displaying cytological features of different subtypes in the same kidney has been described recently. Although rare, synchronous renal primary malignancies can be observed. Identification of the hybrid character of an RCC has important diagnostic, and potentially, prognostic ramifications. Case report: A 55-year-old male admitted to the intensive care unit, due to idiopathic myelofibrosis, with left lumbar pain of 20 days duration. His past medical history was unremarkable otherwise, and there was no family history of a hereditery disease. Serological studies demonstrated minimal increase in serum BUN and creatinin levels. Abdominal CT 2 cm solid mass in the mid-portion of the left kidney and a×revealed a 3.2 1.5×0.9 cm solid mass in the left adrenal gland. Patient underwent a left radical nephrectomy. Findings: The resected left kidney weighed 357 g, with a 10.5×4.5 cm.×2.8×2.7 cm mass in the mid-portion, measuring 17×3.6 Microscopically, the tumor was an eosinophilic variant of chromophobe RCC (ChRCC/eo) with areas of undifferentiated carcinoma (UC). UC with necrosis and high nuclear grade of Furhman (Grade 3), formed multiple foci within ChRCC/eo, and revealed large polygonal cells with condensed eosinophilic/granular cytoplasm with increased Nuclear size, pleomorphism, high mitotic activity, and 4.5×2 cm,×demonstrated transitions with ChRCC/eo. The left adrenal gland, 6.5 2×1.2 cm solid mass, consisted of a tumor displaying cytological×with a 2.7 features of UC and of mucinous tubular carcinoma (MTC). The adrenal vein and periglandular lymphovascular spaces were extensively invaded by UC panCK, CK7, and EMA were diffusely and strongly positive in all tumor subtypes while, CEA E12 only focally, in UC Conclusion: Hybrid RCCsβwas positive only in MTC, and 34 exhibit characteristic histologic features that set them apart from classical RCC. This type of hybrid tumor seems to be associated with a more aggressive behavior, and warrants diagnostic caution. This case is the first of its type reported in the literature, which is a combination of ChRCC/eo, and UC within the same tumor of the kidney and of a synchronous third subtype, MTC, within the synchronous metastases in the ipsilateral adrenal gland.

PP3-105
RETROPERITONEAL LYMPH NODE METASTASES FROM BURNT-OUT PRIMARY TESTICULAR SEMINOMA. REPORT OF TWO CASES

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The burnt-out phenomenon in germ-cell neoplasia is defined by the presence of an extragonadal germ cell tumour with no tumour at the testis. This tumour has spontaneously regressed with no treatment. We report two cases of burnt-out primary testicular tumours revealed by a retroperitoneal metastatic seminoma. Case 1 A 30-year-old man, with a history of cryptorchidy, was admitted for exploration of a left iliac lymph node mass, 7 cm in diameter. A biopsy specimen suggested a metastasis from a germ cell tumour. At clinical examination, the left testis was small. Case 2 A 40-year-old man presented with flank pain. Abdominal CT scan showed a 9 cm mass left retroperitoneal between the aorta and inferior cava. A lymphadenectomy was performed.

Frozen section analysis suggested lymph node metastasis from testis seminoma. Scrotal ultrasonography in these 2 patients revealed a small left testis containing a 5 mm (case 1) and 1 cm (case 2) hypoechogenic area. Chest CT was normal. They had a left radical orchidectomy and the lymphadenomectomy. The macroscopic examination of the pulp (case 1: 34 g , 3x2 cm; case 2: 46g, 3,5x2,5) showed a 8 mm juxtahilar (case 1) and 1.5 cm central (case 2) fibrous scar. Histological examination showed a testicular atrophy with sclerosis of the seminiferous tubules, hyperplastic Leydig cells, and foci of intratubular germ cell neoplasia unclassified type. associated with fibrous scar and calcifications. Lymph node (n=3) examination confirmed a pure seminomatous germ cell metastatic tumour with an epithelioid giant cell reaction associated with lymphocyte infiltrate. Immunohistochemical staining in lymph node specimen showed a cytoplasmic membrane immunoreactivity for placental alkaline phosphatase and CD117. Chemotherapy was administred. Testicular ultrasonography is today the gold-standard technique and any parenchymal echostructure abnormality should be verified histologically

PP3-106
A SEARCH FOR THE USEFULNESS OF ANTIBODY COCKTAIL (AMACR (P504S)+HMW-CK+P63) IN PROSTATIC NEEDLE BIOPSIES WITH A PROPOSAL OF A NEW SCORING SYSTEM

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BACKGROUND We aimed to search for the usefulness of an antibody cocktail, combining alpha-methylacyl CoA Rasemase (AMACR), high molecular weight cytokeratin (HMW-CK) and p63 antibodies, in differentiating prostate cancer (Pca) from high-grade prostatic intraepithelial neoplasia (HGPIN), atypical small acinar proliferation (ASAP) and benign glands. We also tried to evaluate whether to use HMW-CK singly or to use the antibody cocktail. MATERIALS-METHODS: We included 109 prostatic needle biopsies displaying features of ASAP, HGPIN and Pca in H&E stain. Benign glands were detected in all biopsies as a control group. Each lesion were analyzed separately. HMW-CK and p63, as basal cell markers, were expected to be positive in basal cells of benign glands and HGPIN, whereas AMACR was expected to be positive in the tumoral cells. The result of the interpretation of the antibody cocktail were scored from (-1) to (7) based on the percentage and the intensity of staining. We checked the statistical significance of the diagnostic value of the antibodies, individually and together. RESULTS Thirty five lesions of carcinomas were all negative for basal cell markers, whereas those markers were detected to be positive in HGPIN and benign glands. The staining pattern was patchy or diffuse, respectively. In ASAP lesions, immunohistochemistry demonstrated basal cell staining in 67.7% (23/34) and no staining was found in 32.3% (11/34). It did not differ whether we use p63 or HMW-CK as a basal cell marker. We detected strong positivity for AMACR in carcinomas in more than 75% of the tumor. Strongly positive staining for AMACR in more than 75% of cells was seen in 31.4% (17/54) of HGPIN and 26.4% (9/34) of ASAP. Benign glands were negative for AMACR. The immunoreactivity score of carcinomas were 7 in 32 and 6 in 3, whereas it was ≥6 in 16.6% (9/54) of HGPIN and 23.5% (8/34) of ASAP. None of the benign tissues displayed a score of ≥5. We observed a superiority in terms of specificity for antibody cocktail (96.3%) over HMW-CK (85.1%) in ASAP. The sensitivity for both antibodies were 100%. CONCLUSIONS As a result, we suggest that combining AMACR with a basal cell marker may improve diagnostic performance, especially in ASAP lesions. We recommend the use of antibody cocktail as a simple and cost-effective method in routine practice.

Pulmonary Pathology

PP3-107
PLEUROPULMONARY ABNORMALITIES IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a low malignant Non–Hodgkin's lymphoma. Pleuropulmonary abnormalities in patients with MM require a broad differential diagnostic spectrum. Method: We retrospectively analysed the clinical histories and findings of 22 patients with MM in whom bronchoscopies were performed because of pulmonary infiltrates, both alveolar and interstitial. The series included 16 men and 6 women; the median age was 64 y (46 – 83 y). Results: Bronchoscopy enabled differential diagnosis in all pts. and was performed without complications regardless of cytopenias. In 16 pts. (72,7%) infections were diagnosed. Rare manifestations were diffuse pleural and pulmonary infiltrations with atypical plasma cells (n=1), light chain deposition disease (n=1), diffuse alveolar hemorrhage (DAH) (n=1), amyloidosis (n=1) and toxic pneumonitis (n=2). Subclinical DAH was found in 7 pts. (31.8%). In one patient an intercurrent disease was diagnosed. Conclusion: We conclude that the most common causes of pulmonary abnormalities in pts. with MM are infections. However there are rare differential diagnoses, which are presented in more detail.

PP3-108
SOLITARY FIBROUS TUMOUR OF PLEURA- 16-YEARS REVIEW

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Background: Solitary fibrous tumour of pleura (SFTP) originate from primitive submesothelial fibroblasts differentiated to hemangipericytoma-, neurofibroma- and fibroma-like pattern. SFTPs are pedunculated with visceral, parietal and mediastinal pleura. Extrathoracic SFTPs are rare. Method: Analysis was made on 47 SFTPs diagnosed on surgery extirpated tumours during last 16 years. Results: SFTPs was diagnosed in 25 females and 22 males. Patients' age ranged from 27 to 71 years, average 52.1 and predominance in 6. life decade. Eighteen tumours were localized on the right and 13 from the left site and 14 in mediastinum. Twenty tumours originated from visceral, 13 from parietal and 14 from mediastinal pleura. SFTPs Tumour size varied from 30mm to 540mm, mean 117cm. The greatest SFTP measured 4900g, occuping the whole right hemithorax, involving pulmonary tissue, visceral and parietal pleura and ribs. The other giant SFTPs measured 235mm (2430g), 240mm(2850g) and 350 mm (3600g). Predominant morphological pattern according cellularity was: 28 mixed, 11 purely hypo- and 8 purely hypercellular and rarely purely hypo- or hypercellularity. Predominant morphological pattern was: hemangio-19, neurofibro-19 and fibroma-like pattern 9. Three of them were malignant on surgery. Three SFTPs recidived 3 months to 6 years after surgery. Diagnosis of SFTP was precised by histochemical (van Gieson, Masson-trichrome) or by immunohistochemical stains (vimentin, CD34, bcl-2). Preoperative needle biopsy was performed in 17 patients with suggestive diagnosis of mesenchymal tumour of variable morphological pattern and unpredictive biological behavioring. Conclusion: Diagnosis of SFTPs on preoperative biopsy can be estimated by percutaneous needle biopsy applying CD34. Precise diagnosis of SFTPs is useful for planning surgery. Hypercellularity, increased mitotic activity, nuclear polymorphism and invasion of tumour capsule are suggestive for suspicion on malignancy. Tumour size is not

mandatory for malignancy. On gross examination of tumour node it is necessary to obtain numerous tissue samples specially with tumour capsule for precise the biological behavior of SFTP.

PP3-109
PULMONARY ANGIOMYOLIPOMA: CLINICOPATHOLOGICAL ANALYSIS OF 2 NEW CASES AND 10 PREVIOUSLY REPORTED CASES

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Background: Angiomyolipoma is a rare mesenchymal tumor that most often arises in the kidney, but can also occur in extrarenal locations. Angiomyolipoma of lung is very rare and to date only 10 cases have been reported in the literature. Method: Three cases of pulmonary angiomyolipoma were identified in the registry of the Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology, from 1977 to 2007 (one of which was previously reported). The clinical, radiologic and pathological findings of these three cases were reviewed and were compiled with findings abstracted from the cases reported in the literature. Results: Twelve patients included 4 males and 8 females (male:female, 1:2) with a mean age of 52 years (range, 28 to 68). Pulmonary angiomyolipoma occurred de novo in 10/12 (83%) patients and in association with tuberous sclerosis in 2/12 (17%) patients. Pulmonary lesions presented as isolated findings in 7/12 (58%) patients, but were also seen in association with renal (3/12 patients, 25%), hepatic (2/12 patients, 17%) and splenic (1/12 patient, 8%) angiomyolipoma. Radiologically, tumors were well-circumscribed nodules with fat density that were located in the lung parenchyma in 10/12 (83%) patients and as an endobronchial lesion in 2/12 (17%) patient. Tumors ranged in size from 0.4 to 9.5 cm (mean, 2.2 cm). Microscopically, all tumors demonstrated variable amounts of mature adipose tissue, smooth-muscle cells and tortuous, small- to medium-sized, thick-walled blood vessels. Minor histologic features included: epithelioid smooth-muscle cells containing melanin pigment, histiocytes, multinucleated giant cells, plexiform vascular channels, atypia and mitosis. Tumor smooth-muscle cells were immunoreactive with HMB-45 (6/9, 67%), actin (6/7, 86%), desmin (2/4, 50%) and S-100 (2/6, 33%). Conclusion: Pulmonary angiomyolipomas are rare tumors with only 12 cases known to date. They show a female gender bias. Pulmonary angiomyolipomas typically arise de novo as parenchymal nodules. The combination of thick-walled vessels, smooth-muscle cells and adipose tissue is distinctive and aids separation from other benign and malignant pulmonary mesenchymal lesions.

PP3-110
EXTRAMEDULLARY HEMATOPOIESIS IN PULMONARY SPINDLE CELL TUMORS: RARE AND UNUSUAL ASSOCIATION

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Background: Extramedullary hematopoiesis is defined as development and growth of hematopoietic tissue outside the bone marrow. It tends to occur as a compensatory phenomenon in the organs of reticuloendothelial system. Involvement of other organs and some tumors such as pilomatricoma and hemangioblastoma is recognized. Only one case of pulmonary carcinoid with intratumoral hematopoiesis is reported in the English language literature. Method: Two cases of lung tumors with extramedullary hematopoiesis were identified in the registry of the Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology. Herein, we describe the pathological and immunohistochemical features of these two cases. Results: A 72 year-old male presented with 3.5 cm right middle lobe, pleural based solitary fibrous tumor. The tumor was composed of CD34 positive spindle cells with variable collagen deposition. Scattered nests of extramedullary hematopoiesis were distributed throughout the lesion. They exhibited trilineage hematopoiesis and contained numerous dysplastic megakaryocytes. A 64 year-old male presented with a 7 cm peripheral left lower lobe intermediate grade sarcoma with myxoid features. Tumor cells were immunoreactive for smooth muscle actin, muscle specific actin and vimentin, while the pancytokeratin, CAM5.2, EMA, desmin, HMB-45, S-100, CD34, Factor VIII were negative. Hematopoietic cells consisting of megakaryocytes, erythroblasts and rare myeloid precursors were present as small clusters throughout the tumor. No hematopoietic cells were identified in vessels or adjacent lung parenchyma in either case. Megakaryocytes and erythroid precursors were immunoreactive with Factor VIII and hemoglobin respectively. Conclusion: Tumors of the lung displaying foci of intratumoral extramedullary hematopoiesis are rare. However, awareness of this association is helpful in avoiding misdiagnosis of a hematopoietic malignancy and upgrading of spindle cell tumors based on the dysplastic megakaryocytes which resemble pleomorphic tumor cells.

PP3-111
INFLAMMATORY MYOFIBROBLASTIC TUMORS OF THE LUNG ARE NEGATIVE FOR HHV-8

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Background: Inflammatory myofibroblastic tumors are uncommon lesions composed of spindled myofibroblasts within a variable fibroinflammatory background. Although the true nature of these lesions has not been fully elucidated, identification of consistent cytogenetic alterations in the anaplastic lymphoma kinase (ALK) gene suggests that they may represent a neoplastic proliferation. Furthermore, few cases of inflammatory myofibroblastic tumor have been reported to harbor human herpesvirus (HHV)-8 infection, implicating HHV-8 in its pathogenesis. In this study, the largest to date series of pulmonary inflammatory myofibroblastic tumors were tested for the presence of HHV-8 by immunohistochemical and molecular methods. Method: Twenty cases with classical features of inflammatory myofibroblastic tumor were selected for the study. Immunohistochemical studies for latent nuclear antigen of HHV-8 (clone 13B10, 1:50, Vector Laboratories, Burlingame, CA) and for ALK (clone ALK-1, 1:100, Dako, Carpinteria, CA) were performed according to the manufacturers' instructions. Four open reading frames (ORF) of HHV-8, including ORFK2, ORF16, ORF26, ORF72, were targeted with real time PCR.

Results: The study cohort included 9 men and 11 women with a mean age of 37 years (range, 1 to 81). Microscopically, the tumors were composed of predominantly spindled myofibroblasts. On immunohistochemical studies, 4/20 cases (20%) demonstrated diffuse cytoplasmic positivity with ALK-1. Imunohistochemical staining for HHV-8 was negative (0/20, 0%) in all cases. All inflammatory myofibroblastic tumor cases tested with real time PCR were negative (0/20, 0%) for all four ORF, while 10/10 (100%) samples of Kaposi sarcoma, serving as positive control, were positive. Her2 gene, tested for the presence of amplifiable DNA in the tissue lysate, was positive in all (20/20, 100%) of the inflammatory myofibroblastic tumors. Conclusion: This study documents the absence of HHV-8 in a series of pulmonary inflammatory myofibroblastic tumors and suggests that further investigation is needed in elucidating other potential etiologic factors for this lesion.

PP3-112
IDENTIFICATION OF RELEVANT LUNG ADENOCARCINOMA BIOMARKERS BY MASS SPECTROMETRY BASED N-GLYCOPROTEIN PROFILING OF PLEURAL EFFUSIONS

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Background: Late stage lung adenocarcinoma is frequently accompanied by pleural effusion. Tumour cells in the effusion may be the source of proteins, which are potentially detectable in tumour tissue or serum. In particular, N-glycosylated proteins (N-GP) can be secreted into body fluids and are therefore considered potential biomarkers. We aimed for establishing a mass spectrometry based N-GP effusion profile for cytology specimens. Methods: Malignant pleural effusions of 5 patients with lung adenocarcinoma and 5 non-malignant controls were used for N-GP capturing by solid phase extraction. Following trypsin digest and PNGase F release, a liquid chromatography separation connected online to a tandem mass spectrometer was performed. The resulting peptide spectra were screened against IPI and NCI databases. Results: Concentration differences between malignant and non-malignant effusions were found for total protein, LDH, cholesterol, glucose, CA-125 and CEA (p's < 0.05). 174 non-redundant N-GP were detected in the total of 10 samples with probability ≥ 0.9, 29 of them exclusively in malignant effusions. The overall specificity for the N-glycomotif was 89%. Identifications were compared with serum of 4 healthy donors and A549 cell culture supernatant as well as published data sets. Specific protein markers were correlated with their corresponding immunoreactivity in either effusion fluid (CA-125, interferon gamma) or tumour cells or tissue (periostin, CD166), respectively. By applying strict criteria and using data mining, a potential lung adenocarcinoma marker signature of 6 proteins, including periostin, CD166, multimerin 2, lysosome-associated membrane glycoprotein 2, latent TGF beta binding protein 2 and surfactant protein A, was elaborated. Conclusion: The N-GP capturing and LC-MS/MS protocol enables the confident identification of several relevant biomarkers for lung adenocarcinoma.

PP3-113
VALUE OF VARIOUS CYTOHISTOLOGICAL TECHNIQUES FOLLOWING FIBEROPTIC BRONCHOSCOPY IN THE DIAGNOSIS OF LUNG CANCER

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Introduction: The aim of the present study was to evaluate the value of various diagnostic techniques following fiberoptic bronchoscopy in the diagnosis of lung carcinoma. Material and methods: A total of 2005 patients underwent diagnostic bronchoscopic procedures during a 3-year period for various neoplastic and nonneoplastic lung diseases. Histologic examination of specimens obtained by forceps biopsy and cytological evaluation on brushing, washing and post-bronchoscopic sputum smears were performed. The diagnostic value of the procedures was analyzed by Mc Nemar's exact test. Results: Eight hundred thirty patients had at least one of the techniques positive for lung malignancy (41,39%). Mean age 67.46; range 22-91 years. %). Of the above 830 tumors, 302 were diagnosed as squamous cell carcinoma (36.4%), 134 as adenocarcinoma (16.1%), 11 as BAC (1.3%), 148 as small cell lung carcinoma (17.8%), 191 as non- small cell lung carcinoma (23%), 8 as mixed (1.0%),12 as metastatic (1.4%), 4 as carcinoids (0.5%) 1 as lymphoma (0.1%) and 19 as suspicious for malignancy (2.3%). Forceps biopsy specimens gave positive results in 34.3% of our malignant cases, brushing in 52.8%, washing in 69.4% and post-bronchoscopic sputum in 28%. Collection of brushing specimens in addition to washing and post-bronchoscopic sputum increased the diagnostic yield of bronchoscopy from 78.8% to 87.5% (p=0.0001). Furthermore, the addition of forceps biopsy increased the diagnostic yield of the combination of the three cytological techniques from 87.5 to 98.8% (p<0.0001). In conclusion, the best diagnostic yield for the diagnosis of lung cancer, in our material which included visible and non-visible pulmonary lesions, was obtained with the association of brushing and bronchial washing specimens. Furthermore, the application of both cytology and histology can achieve better results.

PP3-114
PROGNOSTIC SIGNIFICANCE OF VASCULAR AND INTERSTITIAL REMODELING PROCESSES IN NONSPECIFIC INTERSTITIAL PNEUMONIA: SYSTEMIC SCLEROSIS VERSUS IDIOPATHIC

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Aims and Methods: Pulmonary biopsy specimens were examined from 40 patients, 22 with idiopathic nonspecific interstitial pneumonia (NSIP) and 18 with nonspecific interstitial pneumonia associated with systemic sclerosis (SSc). We compared the septal and vascular matrix remodeling, vascular grade, pulmonary function tests and survival between the two groups. Results: The content of septal collagen and elastic fibers, as well as the elastic fibers in the vascular interstitium, were higher in the SSc group (p=0.01, p=0.001 and p<0.0001, respectively). Among pulmonary function tests the DLCO/VA was affected to a greater extent in the SSc group (59% of the predicted value in SSc and 97% in the idiopatic group). There were no differences in the collagen content of the vascular interstitium, vascular grade, or survival between the two groups. Conclusions: Although the fibrotic process is more intense in the SSc group, it does not affect the

prognosis of these patients, as it has been described in idiopatic lung fibrosis. It seems that the fibrogenic process has a minor role in SSc when compared with the idiopatic group. Because the elastotic process is higher in the SSc group, this might suggest that autoimmune inflammatory mechanisms affecting the elastic fiber system could play a greater role in the pathogenesis and pulmonary remodeling process of SSc-NSIP than in idiopathic-NSIP.

PP3-115
PARENCHYMAL AND VASCULAR INTERACTIONS IN THE PATHOGENESIS OF NON SPECIFIC INTERSTITIAL PNEUMONIA IN SYSTEMIC SCLEROSIS AND IDIOPATHIC INTERSTITIAL PNEUMONIA

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Background: Interstitial lung disease is a well recognized prognostic factor in systemic sclerosis (SSc). As the prognosis in nonspecific interstitial pneumonia (NSIP) has been described to be better in collagen vascular disorders compared to the idiopathic forms, we hypothesize that the mechanisms of repair and remodeling are different between these two forms of the disease. Objectives: To compare the mechanisms of repair and remodeling between SSc associated nonspecific pneumonia and the idiopathic form, its impact on pulmonary function tests and survival rates. Methods: We analyzed 18 biopsies from patients with NSIP associated with SSc and 22 with idiopathic NSIP and compared the epithelial and vascular densities as well as vascular activity by immunohistochemistry with antibodies directed against Cytokeratin-7, Surfactant protein-a, CD34, and VCAM-1. Results: Epithelial cell density was lower in SSc-NSIP when compared with idiopathic-NSIP ($p<0.0001$). Type II pneumocytes and Clara cells were reduced in idiopathic NSIP ($p=0.02$) . A decrease in microvessel density was found in SSc-NSIP compared to idiopathic-NSIP ($p<0.0001$). The vascular activity measured by VCAM expression was higher in NSIP-SSc when compared to the idiopathic group ($p<0.0001$). The DLCO/VA in SSc-NSIP was more compromised . A direct association between vascular density and DLCO/VA was found ($p=0.02$). There was no difference in the survival rate between the two groups after a follow-up of 36 months. Conclusions: Alterations in the epithelium and vasculature seem to differ in the pathogenesis of SSc-NSIP when compared to the idiopathic form of the disease. Further studies may be required to assess the significance of these findings and explore if they can provide prognostic and/or treatment information. Financial Support: FAPESP,CNPq.

PP3-116
AIRWAY COLLAGEN AND ELASTIC FIBERS REMODELING IN IDIOPATHIC INTERSTITIAL PNEUMONIAS

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Background: Structural alteration of the airways and lung parenchyma, "remodeling", is a recognized feature of pulmonary fibrosis. In this study, we sought to validate the importance of bronchiolar remodelling and to study the relationships between bronchiolar collagen/elastic system and survival in the major histological pattern of idiopathic interstitial pneumonias (IIPs). Material and Methods: We examined bronchiolar collagen/elastic system fibres in 9 non-specific interstitial pneumonia (NSIP), 24 idiopathic pulmonary fibrosis (IPF), 5 bronchiolites obliterants (OP) and 18 acute interstitial pneumonia (AIP) cases. We used the Picrosirius-polarization method and Weigert's resorcin-fuchsin histochemistry to evaluate the amount of bronchiolar collagen/elastic system fibres. Results: The bronchiolar measurement of collagen fibres content was higher in AIP ($p=0.08$) than lungs of OP, UIP and NSIP. In addition, was a not difference of elastic fibres inter groups. Conclusion: We concluded that a not differences of progressive bronchiolar fibroelastosis occurs in IIP histological patterns, probably indicating who not interview in to parenchymal injury.Financial Support: FAPESP,CNPq.

PP3-117
TYPE V COLLAGEN PARTICIPATION IN PATIENTS WITH SYSTEMIC SCLEROSIS: PRELIMINARY STUDY

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Background: Systemic sclerosis (SSc) is a polymorphic and heterogenic systemic disorder with inflammation, fibrosis and vascular damage. Recently, the pathogenesis of SSc has been extensively studied regarding its autoimmunity aspects related to extracellular matrix remodelling, with an emphasis on the collagens at the inflammatory site. The present paper describes the importance of type V collagen morphologic disorganization, distribution and quantitation in pulmonary interstitium in patients with SSc. Methods: We used immunofluorescence and morphology analise to evaluate and quantified the deposition of collagen I, III and V, and correlation with the active alveolar septal, bronchiolar and arterial interstitum remodeling in open lung biopsies of 11 SSc. Results: The interstitum of alveolar septal, bronchiolar and vascular changes were characterized by morphologic disorganization of fibrillar collagen with diverse disarray, thickness and strong green birefringence of the type V collagen. The quantification of alveolar septal, bronchiolar and vascular collagen I was higher in all groups. In addition, the increase of collagen V in vascular interstitum was major than to collagen III ($p=0.05$), in contras the collagen III septal and bronchiolar interstitum was major than to collagen V. Conclusions: We concluded and suggested that the collagen type V fibrils are increased, in disarray and combine to other collagen (I and III) make larger than normal lungs, creating an abnormal spatial organization principally in vascular interstitium. Financial Support: FAPESP.

PP3-118
STATISTIC ANALYSIS OF MORTALITY AND DISEASE INCIDENCE IN CHRONIC LUNG DISEASES AMONG THE POPULATION OF SEMIPALATINSK REGION WITH LONG-TERM RADIOACTIVE AND POLLUTED ENVIRONMENT

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Work is aimed at examining the prevalence of chronic pulmonary pathology, characteristic of mortality and disease incidence in chronic lung disease (CLD) among the population of Semipalatinsk region having been subjected to long-term radioactive and polluted environment. Public health authorities 1969-2003 reports on spread, mortality and disease incidence of respiratory apparatus among the population of the Semipalatinsk region have been analyzed. 7274 habitants of the Semipalatinsk region have been screened and questionnaired and frequently revealed CLD types in the Semipalatinsk region have been epidemiologically researched. 631 autopsy notes with CLD diagnosis of Morbid Anatomy Bureau of Semipalatinsk for 1996-2003 years period has been studied. Research has revealed tendency to increase in disease incidence and CLD mortality in the course of growth of exposure rate and close location to testing area. Conclusion: tendency to increase in CLD incidence within 1992-2003 years period, i.e. after nuclear tests cessation has been revealed that proves the radiation value as etiopathogenetic factor.

PP3-119
SUDDEN DEATH CASES DUE TO TUBERCULOSIS
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Aim:in this study we investigate the sudden death cases caused by tuberculosis with different localisations. Materials and methods: We performed a retrospective analysis on the forensic necropsies from IML Timisoara in a period of four years 2002 - 2005. There were 36 cases of death by pulmonary tuberculosis (1.23% of the cases). Results: the majority of the victims, 94.44% were males. 27.77% of the victims were aged between 40 and 49 years. Homeless victims' cases represented 27.77% of the cases, 73.33% of these not being hospitalized for this disease. On 18 of the cases (50%) we found fibrocavitary form, 12 of the cases (33.33%) has fibronodular form, 4 cases (11.11%) has an ulcerative form and 2 case was of miliar form (5.55%). Some cases presented unassociated pulmonary tuberculosis, some presented pulmonary tuberculosis associated with broncho-pneumonia (12 cases), 1 case presented tuberculosis associated with pulmonary carcinoma, some presented pulmonary tuberculosis associated with tuberculosis pericardic and hepatic granular tuberculosis (1 single case) and an association of pulmonary tuberculosis and hepatic tuberculosis granulia. In 61.11% of the cases (22 cases) the pulmonary tuberculosis was associated with hepatic steatosis or cirrhosis and 4 cases (45%) had increased alcohol rate. We did not observe any evidence suggesting the existence of HIV. Conclusions: The study shows that sudden death caused by pulmonary tuberculosis mainly affects male alcohol consumers and social cases. It is necessary to observe. the possible cohabitation of HIV/bK.

PP3-120
A MIXED SQUAMOUS CELL AND GLANDULAR BRONCHIAL PAPILLOMA: A CASE REPORT
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Bronchial papillomas are very rare representing less than 0.5 % of lung tumours. The histological types are squamous, glandular and mixed. Papillomas should be differentiated from malignancies by endobronchial biopsy, because complete resection appears to be curative. We describe a case of bronchial mixed squamous cell and glandular papilloma of the bronchus diagnosed by frozen section. The case is a 51 years old male smoker, who was admitted with dyspnea and hemoptysis. Physical examination revealed no abnormalities. Thorax computed tomography slices showed a minimal consolidated lesion in left lower lobe. In bronchoscopic examination a swinging polypoid tumour in left main lower bronchus was detected. Bronchoscopic biopsy was not diagnostic. A left thoracotomy was performed for diagnosis and treatment. The lesion which was 1x1.5x 2 cm in size was removed and a frozen section examination was performed. The lesion was diagnosed as bronchial papilloma with frozen section. Postoperative histopathological examination confirmed the diagnosis as "mixed squamous cell and glandular papilloma". This extremely rare encounter of mixed squamous cell and glandular bronchial papilloma is presented with the review of the literature.

PP3-121
PULMONARY MALT LYMPHOMA: 3 CASE REPORTS
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Primary pulmonary lymphomas are relatively rare tumours that comprise less than 1 % of all lymphomas. Approximately 70-80 % of primary pulmonary lymphomas are marginal zone B-cell lymphomas of Mucosa-Associated Lymphoid Tissue (MALT) type.In this paper 3 MALT lymphoma cases are presented. Case I: A 52 years old male patient was admitted with a solitary pulmonary nodule of 2 cm in diameter. Transthorasic needle aspiration was performed. Cytologic examination revealed atypical lymphocytes which reminded a low grade lymphoma. A thoracotomy was performed and the lesion was removed with wedge resection. Case II: A 56 years old male patient was referred with solitary pulmonary nodule of 2.5 cm. in diameter. The lesion was removed with thoracotomy and frozen section was performed which revealed tumour of lymphoid tissue. Histopathological examination of both cases revealed marginal Zone B-cell lymphoma of the Malt type. Case III: A 37 years old female was admitted with cough and dyspnea. CT examination showed multiple bilateral patchy consolidations. Transbronchial biopsy was negative. VATS biopsy was performed. Histopathological examination revealed marginal zone B-cell lymphoma of the MALT type. All cases were referred to medical oncology clinic. Pulmonary lymphomas could be found as solitary or multiple nodules in radiographic examination, so it should be reminded in diagnosis of solitary pulmonary nodule and interstitial lung disease.

PP3-122
DIFFERENT HISTOLOGICAL PATTERN IN SYSTEMIC SCLEROSIS WITH INTERSTITIAL LUNG DISEASE (ILD)
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Background: The new histological pattern of idiopathic interstitial pneumonia (IIP) named Centrilobular Fibrosis (CLF) characterized by an aggressive bronchocentric scarring, basophilic intraluminal contents, and consequent parenchyma involvement may be relevant in systemic sclerosis (SSc). In fact, the high frequency of esophageal involvement in SSc patients is compatible with the suggested mechanism of CLF proposed by the authors, which is the pulmonary aspiration caused by the gastroesophageal reflux (GER). Materials and Methods: Twenty-eight consecutive patients who fulfilled the ACR criteria for SSc and had ILD on HRCT had been recruited to an open-lung biopsy as part of an ongoing prospective treatment protocol. Sections had been analyzed by two pathologists specialized in lung diseases and classified according to the new consensus classification of IIP and to the diagnostic criteria for CLF. Results: NSIP and CLF pattern had been the predominant patterns in SSc patients, observed in 67.8% and 21.4%, respectively. Intraluminal basophilic content has distinguished the two main groups. In fact, all 6 patients with CLF had this

feature whereas in only 6 of the 19 NSIP patients this parameter had been observed (p=0.007). Higher frequency of foreign bodies and bronchocentric distribution of the lesions in patients with CLF had been observed when compared to those with NSIP, but not reach statistical significance (p>0.05). As expected, peripheral injury had been found absent in 100% of CLF cases, compared with a frequency of 57.9% in the NSIP, though it had not reaching statistical significance (p>0.05). Other three characteristics had been found in the totality of the CLF patients: epithelium necrosis, basement membrane exposure and bronchiectasia. Conclusion: This is the first report of CFL in ILD of SSc patients, characterized by an intraluminal basophilic content, high frequency of foreign bodies and a bronchocentric distribution of the lesions. Since chronic aspiration had been suggested as the main pathophysiological mechanism for this severe form of lung involvement, the identification of this subgroup of patients will certainly contribute for a more appropriate therapeutic approach. Financial Support: FAPESP.

PP3-123
CILIARY DYSMORPHOLOGY: ULTRASTRUCTURAL FINDINGS IN 12 PATIENTS

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Background: Various ciliary abnormalities are found in patients with nonneoplastic diseases,such as unexplained recurrent upper and /or lower respiratory tract infections in children with or without Kartegener ‘s triad (sinusitis,bronchiectasis and situs inversus). Primary ciliary dysmorphology is an autosomal resessive disorder in childhood. Abnormal cilia which have specific electron microscopic features can be detected by transmission electron microscopic study. Method: Herein, we investigated 37 children with severe chronic /recurrent upper and lower respiratory tract problems for presence of primary ciliary dysmorphology. A plastic nasal brush was used to collect the tissue samples from nasal mucosa. Nasal brushing materials were evaluated by transmission electron microscopic. Other possible etiologic causes of severe and recurrent upper and lower respiratory tract problems were also evaluated by immunological tests, including atopy,respiratory function test, sweat test,reflux sintigraphy and radiologic studies. RESULT: Primary ciliary dysmorphology was shown in 12 of 37 children electronmicroscopically. Mean age of the patients was 11 years at diagnosis. Five of them were male and seven of them were female. Family history of cousin marriages was determined in 4 patients. Six patients had bronchiectasis ,6 patients had chronic sinusitis, 1 patient had gastroeosophageal reflux, 2 patients had situs inversus (including Kartegener’s triad). Ultrastructural examination of samples from nasal brushing revealed at least one of the following abnormalities: 1)Loss of normal 9+2 microtubules pattern , 2)Compound cilia, 3)Multiple axonemes in a single cilium, 4)Dysorganization of axonemes including loss of dynein arms, 5)Increasing in number of intracellular cilia. Conclusion: Primary ciliary dysmorphology is a cause of recurrent upper and /or lower respiratory tract infections in approximately one third of the patients. Electronmicroscopic study of nasal brushing is the valuable method in diagnosis of PCD patients with or without Kartegener’s triad and it may be recommended for unexplained recurrent upper and /or lower respiratory tract infections

PP3-124
EXPRESSION OF THE c-KIT AND Ki–67 PROLIFERATION INDEX IN PATIENTS WITH LUNG CANCER

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Background: Kit is a transmembran tyrosine kinase receptor which is the product of protooncogene c-kit. Stem cell factor, ligand of c-kit, is co-expressed in various solid tumors, including carcinomas of the lung. The aim of this study was to determine the expression of c-kit and Ki-67 labelling index and the differences between histopathologic subtypes. Methods: In this study, resection materials of 75 lung carcinomas were used. Sections were taken from parafin-embedded blocks and stained immunhistochemically with c-kit and Ki-67 antibodies. Cytoplasmic and membranous staining density and percentage of stained areas were evaluated semiquantitatively. Mann-Whitney U and chi-square test were used for statistical analyses. Clinical data, information on tumor stage and histomorphologic details were reviewed. Results: Seventy-five patients included 70 males and 5 females (male:female, 14:1). Tumor samples of 30 squamous cell carcinomas, 30 adenocarcinomas and 15 small cell carcinomas were studied. Membranous or cytoplasmic c-kit immunoreactivity was documented in 9 of 15 small cell carcinomas (60%), 26 of 30 squamous cell carcinomas (86%) and 25 of 30 adenocarcinomas (83%). The average of Ki-67 labelling index was found 82% in small cell carcinomas, 62% in squamous cell carcinomas, 34% in adenocarcinomas and statistically significant differences were shown between histologic subtypes (p=0.000). No significant association was found between c-kit immunoreactivity and Ki-67 labelling index (p=0.706). Conclusions: C-kit is expressed in a high percentage of lung tumors; however there is no association between c-kit expression and histologic tumor subtypes. Ki -67 labelling index is higher in small cell carcinomas. Among non small cell lung carcinomas, Ki-67 labelling index is higher in squamous cell carcinomas than adenocarcinomas. There is no correlation between c-kit expression and Ki-67 labelling index in lung tumors.

PP3-125
ROENTGENOMORFOLOGIC PREDICTORS OF HORMONAL THERAPY RESPONSE IN PULMONARY SARCOIDOSIS

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Sarcoidosis is systemic, relatively high-quality granulomatosis of unknown etiology, which is characterized by the accumulation of T-lymphocytes and mononuclear phagocytes, by the formation of the non-secreting, non-caseous epitelioid-cell granulomas and by the disturbance of the normal architecture of the diseased organ. There is a large quantity of sarcoidosis classification, which do not always accurately reflect the nature of clinical and roentgenological data with the answers to the ongoing therapy. Aim. The aim of the study was to investigate correlation of the X-ray and morphological variants of lung sarcoidosis depending on the nature of granulomatous inflammation and special features of answers to the anti-inflammatory hormonal therapy. Materials and methods. High resolution multispiral CT scan and open lung biopsies of lung and lymph nodes have been studied from 40 patients with sarcoidosis. Results. Patients were divided into two groups according to the localization of sarcoid granulomas. First group (27 patients) had perivascular and subpleural arrangement of granulomas – places of pulmonary lymphatic collectors, the second group (13) – did not have subpleural and perivascular granulomas , granulomas were localized predominantly in

interstitium of respiratory part of the lung. Positive response to therapy has been found in 22 patients of the first group(80%) and 5 patients (20%) didn't show negative dynamics. In 2 group positive dynamics was observed only in 4 patients (34%) and 9 (66%) didn't show any. Conclusions. Hormonal therapy response in patients with sarcoidosis correlates with granuloma location and is better when sarcoid granulomas are accumulated in lymphatic collectors of the lung.

PP3-126
GEOGRAPHY OF MESOTHELIOMA AND ASBESTOS EXPOSURE

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BACKGROUND The etiological relationship between exposure to asbestos and malignant mesothelioma is well established. However, it remains uncertain if the effects of asbestos are the same in all the populations. We collected the available data about the geographical distribution of mesothelioma. METHODS Some 300 researchers (including epidemiologists, pathologists, clinicians, etc.) were interviewed by a questionnaire to obtain information on mesothelioma incidence, and on the percentage of asbestos-related mesotheliomas in the various countries. In addition, literature data on mesothelioma were reviewed. RESULTS 1) Reliable data on mesothelioma incidence/mortality are available for a relatively low number of countries, corresponding to about 15% of the world population. 2) The highest annual crude incidence rates (over 25 cases per million) are reported or estimated for few countries (Australia, Belgium, UK). Intermediate rates (11-25 cases per million) are estimated for some countries of Europe (France, Germany, Italy, Scandinavian countries, The Netherlands), and New Zealand. Crude incidence rates lower than 11 cases per million are estimated for many countries of Europe (central Europe, Iberian peninsula, Ireland), some countries of America (Canada, USA), Asia (Cyprus, Israel, Japan, Turkey), and Africa (Morocco, Tunisia). 3) A parallelism between mesothelioma incidence and asbestos consumption in previous decades is observed in a large part of countries. 4) Marked differences in mesothelioma incidence from one region to another are seen in several countries (Croatia, Italy, Japan, Spain, Sweden, UK, etc.), with case clustering in areas with shipyards and asbestos-cement factories. 5) Very low incidences of mesothelioma are reported from some countries with high asbestos production and/or consumption, such as Russia, Thailand, South Korea. 6) Major difficulties are encountered in the histological diagnosis of mesothelioma. In some countries the original diagnosis of mesothelioma has not been confirmed by expert panels in a high proportion of cases. In addition, major difficulties are often encountered in mesothelioma registration. CONCLUSIONS Differences in previous asbestos exposure may explain a large part of the differences in mesothelioma geography. The apparent discrepancies between asbestos consumption and mesothelioma incidence have probably to be attributed to different factors (underestimation, low life expectancy, recent history of industrialization), rather than to ethnic differences in susceptibility to asbestos.

PP3-127
CONGENITAL MALFORMATIONS OF THE LUNGS AS POSSIBLE PRECURSOR OF CANCER

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Aim. A retrospective study was carried out on 110 cases with malignant lung tumors arising within congenital malformation areas. Matherial. There were 91 males / 82 % / and 19 females / 18 % / with ages ranging from 23 to 65 years. In 18 cases / 22% / congenital lung malformations had been revealed 1-15 years earlier lung cancer development. Methods included routine histology, immunohistochemistry of oncomarkers (p53, bcl-2, c-fos, c-myc, K-ras, c-erbB2, TTF-1, CK 8,19, AE1/AE3, Ki-67, PCNA, EGF, TGF-β), PCR for p53 mutations. Results. Correlations between type of congenital malformation and histological variant of lung cancer were established. Morphologic analysis revealed five groups of patients: 80 cases / 73% / with different types of adenocarcinoma / papillary, solid adenocarcinoma with mucin productions, bronchioloalveolar adenocarcinoma / inside congenital cystic adenomatoid malformations of the lungs; 9 cases / 8% / - different grade squamous cell carcinoma incide bronchogenic cysts; - 9 cases / 8% / - different grade squamous cell carcinoma or adenocarcinoma incide simple hypoplasia; - 7 cases / 6.5% / - different grade squamous cell carcinoma or adenocarcinoma inside congenital bronchiectasis; 5 cases / 4.5 % / - adenocarcinoma or carcinoid inside intralobar sequestration. Precancer lesions in congenital malformation and lung cancer cells had similarities in expression of p53, bcl-2, c-myc, K-ras, CK19. p53 mutation were found in lung cancer cells, as well as in epithelial cell of precancer lesion in congenital malformation. Conclusion: obtained results may proof contribution of congenital malformations in the development of and lung cancer.

PP3-128
VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ITS RECEPTORS (VEGF-R1-Flt-1, VEGF-R2-KDR/Flk-1) AS PROGNOSTIC INDICATORS IN SMALL CELL LUNG CARCINOMAS (SCLC)

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Background: Tumor angiogenesis is a highly regulated process influenced by the host microenvironment and mediators. VEGF and its receptors (VEGF-R1-Flt-1, VEGF-R2-KDR/Flk-1) are good markers of vascular proliferation but their expression and relevance to tumor spread in SCLC is less clear. The present study aims to investigate angiogenesis and the expression of VEGF, VEGF-R1-Flt-1 and VEGF-R2-KDR/Flk-1 in SCLC and to establish their interrelationship and prognostic influence. Method: Immunohistochemical study of 50 SCLC was performed on paraffin sections with VEGF, VEGF-R1-Flt-1 and VEGF-R2-KDR/Flk-1 antibodies (DBS California-Menarini Hellas). Mean vascular density (MVD) was assessed and rectospectively correlated with the clinical outcome. Results: A group of 50 patients (5 women, 45 men, mean age 64,3 years) included 25 with limited and 25 with extensive stage SCLC. 6%, 34% and 40% of tumor specimens presented high expression of VEGF, VEGF-R1 and VEGF-R2 respectively. 1/3 specimens presenting high VEGF expression, 18/20 having high VEGF-R2 expression and 12/17 with high VEGF-R1 expression corresponded clinically to metastasis. Mean survival for patients with high VEGF expression was 8,3 months and with low expression was 10,83 months. Mean survival corresponding to high VEGF-R1 and VEGF-R2 was 10,94 and 6,75 months respectively and to low was 10,35 and 9,41 months respectively. Conclusion: VEGF is expressed less than its two receptors (VEGF-R1-Flt-1, VEGF-

R2-KDR/Flk-1) in SCLC. VEGF-R1 and VEGF-R2 expression are more closely correlated with metastasis than VEGF although the latter and VEGF-R2 seem to be positively correlated with poor patient outcome.

PP3-129

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ITS RECEPTORS (VEGF-R1-Flt1, VEGF-R2-Flk1) IN NON SMALL CELL LUNG CARCINOMAS (NSCLC): CORRELATION WITH LYMPHANGIOGENESIS AND CLINICAL OUTCOME

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Background: Angiogenesis is an essential process in tumor growth and metastasis. VEGF and its receptors are considered to be good angiogenic markers but their correlation with lymphangiogenesis and patient outcome in NSCLC is yet to be defined. The present study aims to evaluate angiogenesis and the expression of VEGF, VEGF-R1-Flt1 and VEGF-R2-Flk1 in NSCLC, to correlate them with lymphangiogenesis (CD105 expression) and clinical outcome. Method: 96 NSCLC specimens were analyzed immunohistochemically using VEGF, VEFG-R1-Flt1, VEGF-R2-Flk1 and CD105 antibodies (DBS California-Menarini Hellas). Mean vascular density (MVD) and intratumoral lymphatic microvessel density (ILMVD) were determined and rectospectively correlated with clinical outcome. Results: 13 women and 83 men (mean age 64,03 years) were included in our study. 15,6% were in stage I, 46,8% stage II, 40,4% stage III, 2,1% stage IV. 25% presented high VEGF expression, 40,6% high VEGF-R1-Flt1 and VEGF-R2-Flk1 expression and 87,5% high CD105 expression (ILMVD>25). 63 patients presented metastasis, 27% of which had high VEGF expression, 42,9% high VEGF-R1-Flt1 expression, 44,4% high VEGF-R2-Flk1 and 95,2% high ILMVD ($p<0,05$). ILMVD was significantly correlated with the stage of the disease ($p<0,05$) whilst angiogenic factors were not. Conclusion: Lymphangiogenesis proved to be of superior significance in being correlated with clinical outcome and predicting metastasis in NSCLC in comparison with VEGF and its two receptors (VEGF-R1-Flt1, VEGF-R2-Flk1) that seem to be of little significance.

PP3-130

CLINICOPATHOLOGIC FEATURES AND PROGNOSTIC SIGNIFICANCE OF LUNG TUMORS WITH MIXED HISTOLOGIC PATTERN

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Background: Tumors with mixed histology are seen less often than the tumors with single histology in the lung. The prevalence is approximately in the range of 2-4%. The biologic behavior and clinicopathologic characteristics of these tumors have not been well described. We evaluated the clinicopathologic features and prognosis of lung tumors with mixed histology and compared them with the tumors that have single histology. Method: The study group consisted of 39 patients with a mixed histologic pattern and control group consisted of 41 patients with a single histology on the surgical specimen which were diagnosed between 1997-2007 at Ankara Ataturk Chest Disease and Chest Surgery Education and Research Hospital.In the study group three types of tumors were identified: adenosquamous carcinoma, combined neuroendocrine tumors and biphasic tumors (epithelial + mesenchymal malignant components). The combined neuroendocrine tumors were further divided in small cell carcinoma (SCLC)+non-neuroendocrine carcinoma (NNEC), small cell carcinoma+large cell neuroendocrine carcinoma (LCNEC) and LCNEC +NNEC. Clinicopathologic characteristics, pTNM and survival rates were evaluated and compared with the population of resected lung tumors with single histology. Results: There were 20 adenosquamous carcinoma, 9 SCLC+ NNEC, 5 SCLC+LCNEC, 2 LCNEC+NNEC, 3 biphasic tumors as study group and 25 squamous cell carcinoma, 16 adenocarcinoma as control group. There was no significant difference for mean age, sex, smoking history, asbestos exposure and tumor size between study and control group ($p>0.05$). Among adenosquamous carcinomas advanced stage (IIIa or IIIb) was significantly more evident than the single histology group but survival rates were not different ($p=0.328$). Among combined neuroendocrine tumors advanced stages was more evident than adenosquamous carcinomas and single histology group ($p=0.014$). Survival was significantly worse for patients with combined neuroendocrine tumors than for patients with adenosquamous carcinoma and single histology group ($p=0.0002$). Median survival of biphasic tumors was 21 months (range 10-36). Univariate analysis revealed that tumor stage was significantly associated with the survival rate for all groups ($p=0.016$). Conclusion: Tumors with mixed histology are rarely seen in the lung. Among these tumors adenosquamous carcinoma and combined neuroendocrine tumors present more aggressive clinic behaviour than the tumors with single histology.

PP3-131

STUDY OF THE POSSIBLE HORMESIS EFFECT IN AN EXPERIMENTAL PASSIVE TOBACCO MODEL IN THE EVOLUTION OF LUNG CANCER INDUCED BY URETHANE

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Background: In a previous study, we verified an unexpected reduction of the number of pulmonary nodules in mice exposed to the cigarette smoke. One of the possibilities for such event would be a dose response effect called hormesis, which is represented by a J-shaped curve. The aim of the present study was to verify whether passive cigarette smoke might act by hormesis in an experimental model of lung cancer carcinogenesis. Method: 3mg/kg urethane was injected in male Balb-c mice, with 7 to 13 life weeks. After that, they were exposed to passive tobacco smoke twice a day, for five times a week, in a closed chamber. The animals were randomly divided into four groups. G0: only received urethane; G1: exposed to the smoke of three cigarettes per 10 minutes each time; G2: exposed to the smoke of three cigarettes per 10 minutes, twice; G3: exposed to the smoke of six cigarettes per 10 minutes, twice. Autopsy was performed after 16 weeks and the number of nodules and hyperplasia in lung was counted. Results: The statistics analysis showed that there was not significant difference when we compare the mean number of nodules and the hyperplasia between the studied groups ($p=0,146$ and $p=0,513$, respectively). Conclusion: The studied model did not show evidences of hormesis effect. Balb-C mice apparently are resistant to cigarette smoke exposure. Financial Support: FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo.

PP3-132
RESPIRATORY BRONCHIOLITIS – ASSOCIATED INTERSTITIAL LUNG DISEASE WITH FIBROSIS IN YOUNG PATIENTS PRESENTING WITH SPONTANEOUS PNEUMOTHORAX

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Background: Respiratory Bronchiolitis (RB) is a common incidental finding in lung specimens of smokers. It is defined as a bronchiolocentric accumulation of pigmented alveolar macrophages and is usually an incidental histological finding. In association with clinical symptoms such as shortness of breath, abnormalities of pulmonary function tests and/or CT scan findings the diagnosis of Respiratory Bronchiolitis-associated Interstitial Lung Disease (RB-ILD) can be considered. It is known that RB and RB-ILD are associated with a higher prevalence of spontaneous pneumothorax. Method: We retrospectively reviewed tissue of 14 cases of RB-ILD of young patients (age less than 35 years) presenting with spontaneous pneumothorax and requiring surgical pleurodesis. Among the study group there were 3 females and 11 males, the age ranged from 19-34 years with a mean age of 23,9 years. In 8 cases a CT-scan was available. The H&E-stained sections were examined, and of areas with marked fibrosis also a Movat-stain were performed. Results: In 10 of 14 cases (1 female, 9 males) we observed a marked fibrosis, predominantly in subpleural and centrilobular areas. The Movat-stain allowed to discern early fibrosis with immature Collagen Type III and late scarring with mature collagen Type I. Conclusion: We think that Respiratory Bronchiolitis – Interstitial Lung Disease may progress to fibrotic lung disease since the occurrence of Collagene Type I is an irreversible event. This disease therefore deserves more attention and should be mentioned in the pathohistological report.

PP3-133
ASBESTOS: MYTH, HISTORY, RELIGION

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BACKGROUND The most ancient evidences regarding the use of asbestos were traced in Finland and Sweden. In particular, asbestos was used in pottery in Eastern Finland from about 4000 B. C.. In the Mediterranean region the commerce of asbestos in the Classic Age is documented. In ancient era and in the Middle Ages the properties of asbestos were the subject of legends, myths, and theological comments. METHODS In the present study some Latin, Greek, and Italian literature texts were reviewed. RESULTS In the first century, the Latin encyclopaedist Pliny the Elder describes asbestos under various names in his Historia Naturalis. In one of these descriptions, asbestos is defined as a plant which grows in the desert of India. Pausanias, a Greek geographer of the second century, reports the presence of asbestos in the lamp placed in a temple in the Acropolis of Athens. An apocryphal gospel, the Protoevangelium of James, reports that different materials, including asbestos, were used in making the curtain of the Jewish Temple. After the Liber Pontificalis, a book in which the biographies of the first Popes are collected, an asbestos wick was placed on the candle in the middle of the Lateran baptistery in Rome. Asbestos is also quoted by the Christian Fathers of the Church, Basil the Great (339-379), and Augustin of Hippo (354-430). Basil states that the bodies of the three boys of Babylon, who after the tale of the Bible resisted to fire, had the nature of asbestos. An interesting description may be found in the Milione (Travels of Marco Polo) of the Italian traveller Marco Polo (1254-1324). In a region of Central Asia, Marco Polo saw towels that were placed in the fire without burning. In addition, he reports that the Great Khan, the emperor of China, sent an asbestos towel as a gift to Rome; such towel had to be used “to wind the sudarium of our Lord”. CONCLUSIONS The review of the above texts shows that in the past the ‘positive’ features of asbestos were strongly emphasized. This agrees with the fact that asbestos was also used in medicine. Finally, the frequent association of the mineral with places of worship and with religious issues makes asbestos in some way sacred.

PP3-134
MIXED SQUAMOUS CARCINOMA AND OSTEOSARCOMA (CARCINOSARCOMAS) OF THE LUNG HAVE A CGH MAPPING SIMILAR TO PRIMITIVE SQUAMOUS CARCINOMAS AND OSTEOSARCOMAS

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Background: Carcinosarcomas (CS) are malignant tumors with a mixture of carcinomatous and differentiated sarcomatous elements. We selected from the surgical files of the Department of Pathology, University of Navarra three cases of CS composed of squamous carcinoma (SC) and osteosarcoma tissues. Methods: Clinical parameters were recorded. All cases were studied by light microscopy, immunohistochemistry and CGH technique. CGH assay was made in both carcinomatous ans osteosarcomatous areas, using as controls two lung squamous carcinomas and two lung metastatic osteosarcoma. Results: All patients were male and their age was 72, 43 and 58 years respectively. The size of the neoplasms was 7, 5.5 and 5 cm in maximum diameter. Only one patient had lymph node metastasis at presentation. All three patients died of their disease 4, 9 and 14 months after surgery. Pathologically, all cases had similar proportion of both squamous and osteosarcomatous structures by light microscopy. Immunoreactivity was positive for AE3AE1, CAM 5.2, CK-7, EMA, E-cadherine, p53 and CEA in carcinomatous areas and for vimentin and CD-68 in sarcomatous component. Foci of chondroid differentiation were positive for S-100. Chromosomal imbalances using CGH were identified in all tumors from predominant epithelial and mesenchymal areas. Overall, a total of 55 copy number changes were detected with a median of 18 abnormalities per case (range: 12-26): 48 gains, 6 losses and one high level amplifications. We found similar chromosome alterations in carcinomatous areas and lung squamous carcinoma and in osteosarcomatous areas and metastatic osteosarcoma respectively. Conclusions: This study provided an accurate demonstration that the CGH alterations in osteosarcoma component in carcinosarcomas of the lung are similar to CGH changes of soft tissue osteosarcomas. Additional functional studies are needed to determine the biological and clinical significance of the CGH changes and theorize about the histogenesis of this unsual tumor.

PP3-135
IS D2-40 A USEFUL MARKER DISTINGUISHING MALIGN MESOTHELIOMA, PULMONARY ADENOCARCINOMA AND BENIGN MESOTHELIAL PROLIFERATIONS?

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Background: Pulmonary adenocarcinomas, malign mesotheliomas and sometimes benign mesothelial proliferations may show great resemblance histomorphologically. For this reason an immunodiagnostic panel is usually needed for differential diagnosis. Recently D2-40, a lymphatic marker, is suggested to be useful for mesothelial differentiation. Aim of this study is to compare D2-40 immunostaining in malignant mesothelioma, pulmonary carcinoma and benign mesothelial proliferations. Method: In this retrospective study, D2-40 immunostaining was investigated in 37 cases of malign mesothelioma, 36 cases of pulmonary adenocarcinoma and 31 cases of benign mesothelial proliferation. Diagnosis of malign mesothelioma were previously confirmed by a panel including calretinin, CK5/6, CEA. Results: Predominantly membranous immunoreactivity was observed in 51% of malignant mesothelioma and 55% of benign mesothelial proliferations. All of the 36 pulmonary adenocarcinomas were negative. These results were statistically significant ($p<0.001$). Conclusion: We believe that D2-40 may be helpful in differential diagnosis of malign mesothelioma from pleural involvement of pulmonary adenocarcinoma.

PP3-136
PROGNOSTIC SIGNIFICANCE OF OSTEOPONTIN EXPRESSION IN MALIGNANT PLEURAL MESOTHELIOMA

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Background: Malignant pleural mesothelioma (MPM) is an asbestos-related type of cancer with a median survival time of approximately 10 months. Long survivors are usually rare and currently no clear prognostic factor are strongly associated with such status. Recently, in asbestos exposed individuals, high osteopontin (OPN) serum levels were correlated to the presence of mesothelioma, but not with other asbestos-related conditions (pleural plaques or interstitial lung disease) and it has been proposed as a serum marker of mesothelioma in asbestos exposed subjects. Since OPN is a glycoprotein involved in cell-matrix interactions and cell signalling that was shown to be correlated with invasion, metastatic spread and decreased survival in several carcinomas and melanoma, the aim of this study was to analyse the prognostic role of OPN in MPM. Method: From a large database of 395 MPM cases diagnosed between 1989 and 2003 in a single institution, 26 cases with long survival (>24 months, mean 39.2 months) were extracted. The male/female ration was 16/10, median age 63.5, epithelioid versus biphasic histology 24 and 2/26, respectively, and extensive versus limited pleural involvement 17 and 9, respectively. None was surgically resected and treatment was talc pleurodesis associated with cisplatin-based chemotherapy All cases were matched to control cases with comparable clinical-pathological characteristics, except for a fatal outcome within 15 months (mean 8.9 months). OPN expression was detected by immunohistochemistry on archival biopsy specimens, the results quantified scoring extent and intensity of the immune reaction (scale 0-300), and the expression levels compared with survival and other parameters. Results: Tissue immunoreactive OPN was found in all cases, with a median score of 90 (range 5-285). Interestingly, OPN levels were significantly higher in short survivors compared to long surviving patients (Mann-Withney U-test, $p<0.0001$). At univariate analysis, Kaplan-Meier curves showed that patients with low (< median) OPN expression had longer survival compared to those with higher expression levels (38.7 vs 11.5 months, $p=0.00001$), while performance status, age, gender and extent of pleural involvement had no impact on the outcome. Conclusion: As shown in several carcinomas, OPN expression levels in mesothelioma cells have a prognostic significance, being this protein probably involved in tumor progression and dissemination. It could represent a novel target for growth control strategies of this highly aggressive neoplasia.

Hematopathology

PP3-137
MEDIASTINAL B LARGE CELL LYMPHOMA A STUDY OF 11 CASES

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Background : Mediastinal B large cell lymphomas is rare, distinguished from the other locations of lymphoma by its clinical and radiological features, its histopathological appearance and by a poor prognosis. The aim of this study is to specify the clinico-pathological particularities, the management and the behaviour of this entity. Material and Methods : We studied 11 cases of mediastinal B large cell lymphomas diagnosed in the department of pathology during a period of 11 years (1993-2003). Clinical, radiological and biological informations provided from patient files. We used the index performance status (IPS) of the OMS to evaluate the clinical activity level of patients. Slides were reviewed to confirm the diagnosis . An immunohistochemistric study was performed to specify the proliferative index of tumors. The staging of tumors was evaluated according to the clinical staging system of Ann Arbor. Results : Our population was composed of 7 women and 4 men with a mean age of 25 years. The predominant presenting symptom was superior vena caval syndrome. The diagnosis was confirmed with an expression of CD20 by tumor cells in all cases. The IPS was low in 7 cases, which were stage IV of Ann Arbor system. Patients were treated chemotherapy associated to radiotherapy in three cases. Remission was noted in four cases and recurrence in three cases. No response was seen in four cases. Conclusion : Mediastinal B large cell lymphoma occurs preferentially in young females, most commonly revealed by superior vena caval syndrome. The diagnosis should be confirmed by histopathology and immunohistochemistry. A good response is a rule to radiation therapy and chemotherapy but recurrences are frequent.

PP3-138
HETEROGENEITY OF MANTLE CELL LYMPHOMA

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Background: the aim of this study is to investigate the heterogeneity of mantle cell lymphoma (MCL) in morphology, immunohistochemistry, FISH and molecular biology. Material and Methods: in the archive of Department of Pathology University Hospital Brno we found 27 cases of MCL in lymph nodes. Classical histology, immunohistochemistry, FISH method and from native tissues FASAY (Functional Analysis of Separated Alleles in Yeast) were performed. Results: in series of 27 cases of MCL were 17 men and 10 women. Most patients were in age of 61-80 years. Morphologically, diffuse infiltration with medium size lymfoid cells was found. Mitotic rate (number of mitoses/10HPF) in first category (0-5 mitoses) in 13 cases; second category (6-10 mitoses) in 8 cases; third category (11-20 mitoses) in 4 cases; fourth category (21-30 mitoses) in two cases. Highly characteristic marker for MCL, hyalinne deposits, was recognized in 20 cases. FISH for t(11;14) was performed in all cases and 51-100% of tumour cells were positive. Immunohistochemistry revealed positivity with marker CD 5 in 19/27 cases (30% cases were negative); CD 20 in 27/27; CD 43 in 23/27; cyclinD-1 in 19/27 (30% cases were negative); bcl-2 in 26/27; p53 in 22/27. The proliferation marker Ki-67 was detected in approx. 20% of cells in 16 cases and 30-60% of cells were found in 11 cases. For status p53 by FASAY method, 12 native tumour tissues were examined. In the two of them, abberation in the p53 gene was revealed and missense p53 mutations R282W and C288S were detected by DNA sequencing. Conclusions: our results show that MCL was uniform in morphology and in type of infiltration, which was in all cases diffuse. Very useful marker for diagnosis seems to be hyaline deposits stcattered in the lymphoma tissue. Number of mitoses enable us include 6 cases of the blastoid variant of MCL. In immunohistochemicstry, CD 5 and cyclinD-1 markers were negative in 30 cases. Abberation of p53 gene is rare in MCL. This work was supported by grant NR/9305-3 of The IGA MZ CR.

PP3-139
PROLIFERATION MARKERS IN ZAP70+ AND ZAP70- B CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: B cell chronic lymphocytic leukaemia (B-CLL) is a low grade lymphoproliferative disorder. Generally, most patients are asymptomatic with a long survival, but there exists a subset of cases with a more aggressive course. The expression of protein ZAP70, often associated with non mutated status of IgVH genes, and expression of CD38 molecule divide B-CLL into prognostically relevant subgroups. In this study we correlated these subgroups with the expression of various proliferation markers (CD71, proliferation index (PI) and Ki67). Methods: We analyzed 50 samples from 40 patients with B-CLL/SLL (bone marrow, lymph nodes, peripheral blood and tonsils). The expression of ZAP70 and CD38 molecules on CD19+CD5+ tumor cells were assessed in cell suspensions by means of flow cytometry, as well as the expression of CD71 (transferrin receptor). The PI was defined as a number of cells in S and G2/M phase of cell cycle determined by flow cytometry analysis. Ki67 was detected immunohistochemically in formalin-fixed paraffin-embedded material if available. Results: 32 samples from 26 patients were ZAP70+ and 17 samples from 14 patients were ZAP70-. The expression of CD38 subdivided samples into ZAP70+CD38+ (30 %), ZAP70+CD38- (35 %), ZAP70-CD38+ (4 %) and ZAP70-CD38- (31 %). The higher proportion of sample infiltration, higher CD71 expression and higher proliferation was found in ZAP70+ patients compared to B-CLL without expression of ZAP70. The information on status of CD38 brought no significant difference. Conclusion: B-CLL is clinically as well as biologically a heterogenous disorder. This fact is reflected by description of various markers which divide the disease to subcategories with different prognosis. In the investigated set of patients we showed that the ZAP70 positivity of B-CLL cells is followed by a higher expression of proliferation markers and implicates a heavier tumor infiltration of the tissue. We conclude that the expression of ZAP70 is associated with a higher proliferative activity and thus can be a good marker of more aggressive disease with worse prognosis. This study was supported by Research Project FNM MZO 00064203 n. 6704.

PP3-140
FIRST EXPERIENCES WITH DETECTION OF JAK2 MUTATION IN PARAFFIN EMBEDDED BONE MARROW BIOPSIES OF POLYCYTHEMIA VERA PATIENTS

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Background: Point JAK2 mutation (V617F) was recently identified in association with Ph1- myeloproliferative diseases (MPD) in almost all polycythemia vera (PV) patients and in cca 50% of patients with essential thrombocythemia and chronic idiopathic myelofibrosis. The mutation shows association with typical laboratory and clinical findings, while its prognostic significance remains uncertain. It might be identified by direct DNA sequencing, allele-specific PCR (ASPCR), real-time PCR, DNA-melting curve analysis, etc. In majority of published papers fresh peripheral blood or bone marrow (BM) cell analysis was used, but only limited experiences based on retrospective analyses of BM biopsies are known. The aim of this study was to prove the possibility of DNA analysis obtained from the regular BM biopsies with the aim to implement JAK2 mutation detection into the routine praxis of our hematopathology center. Methods: The paraffin-embedded BM biopsies of PV patients diagnosed in the period January 2004 – March 2007 were selected from the files and reevaluated histologically and clinically. BM sections were deparaffinized in graded alcohols and xylene and digested with proteinase K over two nights. DNA was extracted using the Wizard® genomic DNA purification kit (Promega). Detection of the V617F mutation was performed by allele-specific hot start multiplex PCR with one common reverse and two separate forward primers. In the presence of the V617F JAK2 mutation two PCR products of different sizes were amplified, while in the presence of wild-type DNA only one fragment is amplified. Results: BM biopsies of 53 patients fulfilled WHO criterias of PV diagnosis established by BM biopsy and/or by hematologist. In addition, the clinicians were subsequently asked to report the follow up. At the time of abstract submission, results of JAK2 detection of 19 patients were available. BM biopsy showed typical PV morphology in 18 cases and MPD NOS in 1 case and the diagnosis was confirmed also by follow up in 13 of them. JAK2 mutation was detected in 16 of 19 patients and in all of 12 cases sharing both typical morphology and clinical behaviour of PV. Conclusion: Although the importance of JAK2 detection for MPD typing is limited, its diagnostic implementation is usefull and its possible therapeutic significance might not be excluded. Our preliminary results confirmed a high sensitivity of JAK2 detection by using routine BM biopsy material. Further analyses will follow, incl. the atempts to identify its homozygous and heterozygous forms. Supported by Grant UK/84/2007.

PP3-141
OPPORTUNISTIC INFECTIONS IN HIV+/AIDS PATIENTS – COMPARATIVE AUTOPSY STUDY

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Background: Patients with HIV/AIDS have an important immunodepression, which makes them susceptible for opportunistic infections which are, in most cases, the main cause of death. Material and method: We made a comparative study of autopsy findings regarding infectious pathology including all AIDS patients submitted to autopsy in our department as follows: group A – patients HIV positive autopsied between 1997-2001 (27 cases) and group B – 19 cases between 2002-2006. Results: The cause of death in our two groups varied as it follows: • group A: - opportunistic infections (single or combined) - 22 cases (7 cases of each tuberculosis and systemic candidosis, 4 cases of pneumocystosis, 2 cases of cryptococcosis, 1 case of each Cytomegalovirus infection and systemic aspergillosis). - nonspecific infections - 4 cases - other causes – 1 case (non-hodgkinian malignant lymphoma) • group B: - opportunistic infections (single or combined) – 13 cases (3 cases of tuberculosis, 2 cases of each pneumocystosis, toxoplasmosis, progressive multifocal leukoencephalopathy and Cytomegalovirus infection, 1 case of coccidioidomycosis and 1 case of systemic aspergillosis. - nonspecific infections – 4 cases - other causes – 2 cases (one cerebral malignant lymphoma and one myocardial infarction). There is a definite change in the spectrum of lethal opportunistic infections in AIDS related deaths in Romania - a lower rate of tuberculosis in group B comparing with group A, disappearing of lethal systemic candidosis and occurence of other type of opportunistic infections: toxoplasmosis and progressive multifocal leukoencephalopathy due to JC virus infection. The most probable cause of this trend is high active antiretroviral therapy (HAART) which assures a tolerable level of immunity for these patients. The majority of the group B patients who died by opportunistic infections were recently found HIV+ (1-3 months) and HAART was either not used or very recently administrated. Conclusion: Opportunistic infections are an important health problem and a frequent cause of death in HIV positive patients. This study provides informations about changes occurred in the main causes of AIDS related deaths, data that may be useful for improving the medical management of the HIV+/AIDS patients.

PP3-142
BONE MARROW (BM) PATHOLOGY CONTRIBUTES TO "IN SITU" UNDERSTANDING OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IMMUNOPATHOGENESIS: ANALYSIS OF A SERIES OF 92 PATIENTS

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We conducted a detailed analysis of BM biopsy (BMB) samples from a series of 92 CLL patients and explored associations with clinical features and outcome, CD38 expression, and IGHV mutation status. Patient group: M:F=54:38, median age: 66 years, Binet stage-A/B/C: 76/12/4. Peripheral blood immunophenotype: (1) CD38+: 35/92 cases (2) IgG+: 19/92 cases. Mutated/unmutated IGHV genes (M-IGHV/A-IGHV): 59/33 cases. Formalin-fixed, decalcified, paraffin-embedded sections of BMBs were examined: (1) morphologically (H&E); (2) immunohistochemically (ABC technique). The following antibodies were used: CD20, CD79a, CD3, BCL-2, CD5, CD23, SIg/CIg (kappa/lambda/mu/delta/gamma/alpha), CD27, myeloperoxidase, PGM1 (CD68), glycophorin-C, CD61. All cases showed neoplastic lymphocytic infiltration (20-95% of BM cellularity). In all cases, neoplastic lymphocytes expressed CD20/CD79a/CD5/CD23. In the vast majority of cases, the neoplastic population consisted almost exclusively of small lymphocytes. Medium- or large-sized cells were admixed in 4 and 2 cases, respectively. A moderate number of monocytoid-like cells with clear cytoplasm were identified in 3 cases. Finally, 5 cases exhibited lymphoplasmocytoid differentiation in a small proportion of the neoplastic population. Three patterns of neoplastic lymphocytic infiltration were identified: (1) interstitial: 41 cases; (2) nodular/nodular+interstitial: 31 cases; (3) diffuse: 20 cases. Moderate-to-significant reduction of the granulocytic series was observed in 47/92 cases. The remaining cases showed hyperplastic granulocytic series with a left shift and, occasionally, dysplastic changes of mature forms. Hyperplasia of the erythroid and megakaryocytic series -often with dyserythropoiesis and dysmegakaryopoiesis- in a context of normal/decreased hemoglobin levels and platelet counts was observed in 52/92 and 72/92 cases, respectively; the remaining cases showed moderate-to-significant reduction of either series. Significant correlations were identified between: (i) nodular

infiltration and M-IGHV genes (p<0.001); (ii) diffuse infiltration and shorter time-to-progression (p=0.05). In conclusion, BMB examination: (1) permits identification of the rare CLL subtype with plasmocytoid differentiation (WHO); (2) allows evaluation the BM stroma and the hematopoietic marrow and provides important evidence for CLL-associated hematopoietic autoimmunity. Finally, the present study indicates that the favorable prognosis of the nodular pattern of infiltration in CLL may be interpreted in the context of its association with M-IGHV genes.

PP3-143

A CASE OF V617F(+) MYELODYSPLASTIC / MYELOPROLIFERATIVE DISORDER

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Background: Mutations involving the JAK-2 tyrosine kinase have been described in a great majority of Polycytemia Vera(PV)and to a lesser extend in Essential Thrombocytemia(ET) and Chronic Idiopathic Myelofibrosis(CIMF). Myelodysplastic syndromes(remained mostly (-) for JAK-2 mutation except a unique subgroup with persistant presence of anemia, ringed sideroblasts and thrombocytosis. While some of these were referred as Refractory anemia with ring sideroblasts –Thrombocytosis (RARS-T) in the literature some were classified as Myeloproliferative / Myelodysplastic Disorder– unclassifiable (MPD/MDS–u). Here we are reporting a patient with a V617FJAK(+) MPD/MDS-u, characterised by anemia, ring sideroblasts, thrombocytosis,granulocytosis and multilineage dysplasia.Material&Methods: A 53 year old male presented with recurrent gastrointestinal bleeding. He was found to have anemia,thrombocytosis, granulocytosis and hepatosplenomegaly. In contrast to an iron deficiency anemia, expected in a setting of recurrent bleeding, he had macrocytic anemia. Upon completion of his acute management, the patient underwent a bone marrow biopsy in the outpatient setting. His bone marrow biopsy showed hypercellularity(%100) due to granulocytic and megakaryocytic expansion. Erythroid precursors were decreased and there was multilineage dysplasia. Numerous ring sideroblasts were identifed on the Prussion blue stain. The case was classified as MPD/MDS-u and a correlation with JAK-2 mutation was suggested by the pathologist. Real time PCR with melting curve analysis was performed for V617FJAK-2 on genomic DNA isolated from peripheral blood. Results : Our case characterised by anemia, thrombocytosis, ring sideroblasts, leucocytosis and multilineage dysplasia, classified as MPD /MDS–u revealed V617FJAK-2. Discussion : Detection of JAK-2 mutation with different diseases raises the possibility of presence of additional mutations determining the final disease phenotype. V617F mutation is known to render JAK-2 constitutively active resulting in abnormalmyeloproliferation. Therefore, in the myeloproliferative disease PV, JAK-2 mutation may be the sole genetic anomaly, correlating with the promising results obtained with JAK-2 inhibitors. However, in the MPD/MDS category, sole JAK-2 inhibition may not be sufficient in controlling the dysplastic component. Although, effects of JAK-2 inhibitors remain to be tested in clinical practice,further studies are needed to uncover cooperating mutations in this category.

PP3-144

c-MYC GENE TRANSLOCATION AND INCREASED COPY NUMBER PREDICT POOR PROGNOSIS IN ADULT DIFFUSE LARGE B CELL LYMPHOMA (DLBCL), ESPECIALLY IN GERMINAL CENTER-LIKE B CELL (GCB) TYPE

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Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease showing variable clinical presentations, histology, and molecular and cytogenetic features. In this study, c-MYC, BCL2, and BCL6 gene translocation and gene copy number change were comprehensively investigated in adult DLBCLs using interphase fluorescence in situ hybridization (FISH) analysis in formalin-fixed paraffin-embedded tissues, and their clinicopathologic features and prognostic implications were analyzed, especially in the context of germinal center B-cell like (GCB) and non-GCB type of DLBCLs based on immunohistochemistry. C-MYC translocation was observed in 9% (14 of 156), and increased gene copy number (ICN) was in 7.1% (11 of 156) of DLBCLs. Of the 14 cases with c-MYC translocation, 12 had IgH gene as a fusion partner. C-MYC translocation was significantly more common in GCB type DLBCLs than non-GCB type (22% [9 of 41] in GCB vs 4.9% [5 of 103] in non-GCB, p = 0.004). C-MYC translocation or ICN was not associated with Ki-67 labeling index. However, c-MYC translocation or ICN exhibited association with decreased BAX expression and increased BIM expression (p = 0.022 and 0.001, respectively). Clinically, c-MYC translocation was closely related with advanced IPI group (p = 0.005). In univariate survival analysis, c-MYC aberration including translocation or ICN was associated with shorter overall survival (p = 0.012) in adult DLBCLs, especially within GCB type (p = 0.030). BCL2/ IgH translocation in DLBCLs was only observed in 3.4% (5 of 145) in our cohort, which was much lower in comparison with the reports of Western countries. BCL2 ICN was detected in 11.7% (17 of 145), more frequently in non-GCB type DLBCLs than GCB type (16% [15 of 94] in non-GCB vs 2.5% [1 of 40] in GCB, p = 0.038). BCL2 ICN and/or BCL2/IgH translocation was well correlated with BCL2 protein expression (p = 0.031). BCL2 aberration such as ICN or translocation tended to show adverse effect on the overall survival of patients, but without statistical significance. BCL6 translocation was observed in 17.5% (24 of 137), which was most common gene aberration in our study. However, BCL6 translocation had no influence on the prognosis of DLBCL patients. In conclusion, we suggest that the evaluation of c-MYC gene status including translocation and increased gene copy number and the analysis of the results in the context of DLBCL phenotype might be important to predict the prognosis of DLBCL patient and determine the therapeutic strategies.

PP3-145

A SIX-MONTH ART INTERRUPTION IN HIV-INFECTED PATIENTS IMPROVES ADIPOSE TISSUE MORPHOLOGY AND GENE EXPRESSION

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To determine the ability of adipose tissue to recover after six-month ART interruption, subcutaneous abdominal adipose tissue of forty well-controlled HIV-patients with thirty three completed the study was studied at inclusion and after 6 months. Adipose tissue morphology, mithochondrial DNA and gene expression were evaluated. At M6, clinical parameters were not significantly modified, but inflammation markedly improved with a decreased in numbers of macrophages, and of adipocytes or inflammatory

cells stained with TNFα and IL6. The expression of adipogenic factor PPAR gamma and of markers associated with mitochondrial function and biogenesis, COX2 and PGC1α, was improved. Mitochondrial alterations were improved with decreased COX4 mRNA and increased mtDNA and COX2/C0X4 ratio. These data clearly outline the delerious impact of the thymidine analogues on adipose tissue. Even if no modification was clinically noticed, a 6-month treatment interruption allows a partial recovery of adipose tissue alterations.

PP3-146

SIMIAN VIRUS 40 IN KOREAN PATIENTS WITH NON-HODGKIN'S LYMPHOMAS

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DNA sequencies coding for simian virus 40 (SV40) large T antigen have been detected in various human tumors, including non-Hodgkin's lymphomas (NHLs). But the relationship between SV40 and NHLs was controversial and needs to be studied. 89 cases of NHLs and 20 control lymph node tissues were tested for SV40 sequences using PCR amplification and sequencing. Viral sequences were present in two (2.2%) of 89 NHLs and were absent in 20 control tissues. Two SV40 positive cases were both intestinal diffuse large B cell lymphomas, and immunohistochemical staining for SV40 large T antigen was completely negative. These results suggest that SV40 is unlikely to have played a pathogenetic role in most of lymphoproliferative disorders in the Korean population.

PP3-147

UNUSUAL SITES OF B-CLL INVOLVEMENT: BIOPSY DIAGNOSIS OF B-CLL INFILTRATES PRESENTING IN EXTRANODAL AND EXTRAMEDULLARY TISSUES

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Background: Majority of B-CLL/SLL cases with the diagnosis established by a biopsy is based on examination of lymph node (LN) or bone marrow (BM) specimens using WHO classification. Less commonly the patients show extranodal and/or extramedullary B-CLL manifestation, requiring an endoscopic or surgical intervention and biopsy verification of the tumor infiltrate. The aim of this study was to evaluate the possibilities of an appropriate B-CLL/SLL biopsy diagnosis in such cases. Methods: We reviewed records of 716 biopsies of B-CLL/SLL patients performed in the interval 01/2004 – 03/2007 and selected those presenting in other than LN and/or BM localisations. All the cases were reevaluated in sections stained with HE and Giemsa staining and by immunohistochemical detection of CD20, CD23, CD43, CD3 or CD45RO and Ig light chain. In addition, the biopsy register was analyzed to identify all other previous or subsequent biopsies of the patients. Results: From all reviewed biopsies only 15 cases (2,1%) represented infiltrates of other than LN or BM tissues, 3 of them represented a high grade transformation of B-CLL. In 5 patients the disease was diagnosed by tonsillectomy and in 10 cases by involvement of non-lymphoid organs: upper respiratory tract (n=3), trachea (n=1), thyroid gland (n=1), skin (n=3) and soft tissues (n=2). B-CLL/SLL diagnosis was confirmed: a.) by typical morphology and phenotype (n=12), incl. 2 cases of CLL with coincidental diffuse large B-cell lymphoma and classical Hodgkin lymphoma, resp. b.) by previous or subsequent BM (n=6) or LN biopsy (n=3) confirming the B-CLL diagnosis, incl. also cases problematic at the actual time of biopsy. These cases included: 2 limited and partially necrotic specimens insufficient for the complex analysis, especially in relation to confirmation CD23 positivity and 1 skin biopsy of a patient with B-CLL showing diffuse large B-cell lymphoma without residual CLL infiltration. Conclusion: The biopsy examination of B-CLL infiltrates appearing in the extranodal localisation seems to represent a rare event. These cases may cause diagnostic and differential diagnostic problems, while it might be difficult to follow the WHO criteria in limited material from unusual sites of involvement. Also the evaluation of high grade transformation might be problematic. In such cases the diagnosis should be supported by a complex clinical evaluation and/or by a BM and LN biopsy. Supported by Grants of Slovak Ministry of Health Nr. 2005/12-MFN-04, VEGA Nr. 1/4285/07 and Comenius University Nr. UK/83/2007.

PP3-148

PROGNOSTIC SIGNIFICANCE OF Bcl-2, P53 PROTEIN EXPRESSION AND Ki67 PROLIFERATIVE INDEX IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse Large B-Cell Lymphoma (DLBCL) is a high grade neoplasm which has a heterogeneous properties in clinical, morfological, immunophenotypical aspects. DLBCL exhibits varies markedly in response to treatment and prognosis. Clinicopathological and biological factors must be investigated and evaluated to determinate the prognose of the disease. In our study we searched effects of p53 (tumor supressor gene) which is a cell cycle regulator, bcl-2 oncoprotein which is an inhibitor of apoptosis and a cell proliferation marker Ki-67, on prognosis and relations with clinical parameters. Method: Total of thirty five patients who had been diagnosed as nodally located DLBCL at the Izmir Ataturk Training and Research Hospital between January 1999-June 2006 were included in this study. The Ann Arbor classification used to detemine the stage of patients. Patients were evaluated according to the age, sex, stage, B symptoms, extranodal involvement, lactate dehydrogenase (LDH) level and also immunohistochemically; p53 expression, bcl-2 oncoprotein expression and Ki-67 proliferation index. Statistical anlyses were performed using Chi-square, Fishers Exact and Log-rank test. Results: High Bcl-2 expression was identified in 9 patients (%25.7), high P53 expression was identified in 10 patients (%28.6) and high Ki67 expression was observed in 23 patients (%65.7). There was no statistical significant corelation between the p53, bcl-2 expression, Ki 67 proliferation index and age, sex, stage, B symptoms, extranodal involvement, LDH level, overall survival ($p>0.05$). There was no statistical significant relationship between p53, bcl-2, Ki 67 expression and prognosis ($p>0.05$). There wasn't a statistical significant relationship between overall survival and age, sex, stage, B symptoms, extranodal involvement, LDH level ($p>0.05$). Conclusion: As a result in our study we found that bcl-2 and p53 protein expressions, Ki67 proliferation index have no effect on overall survival of patients with DLBCL. The prognostic importance of p53, bcl-2 protein expression, Ki 67 proliferation index in DLBCL which has a biological and clinical heterogenity, can be understood in large series studies which have subclasses and with immunohistochemical markers which have optimal cut off values.

PP3-149
SPLENIC MARGINAL ZONE LYMPHOMA WITH ABERRANT CD5 EXPRESSION: REPORT OF A CASE
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Splenic marginal zone lymphomas (SMZLs) are low-grade B-cell lymphomas that characteristically lack CD5 expression. However, rare cases of marginal zone lymphomas have been reported with CD5 coexpression which show an aggressive clinical course than the CD5- negative SMZLs. We represent here a 44-year-old male with splenomegaly and leucocytosis in laboratory findings. Peripheral blood smears showed monocytoid B cells with villous projections. Flow cytometry of the peripheral blood revealed CD19, CD20, CD22 and FMC7 positivity without CD5, CD23, CD10, CD38, and CD103 positive expression. The spleen histology showed a distinctive pattern of white pulp infiltration by abnormal B lymphocytes with marginal-zone differentiation. Immunohistochemical findings of the spleen were consistent with flow cytometric analysis of the peripheral blood. Mononuclear cells showed CD20 positivity without CD5 and CD23 expression, However bone marrow biopsy revealed an interstitial and nodular lymphoid infiltration with strong CD5 immunopositivity. Clinicians and pathologists must be aware of this unusual aberrant CD5 expression in SMZLs on bone marrow infiltrate in order to reach a correct diagnosis.

PP3-150
CD 30 POSITIVE T- CELL LYMPHOPROLIFERATIVE DISORDER: A CASE REPORT
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Introduction: CD30-positive T-cell lymphoproliferative disorders (CD30+ T-LPD) of the skin include a heterogeneous group of diseases which differ in their clinical and histological presentations. Lymphomatoid papulosis (LyP), primary cutaneous anaplastic lymphoma (C-ALCL) and borderline lesions constitute CD30+ T-LPD. The diagnosis of C-ALCL and LyP is very difficult as many of them have overlapping clinical, histologic, and immunophenotypic features. Most patients within this spectrum of disease have an excellent prognosis with 5-year-survival rates of 100% for LyP and %90 for C-ALCL. Extracutaneus spread and regional lymph node involvement may be seen on patients with multifocal tumor lesions. LyP is generally managed by observation whereas C-ALCL is treated with spot radiation or surgical excision with systemic chemotherapy reserved for cases with large tumor size or extracutaneous involvement. Therefore it is necessary to differentiate these types of disorders from each other. We report here a case of CD30+ T-LPD with multiple skin nodules and no signs of generalization, lymph node or internal organ involvement. Case Report: A 63-year old male represented with increasing number of skin papules and nodules on his belly, arm, back and subcostal region. The largest one 4x3 cm in size enlarged rapidly and became ulcerated. The patient had a history of bladder carcinoma in 1998 without any postoperative chemo- or radiotherapy. The physical examination revealed no lymphadenopathy or organomegaly. Initial biopsy showed a subepidermal dense infiltrate of large lymphocytes with irregular nucleus and abundant clear-eosinophilic cytoplasm intermixed with a few reactive cells. Immunohistochemistry revealed strong and widespread CD30 positivity with expression of T-cell associated antigens. The clinical, histologic, and immunohistochemical findings pointed to a borderline lesion of CD30+ T-LPD. The patient was treated with one dose of CHOP protocol. After the 3 month-follow-up period a rapidly regress of all the nodular lesions was noticed. Conclusion: This report adds to the clinical and morphologic spectrum of the CD30+ T-LPD with uncertain progress and treatment. Accurate recognition of this lymphoma and follow up is needed.

PP3-151
CYTOGENETIC ABERRATIONS OF CHROMOSOME 1 IN B-CELL NON HODGKIN LYMPHOMAS: CORRELATIONS WITH DISEASE SUBTYPE
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Background: B-cell non Hodgkin lymphomas (B-NHL) comprise a variety of distinct entities that are associated with characteristic chromosome (chr) abnormalities. Many B-NHL acquire additional, recurrent cytogenetic aberrations during disease evolution. Abnormalities of chr 1 are considered a frequent secondary event in the cytogenetic progression of certain B-NHL. We undertook this study to analyze the location and frequency of chr 1 abnormalities in a large series of B-NHL to determine differences and common regions of alterations in distinct types of B-NHL. Methods: G-band karyotypes with clonal abnormalities, obtained from tissue submitted for cytogenetic analysis for suspected B-NHL, were reviewed to detect chr 1 abnormalities. All numerical and structural chr 1 aberrations were mapped on an ideogram to identify specific regions of gains and losses and sites of translocations. The B-NHL were classified according to current WHO criteria. Results: Chr 1 abnormalities were detected in 110/275 (40%) cases comprising 103 (37.4%) B-NHL; 58 diffuse large B-cell lymphomas (DLBCL), 21 follicular lymphomas (FL), 13 Burkitt lymphomas (BL), 5 mantle cell lymphomas (MCL), 5 marginal zone B-cell lymphoma (MZBCL), 1 chronic lymphocytic leukemia (CLL), and 7 (2.5%) reactive lymphoid hyperplasias (RLH). Isolated chr 1 abnormalities were detected in 2 B-NHL. Intra or interchromosomal translocations were found in 77/103 (74.7%) B-NHL and 4/7 (57%) RLH. Gains of chr 1 resulting from duplications, triplications, and homogeneously staining regions were observed in 47.6%, 6.8%, and 1.9% B-NHL, respectively and in 37.5% RLH. HSR were only detected in DLBCL. Deletions were identified in 33/103 (32%) B-NHL and 1/7 (14.3%) RLH. Gains were restricted to 1q and losses predominantly involved 1p, only 9 (8.7%) B-NHL showed 1q losses. The single CLL had a 1q translocation. The majority of rearrangement breaks were localized to chr 1q21.1 in all B-NHL subtypes. However, differences in common regions of gain/amplification (1q21-23 in BL and MZBCL and 1q21-31 in FL and DLBCL) and deletion (1p32-34 in MCL and 1p36 in FL and DLBCL) were noted. Conclusions: In addition to a high frequency of secondary chr 1 alterations, we detected primary chr 1 abnormalities in B-NHL and RLH. Common and different cytogenetic aberrations of chr 1 in B-NHL subsets indicate shared as well as distinct pathways of lymphoma progression. Elucidation of genes whose expression levels are altered due to chr 1 copy number changes could help identify tumor suppressor genes and protooncogenes of prognostic relevance.

PP3-152
PRIMARY FOLLICULAR LYMPHOMA OF THE GALLBLADDER
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Primary lymphomas of the gallbladder are extremely rare, and follicular lymphoma has been reported only twice. We report the third case of follicular lymphoma grade 1 limited to the gallbladder , found in the laparoscopic cholecystectomy specimen from a 80 year old woman with symptomatic gallstones. The patient was admitted to the hospital due to the symptoms and signs of acute cholecystitis. Ultrasound examination demonstrated gallstones within the gallbladder, the wall was not thickened. There was no lymph node enlargement in the abdominal cavity. On microscopy, the wall of the gallbladder was extensively infiltrated by lymphoid cells forming neoplastic follicles. The neoplastic infiltrate was composed of small to medium sized centrocytes with cleaved nuclei, inconspicuous nucleoli and scant pale cytoplasm. Additionally, less than to 5 centroblasts per high power field were found throughout the background of cleaved follicle centre-like cells. Immunohistochemical study showed that these cells were positive for B–cell marker CD20, CD79a. The majority of neoplastic cells showed bcl-2 immunoreactivity. Furthermore, they were CD5 and cyclin D1 negative. Our case demonstrates that follicular lymphoma can be limited to the gallbladder and confirm that it can occur in an organ normally devoid of lymphoid tissu.

PP3-153
CYCLIN D1 AND TROP-2/GA733-1 IMMUNOHISTOCHEMICAL INVESTIGATION IN ANAPLASTIC LARGE CELL LYMPHOMAS

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Background: Anaplastic large cell lymphoma (ALCL) is a rare non-Hodgkin lymphoma with special immunophenotypic and molecular characteristics. The protein Cyclin D1 is a major regulator of the G1 to S phase transition of the cell cycle and a well known proto-oncogene. In lymphomas, up-regulated cyclin D1 protein resulting from the translocation t(11;14)(q13;q32) has been reported in MCL, MM and cases classified as B-CLL and prolymphocytic leukaemia. Recently, has been reported that nuclear expression in 7/10 C-ALCL was detected using polyclonal antibody against cyclin D1. Trop-2/GA733-1, a cell surface glycoprotein, is a calcium signal transducer, may play an important role in the control of cell-cell adhesion and is frequently expressed at high levels by human carcinomas. A fusion transcript between CCND1 and TROP2 genes has been isolated from human tumors and has been found to possess potent transforming activity. Moreover, a cryptic promoter in the coding region of CCND1 gene drives the expression of TROP2 in the absence of exogenous promoters. The aim of the present study was to study the expression of cyclin D1 in ALCL. In addition, we tested for first time in paraffin material the mAb Trop-2 in order to examine the expression profile in ALCL. Methods: We utilized 52 FFPE biopsies classified as ALCLs according to the latest WHO criteria. For the study purposes, whole tissue sections and sections from 41 arrayed ALCL cases were used for IHC. The rabbit monoclonal anti-human cyclin D1 (clone SP4) and the mouse monoclonal anti-human cyclin D1 (clone DCS-6) antibodies were used for detection of cyclin D1 protein. An immunoenzymatic doublestaining method was also performed (Envision G/2 System/AP) for Cyclin D1 (SP4 or DCS-6) and CD30 mAb. For Trop-2/GA733-1 investigation the mouse anti-human mAb (551317, BD) was used. Results: None of the ALCL cases expressed nuclear or cytoplasmic cyclin D1 protein with both mAbs. All the ALCL tested cases were also negative for Trop-2 expression. Trop-2 predominantly membrane staining pattern was observed in squamous cells of the epidermis and in the epithelial cells of merocrine sweet glands. In 2 ALCL cases (1 C-ALCL and 1 systemic ALCL) scattered neoplastic cells showed slight nuclear positivity. Conclusions: The absence of Cyclin D1 protein in our study of ALCL cases is in line with previous reports. Trop-2 absence in lymphoma cells may indicate that this protein is not involved on the tumor growth. The nuclear staining in rare tumor cells requires further investigation.

PP3-154
COMBINED SKIN LESION CONSISTING OF BASAL CELL CARCINOMA AND LANGERHANS CELL HISTIOCYTOSIS

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Background. Langerhans cell histiocytosis (LCH) is a neoplastic proliferation of CD1α, S100-protein positive dendritic Langerhans cells (LCs) characterized by a highly variable clinical presentation and biological behavior. The pathogenesis of the disease is unknown. There is an association of disseminated LCH with other malignancies, mainly leukemias and lymphomas, although many of these cases may be related to therapy. Moreover, uncommonly combined lesions consisting of LCH and lymphomas or solid tumors have been reported in patients without previous history of systemic disease. In these cases the development of systemic LCH has not been described. We report a highly unusual case of combined skin lesion consisting of basal cell carcinoma (BCC) and LCH. Case report. A 63-year-old healthy man presented with a skin nodule involving the upper left eyelid. An excision biopsy was performed. The histopathological findings of the excised lesion (greater diameter:1.4 cm), were those of BCC. However, in the reticular dermis, adjacent to the BCC, a diffuse infiltration composed of large cells with moderate abundant cytoplasm and grooved or indented nuclei was observed. An admixture of small lymphocytes, plasma cells and few eosinophils was also present. Immunohistochemically, the large cells were CD1α, S-100, CD68, CD3, CD45 and vimentin positive and EMA, pan-CK, MPO, CD30, B and T-cell markers negative. Discussion. Combined skin lesions consisting of LCH and BCC represent an uncommon condition. We are aware of only one similar reported case localized in the scrotum. The nature of LCs in combined lesions remains elusive with debate as to whether they represent a true neoplasm or an exaggerated reactive proliferation, propably induced by factors released by the adjacent tumor cells. X-chromosome inactivation assays in some of these lesions showed that, at least in a proportion of cases, LCs are polyclonal.

PP3-155
IMMUNOHISTOCHEMICAL EXPRESSION OF CELL CYCLE REGULATORY PROTEINS IN MULTIPLE MYELOMA

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Background. The mechanisms involved in cell cycle deregulation in multiple myeloma (MM) are largely unknown. Therefore, the immunohistochemical expression of G1/S cell cycle regulatory proteins were analyzed in relation to the proliferation profile (Ki67, cyclin A, cyclin B1) and clinicopathological parameters in MM. Materials and Method. Bone marrow biopsy specimens from 45 patients with MM were studied by immunohistochemistry, using antibodies directed against cyclin

D1, cyclin D2, cyclin D3, cyclin A, cyclin B1, Ki67, p53, Rb and p16 proteins. Correlations between continuous variables were calculated using Spearman's coefficient test and overall survival was estimated using the Kaplan–Meier method. Results. Expression (more than 10% of neoplastic cells) of cyclin D1, cyclin D2, cyclin D3, cyclin A, cyclin B1, Ki67, p53, Rb and p16 proteins was found in 17/45 (37.8%), 18/42(43%), 3/43(7%), 11/44(25%), 0/43(0%), 20/44(45.5%), 17/45(37.8%), 35/44 (79.5%) and 37/44 (84.1%) cases, respectively. The expression of Ki67 protein was positively correlated with the expression of cyclin A and cyclin D2 proteins ($p<0.001$ and $p=0.001$, respectively). Moreover, Ki67 and cyclin A expression was increased in parallel with the expression of Rb ($p=0.016$ and $p<0.001$, respectively) and p16 proteins ($p=0.043$ and $p=0.005$, respectively). A positive correlation was also found between Rb and p16 expression ($p=0.003$). Although, cyclin D1 and p53 protein were overexpressed, their expression was not correlated with the expression of Ki67, cyclin A or cyclin B1 proteins. The histological stage was positively correlated with Ki67, cyclin A, cyclin D2 and p53 expression ($p=0.03$, $p=0.04$, $p=0.003$ and $p=0.026$, respectively). Kaplan–Meier analysis showed that the histological stage was significantly associated with patient overall survival ($p=0.036$). The expression of the studied cell cycle regulatory proteins did not show any prognostic significance. Conclusions. The findings of this study suggest that in MM: a) cyclin D1 and cyclin D2 proteins are overexpressed, b) the expression of cyclin D2 is increased in parallel to proliferative activity and may be involved in the deregulation of cell cycle, and c) the expression of Rb and p16 proteins follows proliferative activity suggesting no abnormal loss of these proteins.

PP3-156

MICROVESSEL DENSITY AND EXPRESSION OF THE ANGIOGENIC PROTEINS VEGF, VEGFR1, VEGFR2 AND HIF-1α IN HODGKIN AND REED-STERNBERG CELLS OF CLASSICAL HODGKIN LYMPHOMA

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Background. Few studies have focused on angiogenesis in classical Hodgkin lymphoma (cHL). Therefore, in the present study the microvessel density (MVD) and the immunohistochemical expression of angiogenesis related-proteins in Hodgkin and Reed-Sternberg cells (HRS) of cHL were analysed. Materials and Method. Biopsy material from 95 cases with cHL (mixed cellularity:60, nodular sclerosis:23, lymphocyte depletion:3, unclassified:9) were studied immunohistochemically for the expression of VEGF, VEGFR1, VEGFR2 and HIF1a proteins. In addition, MVD was estimated after immunostaining for CD34 (MVD-CD34) and CD105 (MVD-CD105) proteins and evaluated as the mean (±SD) vessel count/high-power microscopic field (HPF). The results were correlated with clinicopathological data. Results. Expression (at least 10% positive HRS cells) of VEGF, VEGFR1, VEGFR2 and HIF1a proteins was found in 78/90 (86.7%), 32/95 (33.7%), 18/89 (20.2%) and 46/81 (56.8%) cases, respectively. The MVD-CD34 and the MVD-CD105 were 61(±28.7) and 12(±7.65), respectively. VEGF expression was positively correlated with VEGFR2 expression and MVD-CD34 ($p=0.003$ and $p=0.02$, respectively). A similar correlation was found between VEGFR1 expression and MVD-CD34 ($p=0.04$). Moreover, an inverse correlation between the expression of VEGFR2 protein and the clinical stage of the disease was found ($p=0.02$, $r=-0.505$). Conclusion. The present study shows that the VEGF, VEGFR1, VEGFR2 and HIF1a proteins are expressed in HRS cells of cHL. VEGF produced by HRS cells may act in a paracrine and autocrine fashion in cHL and possibly is implicated in the pathogenesis of the disease.

PP3-157

ALTERATIONS OF THE p53, Rb AND p27 TUMOR SUPPRESSOR PATHWAYS IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Background. Diffuse large B-cell lymphomas (DLBCL) display defects in cell cycle and apoptosis regulation. Therefore, the immunohistochemical expression patterns of the proteins p14, p21, Hdm2 and cyclin D2 were analyzed in relation to the previously reported expression of other major cell cycle proteins (p53, Rb, p16, p27, Ki67 and cyclins A, B1, D2, D3 and E), apoptosis-associated proteins (bcl2, bcl-xl, bax, bak, bad and bid) and the B-cell differentiation immunophenotypes. Materials and Method. Seventy nine cases of de novo DLBCL (37 nodal and 42 extranodal) classified according to WHO classification were studied by immnohistochemistry, using antibodies directed against p14, p21, Hdm2 and cyclin D2 proteins. Results and Conclusions. Expression of the proteins p14, p21, Hdm2 and cyclin D2 was observed in 62/71 (87%), 22/76 (29%), 35/74 (47%) and 11/77 (14%) cases, respectively. Immunohistochemical alterations of the p53 [p53-Hdm2-p21-p14], Rb [Rb-p16-cyclin D (D2 or D3)] and p27 [p27-cyclin E] pathways were found in 56/77 (73%), 53/79 (67%), 54/79 (68%) cases, respectively. Concomitant alterations of the p53-Rb, p53-p27 and Rb-p27 pathways were found in 40/77 (52%), 38/77 (50%) and 36/79 (46%) cases, respectively. Three concomitant alterations of p53-Rb-p27 pathways were found in 28/79 (35%) cases. The main findings of the present study were the following: 1) alterations of the p27 pathway were associated with higher expression of Ki67 ($p=0.023$), 2) concomitant alterations of the p53-Rb pathways and the p53-p27 pathways were associated with higher expression of cyclin A ($p=0.015$ and $p=0.021$, respectively) and 3) concomitant alterations of the p53, Rb and p27 pathways were associated with higher expression of cyclin A ($p=0.013$). Since cyclin A supports DNA replication, centrosome duplication and mitosis, these findings indicate that concomitant alterations of the p53, Rb and p27 pathways in DLBCL may have cooperative effects resulting in increased neoplastic cell proliferation. This might explain, at least partially, the association between concurrent aberrations of the p53, Rb and p27 pathways and aggressive clinical behavior in DLBCL.

PP3-158

ANGIOGENESIS AND MYELOMA : A COMPARATIVE STUDY OF CD34 AND CD138 IN PRE- AND POST-TREATMENT BONE MARROW BIOPIES

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Background and aims: Bone marrow (BM) angiogenesis is increased in multiple myeloma and is an important prognostic factor for survival. Given its potential antiangiogenic effect, we

evaluated if thalidomide therapy would affect the BM microvessel density (MVD). Material and methods : We tested both CD34 and CD138 on paraffin sections of bone marrow biopsy using a labelled streptavidin-biotin peroxydase method. MVD and % of CD138 positive cells were compared between pretreatment biopsies and those obtained following therapy. Slides stained with antibodies to CD34 were scanned under low power (x100) to identify three hot spots or areas with the greatest number of microvessels. These three areas were then evaluated at x400 magnification. Large vessels and vessels within the bony spicules and under the periosteum were excluded. Areas of staining without discrete breaks were counted as single vessels. The presence of a lumen or red cells was not required for the identification of microvessels; any highlighted endothelial cell or cell cluster separate from the adjacent microvessels was counted as a distinct vessel. The number of vessels in one x400 field was determined for each hot spot; the average was taken as the MVD. Results : On the basis of the MVD, the patients were grouped into high- and low grades using the mean MVD as the cutoff. The mean MVD for the entire group was 18 (range, 3–54). Good responders (9 patients) with a mean decrease of 60% in CD138 cells (range 30-100%) had a low-grade MVD (mean 15) and a decreasing MVD (pre-treatment MVD : 19, post-treatment MVD : 10). Patients with disease progression (5 patients) had a high MVD (mean 23) with an increasing MVD (pre-treatment MVD : 20, post-treatment MVD : 26). Conclusion : Our results confirm a significant decrease of MVD with thalidomide therapy. Although not conclusive, this result lends further support to the hypothesis that angiogenesis is a relevant therapeutic target in myeloma.

PP3-159
MYELOID SARCOMA OF THE LYMPH NODE WITH EXTENSIVE NECROSIS

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Myeloid sarcoma is a tumour mass of myeloblasts or immature myeloid cells occurring in an extramedullary site. The tumour mass may precede or occur concurrently with acute or chronic myeloid leukemia. A 38-year-old woman was admitted to hospital because of a left axillary lymph node enlargement. She was previously diagnosed as having Acute Myeloid Leukemia-M5 (AML-M5) and was treated with chemotherapy. Fine-needle aspiration cytology of the lymph node was consistent with lymphoma. Excisional biopsy specimen of the lymph node revealed diffuse infiltration of myeloblasts positive for CD43 and myeloperoxidase staining on immunohistochemistry, and changed the diagnosis from malignant lymphoma to myeloid sarcoma. We report an unusual case of acute myeloid leukemia presenting with myeloid sarcoma with a lymph node lesion exhibiting extensive lymph node infarction. A high index of suspicion for myeloid sarcoma is necessary to avoid missing this lesion. The critical factors for detecting this tumor are to be aware of this disease, cooperation between clinician and pathologist and the application of spesific immunohistochemical antibodies to detect the myeloid origin.

PP3-160
EPSTEIN-BARR VIRUS AND HUMANHERPES 8 IN LYMPHOID NEOPLASMS FROM SPAIN

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INTRODUCTION: Epstein-Barr virus (EBV) and Human Herpesvirus 8 (HHV8) are well known lymphotropic oncogenic agents. Our goal was to identify the proportion and the histological types of lymphoid neoplasms that express EBV and HHV8 in tumoral cells of a large series of cases diagnosed in Spain. MATERIAL AND METHOD: Tissue microarrays (TMA) were constructed from paraffined tumoral tissue of lymphoid neoplasms diagnosed in 5 Spanish hospitals from 1998 to 2003. EBER in situ hybridization for EBV and LANA immunohistochemistry for HHV8 were performed on histological sections of the TMA. RESULTS: From 182 obtained biopsies, 24% were Hodgkin's lymphomas (HL) and 76% were non-Hodgkin's lymphomas (NHL). 93,5% of NHL were of B cell origin whereas 6,5% were of T/NK cell. HLs were all HHV8 negative while only one case of NHL was HHV8 positive (an extracavitary PEL in a HIV+ patient). 22,7% of HL and 10,9% of NHL were EBV positive (EBV+). 19,4% (13/67) of the high-grade B-cell NHLs (Diffuse large B-cell, Burkitt and Burkitt-like) were EBV+ while all the low-grade B-cell NHL (0/60) were EBV negative. 11,1% (1/9) of T/NK NHL were EBV+. 10% of the cases were associated with an immunodeficiency and showed an expression significantly higher of EBV than cases from immunocompetents (9.1% vs. 55,6% $p<0.0001$). In the immunocompetent group, there was a significant difference between HLs and NHLs EBV expression (21,4% vs. 4.9%; $p=0.003$) and, by histological subtypes, the high-grade B-cell NHLs were EBV+ in a 9,3% of the cases and, in this group, diffuse large B-cell NHL showed an expression of 7,7% (4/52). CONCLUSIONS: When studied with TMA, the expression of HHV8 in the tumoral cells of lymphoid neoplasms is very infrequent (0.54% of lymphoid neoplasms) and when found, an underlying immunodeficiency should be excluded. By contrast, EBV is expressed in a higher proportions, mostly in HLs and high-grade B-cell NHLs and, in a significantly higher proportion, in the setting of an immunodeficiency. (PI 04-0091, PI 04/1440, 04/1467, 05/1527)

PP3-161
MACROPHAGES AND DENDRITIC CELLS IN THE MICROENVIROMENT OF THYMIC NEOPLASMS. CORRELATION TO VASCULAR DENSITY AND HISTOLOGIC TYPE

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Background: Tumor associated macrophages (TAMs) are key components of the tumor microenvironment and seem to promote tumor growth. Their number is increased in various neoplasms. Dendritic cells (DCs) are basic components of the innate inflammatory response. Their role in tumor microenviroment is unclear. The presence of TAMs and DCs in thymic neoplasms has not been thoroughly evaluated before. This study investigates the presence of TAMs and DCs in thymomas and correlates them with microvessel density (MD). Method: A total of 20 thymomas (4AB, 2B1, 4B2, 6B3 and 4C according to WHO histologic classification and 3T1, 10T2, 4T3 and 3T4 according to TNM staging) were immunostained using antibodies to S100, PGM1, CD34 and CD31 proteins. For all antibodies, areas with the

highest density of positive cells were selected at low power magnification (x40), and the total number of immunostained cells or vessels was counted at high power (X400). At least three high power fields were counted and the mean value was marked as labeling index (LI). Results: LIs for microvessels according to histologic subtype were as follows (mean value±SD): AB 73.25±23.78, B1 54±43.8, B2 42.2±26.9, B3 32.83±24.02 and C 36.5±24.26. Tumors were subdivided for statistical purposes in two groups. Surprisingly, AB, B1 and B2 thymomas demonstrated higher MD compared to B3 and C (58.9±28.75 vs 34.3±22.8, respectively, p=0.049). S100 positive DCs were always identified within the tumor parenchyma. DCs number was higher in AB, B1 and B3 (17.125±12.26) compared to B3 and C (3.3±2.62) thymomas (p=0.003). A positive correlation between DCs and MD was also recorded (p=0.007). PGM1 positive TAMs were diffusely found in the tumor parenchyma in all AB, B1 and B2 tumors. In contrast, 50% of the B3 and C thymomas exhibited a tumor-stroma interface preference for TAMs localization. No statistical correlation between histologic type and TAMs number was seen [AB/B1/B2 45.67±16.67, B3/C 39.10±18.6336 (p=0.432)]. In addition, MD, DCs and TAMs were not correlated to tumor stage. Conclusions: A decrease of DCs is noted in less differentiated thymomas and implies that impairment of the host defense accompanies and possibly supports the progression of thymic neoplasia. TAMs do not seem to play an important role in thymic tumors, albeit their presence at the invasion front in the less differentiated tumors suggests a possible role in tumor invasion. Finally, further studies are needed to elucidate the exact role of vascular density in thymomas.

PP3-162
IMMUNOHISTOCHEMICAL EXPRESSION PATTERNS OF NEURAL AND NEUROENDOCRINE MARKERS, NEURAL GROWTH FACTOR RECEPTORS AND THE TUBULIN-β2 AND -β4 ISOTYPES IN THYMUS

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Background. Increasing evidence suggest that neuroimmune networks play key roles in the thymic histophysiology and pathology. Therefore, we analyzed the distribution of human thymic cells expressing major neural and neuroendocrine markers and the neural growth factor receptors (NGFR) in combination with the expression patterns of various cytokeratins. Additionally, since some tubulin-β isotypes are preferentially expressed in neuronal cells, we analyzed the immunotopographical distribution of thymic cells expressing tubulin-β2, -β3 and -β4. Materials and Method. Twenty cases of normal thymuses were analyzed by immunohistochemistry for the expression of the protein gene product 9.5 (PGP 9.5), chromogranin A (CHRA), synaptophysin (SYN), neuron specific enolase (NSE), tyrosine hydroxylase (TH), CD56, CD57, neurofilaments (NFs) (140-160kDa), the NGFRs (TrKA and p75), the tubulin-β2 and -β4 isotypes and the cytokeratins (CKs) 7, 8, 10, 13, 14, 18 and 19 proteins. Results. Thymic epithelial cells (TEC) expressed PGP 9.5, CHRA, SYN, NSE, TH, CD56, CD57, NFs, TrKA, p75, tubulin-β2 and -β4 isotypes and the CKs 7, 8, 10, 13, 14, 18 and 19. PGP 9.5 was preferentially expressed in cortical TEC whereas SYN, CHRA, NSE, TH and NFs were preferentially expressed in medullary TEC and Hassall corpuscles. Variable levels of expression of tubulin-β2 and -β4 were observed in all TEC subtypes whereas tubulin-β3 was undetectable in TEC. Subcapsular and cortical TEC display higher expression levels of tubulin-β4 and lower expression levels of tubulin-β2 in comparison to those observed in medullary TEC and Hassall corpuscles. Conclusions.The diversity of the immunotopographical distribution and the expression levels of neural and neuroendocrine markers, the NGFRs, the tubulin-β2 and -β4 isotypes and the CKs in the distinct subtypes of TEC may reflect the diversity of their biological functions and/or their different stages of differentiation. The present results provide further immunohistological evidence that numerous neural and neuroendocrine factors may be required for the development and the function of the human thymic microenvironment.

PP3-163
CIRCULATING LEVELS AND PROGNOSTIC SIGNIFICANCE OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS RECEPTOR IN CHILDHOOD ACUTE LEUKEMIA

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BACKGROUND:Vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1 and R2) are major regulators of angiogenesis, which plays a key role in the growth and dissemination of solid tumors and hematologic neoplasms. METHOD: To assess serum VEGF and VEGF-R1 levels and their prognostic significance in newly diagnosed acute leukemias (ALL and AML), we used a radioimmunoassay (RIA) to quantify their levels in stored samples obtained before treatment from 53 pediatric patients. Bone marrow blast percentage was counted at day of diagnosis and 2 weeks after induction therapy . RESULT:s VEGF level was between 2.7 and 138 pg/ml sVEGF-R1 level was between 0.0020 and 0.1836 ng/ml. A reverse correlation was observed between plasma sVEGF and especially sVEGFR1 levels and the rate of complete remission in pediatric patients with acute leukemias. CONCLUSION: Concentration of sVEGF and sVEGF-R1 in plasma should be considered a significant factor in response to treatment and considerable reduction of bone marrow blast percentage. In contrast, there was no relationship between their levels and WBC or blast count at diagnosis, or between VEGF level and such established prognostic factors as age, cytogenetics or FAB category.

PP3-164
PRIMARY LYMPHOMA OF THE THYROID GLAND. A CLINICAL PATHOLOGICAL REVIEW OF 3 CASES SEEN AT THE PATHOLOGY UNIT OF THE GENERAL HOSPITAL OF MEXICO

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Introduction Primary lymphomas of the thyroid gland constitute approximately 8% of all thyroid neoplasms. They are often associated (80% of the time) to Hashimoto and lymphocytic thyroiditis. The most frequent histological types are: large cell diffuse lymphoma B, marginal zone MALT type lymphoma, follicular lymphoma, plasmacytoma and peripheral t/nk lymphoma. Material and methods A retrospective review (2000-2005) was done of all pathological surgical sample records of the thyroid gland. A total of 115 cases of thyroid neoplasms were found: 64 (5.7%) corresponded to thyroiditis, 26 (40%) to lymphocytic thyroiditis, 24 (37.5%) to Hashimoto´s thyroiditis and 14 (21.8%) to unspecified chronic thyroiditis. Three lymphomas were found which had been submitted to the following specific immunohistochemical tests: cd 20, cd79a, cd 45, cd45 RO, cd2, bcl2, bcl6. cd 5, VEB, calcitonin, chromogranin and specific neuron enolase. Results The three lymphoma patients were females 56, 58 and 70 years of age. The symptomatology constituted a gradual and symmetrical growth of

the thyroid gland, with one of the patients having had cervical compression syndrome. Two cases corresponded to large cell diffuse lymphoma of the centroblastic variety. No marginal zone lymphoma cases nor thyroiditis were found, while one Malt type of the marginal zone associated to Hashimoto thyroiditis having LBDCG transformation zones was found. All were positive for cd 20 and two for bcl2 and negative for the rest of the antibodies. Conclusions Primary thyroid lymphomas were found in 2% of the cases we reviewed and are considered infrequent. The associated diseases, lymphoid and Hashimoto thyroiditis were also found to be infrequent (5.7%). This was probably due to the low frequency of primary thyroid lymphomas in our series.

PP3-165
IMMUNOEXPRESSION OF Skp2 AND CUl1, MOLECULES IMPLICATED IN p27Kip1 DEGRADATION IN AGGRESSIVE AND INDOLENT B-CELL LYMPHOMAS

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Background: Reduced levels of p27Kip1 (p27) are frequently observed in human cancers and associated with aggressive tumor behavior and poor prognosis. There is evidence that Skp2, a member of ubiquitin-ligase SCF (Skp1-Cul1-Rbx1-F box) complex, promotes p27 degradation. Moreover, Skp2 deregulation has been implicated in oncogenesis and lymphomagenesis. The aim of this study was to investigate the expression of Skp2 and Cul1, components of ubiquitin-ligase SCF complex, in aggressive and indolent B-cell lymphomas, and compared them with p27 expression and proliferation index (PI). In addition, the prognostic significance of Skp2 and/or p27 expression was studied in cases with available clinical data. P21Cip1 (p21) expression was also examined, because p21 is a good substrate for the ubiquitin-ligase SCF complex. Method: Formalin-fixed, paraffin-embedded tissue from 126 B-cell lymphomas [64 aggressive (DLBCLs) and 62 indolent (18 SLLs, 14 FLs, 11 MCLs, 19 MZLs)] were immunostained with antibodies for Skp2, Cul1, p27, p21 and MIB-1 using Envision detection kit. Clinical data for statistical analysis were available for 87 cases (40 aggressive, 47 indolent). The median follow up period was 33 months (22 mo for aggressive, 40 mo for indolent). Results: The mean value of Skp2 immunoexpression in aggressive and indolent lymphomas was 33.2% and 7.7%, respectively ($p<0.001$). The corresponding values for Cul1 were 36.4% and 14.7% ($p<0.001$), for p27 32.5% and 62.6% ($p<0.0001$), for p21 13.7% and 6.6% ($p=0.025$) and for MIB-1 68.7% and 27.1% ($p<0.001$). Skp2 showed an inverted correlation with p27 ($p=0.035$) and a positive correlation with Cul1 ($p=0.002$) and PI ($p=0.007$) in aggressive lymphomas. In addition, Skp2 had a similar correlation with p27 ($p=0.025$) and PI ($p<0.001$), but not with Cul1, in indolent lymphomas. There was also an inverted correlation between the expression of p27 and PI ($p=0.008$). Overall probability of survival was 35% for the whole group. This was 51.7% and 31.5% for patients with aggressive and indolent lymphomas, respectively ($p=0.074$). Skp2 and p27 expression did not correlate patients' prognosis. Conclusion: Our findings demonstrate a significant positive correlation between Skp2 expression and proliferation index and suggest the p27 degradative function of Skp2 in aggressive and indolent lymphomas. The positive relationship between Skp2 and Cul1 expression in aggressive lymphomas indicates their implication in p27 degradation.

PP3-166
PRIMARY BREAST LYMPHOMAS. A FOUR CASE REPORT

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Introduction The lymphomas that affect breasts may be primary or infiltrates as systemic manifestations of the disease. The most common histological types are large cell diffuse B lymphomas and extra-nodal B mucosa-associated lymphatic tissue lymphomas (marginal zone or extra-nodal lymphomas). Material and methods This is a report of four cases with primary breast lymphomas. The criteria for diagnosis were based on Wiseman´s and Liao´s proposal which includes: 1) specimen biopsy that involves a lymphoma in close relation to breast parenchyma and 2) no evidence of systemic disease at diagnosis. The clinical and histological data were reviewed, as well as immunohistochemical reactions. Results The average age of the patients was 29 years old. Three patients had problems with their right breasts and another bilaterally. There was progressive increase in volume, pain at palpation and skin affection, in addition to purulent secretion from the nipple. No affected lymph ganglia were palpated in three of the patients. Three of the patients died a few months (2-5) after diagnosis with infiltrations to their central nervous system. All four were large cell diffuse lymphomas of immunophenotype B, three were centroblastic, one had clear cells and another had extensive areas of necrosis with a starry-skied focal pattern simulating Burkitt´s lymphoma. They were positive to cd 20, cd 79 a, bcl2, bcl6, and cd 10. Conclusions Primary lymphomas of the breasts are infrequent. In our cases, the lymphomas were seen in young women. One was associated to pregnancy and another to AIDS. The differential diagnosis should be done with scarcely differentiated lobule and duct carcinoma for which immunohistochemistry reactions are very useful.

PP3-167
LANGERHANS CELL SARCOMA EXPRESSING CD56 IN A 60 YEAR OLD PATIENT WITH MYELODYSPLASTIC SYNDROME

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BACKGROUND: Langerhans cell tumors as currently defined in the WHO classification are a rare group of neoplastic proliferations showing CD1a and S-100 protein expression and displaying Birbeck granules by ultrastructural examination. Langerhans cell sarcoma (LCS) is the aggressive counterpart showing overtly malignant cytologic features and increased mitotic activity. The association of myeodyplastic syndrome with Langerhans cell tumors is extremely rare. Recently it has been suggested that CD56 expression is related to aggressive biological behavior in Langerhans cell tumors. We report a case of LCS expressing CD56 in a 60 year old patient with myelodysplastic syndrome initially diagnosed in the lymph node diagnostic material. METHODS: Formalin fixed, paraffin-embedded tissue sections were stained with H&E and immunohistochemically according to established protocols. RESULTS: Histological examination revealed effacement of the normal structure by a neoplastic proliferation showing nodular and sinusoidal growth pattern. Tumor cells ranged between round and oval displaying a lobulated nuclei with delicate nuclear membrane, chromatin abnormalities and prominent nucleoli. Distinct features of Langerhans cell tumors with the characteristic

nuclear grooving also were encountered, although infrequently. Eosinophils were rarely scattered. The mitotic rate was 10/10 HPF. Immunohistochemically, tumor cells were strongly positive for CD1a and S-100 protein and slightly positive for vimentin. CD56 was strongly positive. Only very weak CD68+ cells were seen. The proliferation marker Ki67 (MIB-1) reached 15%. Tumor cells failed to express CD3, CD20, CD21, CD35, CD30, CD34, pancytokeratin, HMB-45, Melan A and chromogranin. CONCLUSION: Our report further supports the association of Langerhans cell tumors with myelodyplastic syndrome, reinforcing the hypothesis of a common stem cell disorder. CD56 expression could enhance the prediction of the potential of an aggressive biological behavior in Langerhans cell tumors with borderline morphological features of malignancy.

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EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF SURVIVIN AND P 53 IN HODGKIN LYMPHOMAS

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Aim: Apoptosis represents a critical pathway whose abnormalities are linked with carcinogenesis. p 53, tumor supressor gene and inducer of apoptosis, suvivin a member of the inhibitors of apoptosis protein family are important regulators whose abnormal expression or mutation is correlated with several human cancers. To investigate their relationships to carcinogenesis and/or prognosis of Hodgkin lymphomas we analyzed their immunohistochemical expression in Hodgkin lymphomas. Material-Method: Paraffin sections from 56 Hodgkin lymphomas (28 noduler sclerosis, 18 mixed cellularity, 4 lymphocyte-rich, 1 nodular lymphocyte predominant, 5 classical-not specified) were examined with monoclonal antibodies anti-p53 and survivin using streptavidin-biotin immunohistochemical tecnique. Survivin was evaluated for both staining density and percentage of staining. Staining density evaluated as fallows:negative, mild, moderate, severe; percentage evaluates as fallows: +,<25%; ++, 25-50%; +++, 50-75%; ++++, >75% of Hodgkin/Reed Sternberg cells positive. p 53 was evaluated as fallows: negative <10%; +,10-25%; ++, 25-50%, +++, 50-75%;++++ ,>75% of Hodgkin/Reed Sternberg cells positive. Results: p 53 protein was detected in 20 cases. Survivin expression was detected in all cases with variable values. Survivin expression showed significant positive correlation with only age of the patients($p<0.05$). Expression of survivin and p53 did not showed significant correlation with subtypes, stage, survival time and recurrences in Hodgkin lymphomas($p>0.05$). Conclusion: Altough survivin and p53 expression was detected in Hodgkin lymphomas, these results showed that these markers not considered as a prognostic factors for Hodgkin lymphomas.

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EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF COX-2 AND P 53 IN HODGKIN LYMPHOMAS

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Aim: Cyclooxigenase(COX) is a rate-limiting enzyme that plays an important role in the inflammatory process and catayses the conversion of arachidonic acid to the prostaglandins and tromboxanes. One of two isotopes, COX-2 is usually not detectable in normal tissues. COX-2 is associated various human cancers especially colorectal adenocarcinomas and it is related to tumor progression. p 53, tumor supressor gene and inducer of apoptosis whose abnormal expression is correlated with several human cancers. To investigate their relationships to carcinogenesis and/or prognosis Hodgkin lymphomas we analyzed their immunohistochemical expression in Hodgkin lymphomas. Material-Method: Paraffin sections from 40 Hodgkin lymphomas (20 noduler sclerosis, 14 mixed cellularity, 2 lymphocyte-rich, 4 classical-not specified) were examined with monoclonal antibodies anti-p53 and COX-2 using streptavidin-biotin immunohistochemical tecnique. COX-2 was evaluated for both staining density and percentage of staining. Staining density evaluated as fallows:negative, mild, moderate, severe; percentage evaluates as fallows: +,<25%; ++, 25-50%; +++, 50-75%; ++++, >75% of Hodgkin/Reed Sternberg cells positive. p 53 was evaluated as fallows: negative <10%; +,10-25%; ++, 25-50%; +++, 50-75%;++++ ,>75% of Hodgkin/Reed Sternberg cells positive. Results: p 53 protein was detected in 14 cases. COX-2 expression was detected in all cases except one with variable values. Expression of COX-2 and p53 did not showed significant correlation with subtypes, stage, survival time and recurrences in Hodgkin lymphomas($p>0.05$). Although it was not statisticaly significant, COX-2 expression was higher in nodular sclerosis and mixed cellularity subtypes compared to lymphocyte rich and classical-not specified subtypes($p=0.054$). Conclusion: The expression of COX-2 and p53 is confirmed in H&RS cells of classical Hodgkin lymphoma. But, due to absence of association with stage, survival and recurrences of the disease these markers not considered as a prognostic factors for Hodgkin lymphomas.

PP3-170
HISTOLOGICAL ASPECTS OF FOLLICULAR DENDRITIC CELL SARCOMA AND PITFALLS IN ITS DIAGNOSIS

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Follicular dendritic cell sarcoma (FDCS) is a neoplastic proliferation of spindle to ovoid cells with morphologic and phenotypic features of follicular dendritic cells. It is a rare neoplasm with most studies representing single case reports or small series. The aim of this presentation is to present a rare case of FDCS in 27 year-old woman, previously diagnosed and treated as B cell immunoblastic non-Hodgkin`s lymphoma. The first diagnosis was made on a cervical lymph node biopsy five years ago, when she was treated with five cycles of CHOP regimen and complete remission of the disease followed. Four years later new tumor mass in the left midneck region appeared and was surgically treated. On the dissection one could barely recognize destroyed architecture of lymph node by infiltrative fleshy ten grey tissue that even involve subcutaneous and dermal tissue. There was no ulcerative lesion. Histological examination of the tissue sections stained with HE, Giemsa, Reticulin, PAS and Van Gieson revealed spindle to ovoid cell proliferation arranged in fascicles and storiform pattern. There were areas with plexiform architecture and myxomatous degenerative changes. In some of the cells there were PAS negative clear vacuoles. The cells had elongated oval nuclei with granular chromatin and small but distinct nucleoli enclosed in lentiform and syntitial slightly eosinophylic cytoplasm. There were fluid-filled cystic spaces with angiomatous features and only small solid areas with large

pleomorphic cells were found. Mitotic index was low. These tumor cells enclosed fibrous trabeculae with blood vessels surrounded by cuff of lymphocytes. Epidermal layer of the skin was intact but there was infiltration in the lower papillary dermis. Immunohistochemical analysis revealed positivity of the cells for Vimentin, HLA DR, CD21, CEA (nonspecific?) and spotty positivity for ESA. The stainings for cytokerains, S100, HMB45 and lymphocytic subpopulations were negative. Ki67 evaluated low proliferative index of 15%. Based on HLA DR and CD21 expression and the morphological appearance we made a diagnosis of FDCS and revised the previous diagnosis of B immunoblastic lymphoma. We found the same immunophenotype of the neoplastic cells that morphologically resemble large immunoblastic cells. However they were CD20 negative and CD21 positive. We can conclude that FDCS could have pleomorphic expression that ranges from lymphoid like appearance to spindle cell like or neurogenic-like neoplastic lesion. Immunohistochemical analysis is very helpful in distinguishing various entities.

PP3-171
HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED LYMPHOMAS. RECLASSIFICATION WITH TMA-BASED IMMUNOHISTOCHEMISTRY AND MOLECULAR BIOLOGY, ITS ASSOCIATION TO EPSTEIN-BARR VIRUS INFECTION AND EXPRESSION OF NUCLEAR FACTOR KAPPA-B AS PROGNOSTIC FACTORS

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Objectives: To know the clinical and pathological features of human immunodeficiency virus (HIV)-associated lymphomas, its association to Epstein-Barr virus (EBV) infection and expression of nuclear factor kappa-B (NF-kB) as prognostics factors. Material and Methods: HIV-associated lymphomas were selected in a period of 18 years. All the cases were reviewed, and they were reclassificated on the basis of morphology, TMA-based immunohistochemistry and molecular biology. Clinical features were obtained from the clinical records. Results: Fifty-seven cases were obtained. The average age of presentation was 40 (+/- 10) years, 95% were men and 5% women; 92.9% cases were in C3 stage. Seventy-eight patients presented B symptoms. The length of symptoms was from 0 to 48 months, with average of 6 months. The most frequent anatomic localization was nodal in 22 cases, oral cavity, CNS, colon, and rectum in 6 cases each one, and pulmonary in 5 cases. Bone marrow infiltration was present in 13 (23.2%) cases. Twenty-nine cases were in clinical TNM-stage IVB (51.7%). Thirty-six cases were reclassificated; 21 cases were excluded due to insufficient material. Twenty five cases (69.4%) corresponded to diffuse large B-cell lymphoma; of which, 48% were post-germinal origin, 40% centrofolicullar and 12% indeterminate origin. Six cases (16.6%) were reclassificated as Burkitt lymphoma; 3 of them displayed extensive plasmacytoid differentiation. Three cases (8.3%) corresponded to plasmablastic lymphomas and two cases (5.5%) were Hodgkin lymphoma. Twenty-one cases were EBV-associated (EBER positive), 19 cases were positive to NF-kB, and 12 were both positive. The global survival was of 15 months; in the cases that were EBV-positive, the average survival was of 17.3 months, and 14.5 months when NF-kB was positive. When both of them were positive, the survival decreases to 12.6 months. Conclusions: HIV-associated lymphomas are relatively frequent in our population. The prognosis is poor and the survival is short; that is the reason because is necessary to use the current and precise classification in those neoplasms. The VEB and NF-kB expression in HIV-associated lymphomas did not have significant impact on the prognosis; nevertheless, they constitute an important mechanism in the oncogenesis of this neoplastic process.

PP3-172
EPSTEIN-BARR VIRUS TUMORAL CELL EXPRESSION AND ITS IMPACT ON OVERALL SURVIVAL IN NON-HODGKIN LYMPHOMAS IN TARRAGONA, SPAIN

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BACKGROUND: Epstein-Barr virus (EBV) expression has been associated with an adverse prognosis in non-Hodgkin lymphomas (NHL) in few studies. AIM: To define the prognostic impact of EBV tumoral cell expression in a serie of NHL from Tarragona area in Spain. MATERIAL AND METHODS: We revised all the NHL cases diagnosed at our pathology department from the year 1990 to 2000 and we identified 265 NHL (rejecting bone marrow biopsies). After excluding the immunodeficient patients and cases without available paraffined tissue, 256 NHL cases were studied with EBER in situ hybridization. RESULTS: 24 cases (9.4%) were EBER positive (EBER+) [1 Burkitt (25%), 11 DLBC (12.36%), 1 Follicular lymphoma (2.86%), 3 small lymphocytic lymphoma (8.11%), 5 peripheral T-cell lymphomas of unspecified type (38.46%) and 3 extranodal NK/T-cell lymphoma of nasal type (100%)]. The EBER+ patients demonstrated substantially poorer overall survival (OS) [EBER+ vs. EBER-; 27 months (95% CI, 14-41 months) vs. 79 months (95% CI, 64-94 months), respectively, P=0.0013]. When studied by histological types, EBER+ remained statistically significant for poorer OS only for DLBC [EBER+ vs. EBER-; 23 months (95% CI, 3-43 months) vs. 60 months (95% CI, 47-72 months), respectively, P=0.0452] and for small lymphocytic lymphoma [EBER+ vs. EBER-; 23 months (95% CI, 12-34 months) vs. 88 months (95% CI, 59-118 months), respectively, P=0.0256]. Globally, in multivariate analysis for all LNH, EBER+ patients showed substantially poorer OS with 2.36-fold risk for death (95% CI, 1.3-4.3) while a high IPI status showed a 4.0-fold risk (95% CI, 2.6-6.0). When analysed by histological type, only the IPI status remained as a poorer independent survival factor. CONCLUSIONS: In our area, EBV expression in the tumoral cells of immunocompetents NHL patients appears to be associated with a poor OS, specially in DLBC and small lymphocytic lymphomas. However, in Cox multivariate analysis, EBV status did not affect survival with statistical significance in DLBC and small lymphocytic lymphomas. The absence of this signification could be explained, at least partially, by the small number of the EBV positive cases in this series that could affect the results. Further investigations in a larger series, in our area, are required.

PP3-173
PROMOTER HYPERMETHYLATION OF GSTP1, SHP1, DAPK, p16, RB, AND p53 GENES IN DIFFUSE LARGE B-CELL LYMPHOMAS: PREVALENCE AND PROGNOSTIC SIGNIFICANCE IN TUNISIAN PATIENTS

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Background: Promoter hypermethylation is an important mechanism of inactivation of tumor suppressor genes in various human cancers including lymphomas. Diffuse large B-cell lymphomas (DLBCL) are heterogeneous in their clinical presentation, morphology, and molecular alterations. The aim of this study was to determine the prevalence and clinicopathological significance of gene promoter methylation in DLBCL in Tunisian patients. Methods: Sixty DLBCL cases diagnosed at the laboratory of Pathology at Farhat-Hached University Hospital of Sousse (Tunisia) were investigated for the methylation status of a panel of six tumor suppressor genes potentially involved in B-cell malignancies, comprising GSTP1, SHP1, DAPK, p16, RB1, and p53 by methylation-specific polymerase chain reaction. Effect of each gene methylation status on patient's overall survival were estimated by the Kaplan-Meier method and compared with the log-rank test. Multivariable survival analyses were performed with the Cox proportional hazards model. Results: Aberrant promoter hypermethylation of DAPK, GSTP1, SHP1, p16, RB1 and p53 was detected in 75%, 70%, 65%, 53%, 20% and 3.3% of cases, respectively. All cases demonstrated aberrant hypermethylation in at least one gene. There was no significant correlation between overall gene hypermethylation status and patient's age, gender, or clinical stage. Regarding the clinical follow-up data, available for 17 patients, promoter hypermethylation of DAPK and p16 were significantly associated with shortened survival (P=0.007 and P =0.016, respectively) and remained poor prognostic factor in multivariate analysis (P = 0.013 and P = 0.039, respectively). Conclusion: our results indicates that promoter hypermethylation is a frequent event in diffuse large B-cell lymphomas in patients from Tunisia, and that DAPK and p16 promoter regions may serve as a potential prognostic marker for predicting survival in patients with diffuse large B-cell lymphomas.

PP3-174
APPENDICEAL LYMPHOMA PRESENTING AS ACUTE APPENDICITIS: A CASE REPORT

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Malignant lymphoma comprises 1-4% of the malignant neoplasm of the gastrointestinal tract, but appendiceal lymphomas are very rare, also acute appendicitis is an extremely rare initial presenting of appendiceal lymphoma. We describe a case of a 14-year-old male, who had lymphoma of appendix presenting as acute appendicitis. His symptoms were vomitting and pain in the right lower quadrant of the abdomen. Due to the rebound and tenderness of Mc Burney's point on physical examination, appendectomy was performed as a result of the clinical diagnosis of acute appendicitis. Histopathologic evaluation showed diffused proliferation of large and medium size lymphoma cells on mucosa and submucosa layers with acute inflamation on muscular and serosa layers. Immunohistochemical examination revealed that the lymphoma cells were positive for B cell markers. This case was diagnosed as a diffuse large cell lymphoma of the B cell type according to the WHO classification. Despite the fact that lymphoma of appendix is rare, clinically physicians and surgeons have to be aware of the differential diagnosis of acute appendicitis, because, all of cases clinically as acute appendicitis, it is not always true acute appendicitis.

PP3-175
PEARSON SYNDROME: A CASE REPORT AND CLINICOPATHOLOGIC REVIEW

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Pearson marrow-pancreas syndrome is very rare fatal disorder that involves the hematopoietic system, exocrine pancreas, liver, kidneys in early infancy. Due to the diversity of clinical symptoms, the diagnosis can be difficult. We report on a 6 months-old boy who had presented with macrocytic anemia, neutropenia, thrombocytopenia, fever, hepatosplenomegaly and slight lactic asidosis.On bone marrow aspiration, his bone marrow was characterized by marked vacuolization of erythroblasts and myeloid precursors. Molecular analysis was made from the patient's blood leukocytes to detect a deletion of mitochondrial DNA. When a neonate's or infant's bone marrow aspiration exhibits vacuolization of myeloid and erythroid precursors and also clinically the patient has metabolic acidosis of unknown cause, the possibility of Pearson syndrome should be considered.

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IMMUNOHISTOCHEMISTRY IN HODGKIN'S LYMPHOMA: DIAGNOSIS CHANGES BEFORE AND AFTER APPLICATION, TO IDENTIFY MORPHOLOGIC AND BIOLOGIC GREY ZONE LYMPHOMAS

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BACKGROUND: There is a diagnostic grey zone between Classic Hodgkin lymphoma (CHL) and some histologic types of non-Hodgkin lymphoma (NHL), including anaplastic large cell lymphoma (ALCL) , T-cell-rich B-cell lymphoma (TCRBCL) and mediastinal large B cell lymphoma. Some morphological and/or phenotypic features are common in both CHL and those particular NHL types. METHOD AND RESULT: In this study, the cases of HL, diagnosed and subtyped without immunohistochemistry (IHC) were reinterpreted after the performance of IHC and the cases shareing common features with NHL were isolated. In addition the cases confused with HL in the morphological and/or biological grey zone but diagnosed after IHC as ALCL and TCRBCL were taken in the study group and together with HL, and all were reevaluated. After the reinterpretation of 508 cases, the diagnosis of CHL was reestablished in 390 cases out of 429 (90,9 %).In 35 cases (8,1 %) the diagnosis was changed to NHL. NHL subtypes were ALCL in 11 cases,TCRBCL in 21 cases, PTCL (peripheral T-cell lymphoma) in 1 case, DLBCL(diffuse large B-cell lymphoma) 1 case and B cell ALCL in 1 case. In 2 cases the differentiation between HL and TCRBCL, in 2 cases the differentiation between ALCL and HL couldn't be achieved. Out of 11 cases in which the differential diagnosis between HL and NHL couldn't be achieved initially, the diagnosis was established as ALCL in 3 cases(27,3 %) and as HL in 7 cases (63,6%) after the reevaluation of the cases. In 1 case the differentiation between NLPHL and TCRBCL couldn't be made. In 31 cases of TCRBCL,the initial diagnosis wasn't changed. Out of 37 cases initially diagnosed as ALCL the differentiation between ALCL and HL couldn't be achieved in 1 cases . CONCLUSION: The grey zone between HL and NHL can either be a biological grey zone as a result of common pathogenesis or it may be a morphological grey zone due to the morphological similarities.In conclusion, as the biology and the origin of RS cells were cleared out, the differentiation of HL from other aggressive lymphomas such as TCRBCL or ALCL became possible at least to an extend. However, in cases displaying CD 15 negativity and high ratios of CD20 positivity, the differential diagnosis was still troublesome.

PP3-178

CLINICAL ROLE OF FLOW CYTOMETRY IN REDEFINING BONE MARROW INVOLVEMENT IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) – A NEW PERSPECTIVE

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Aims: The clinical role of flow cytometry in staging bone marrows in Diffuse Large B-cell Lymphoma (DLBCL), especially its impact on outcome, remains ambiguous. The aims of this study were to determine the contribution of flow cytometry to conventional staging, and to study impact on survival. Methods and results: One hundred and thirteen cases of DLBCL diagnosed at The Canberra Hospital from 1996-2005 were identified for this retrospective study. On blinded analysis of Bone marrow (BM) aspirates, trephine biopsy slides, and flow cytometry data, involvement on morphology (M) was detected in 23 (20.3%) cases and on flow cytometry (F) in 25 (22.1%) cases with discordance noted in 18 cases (15.9%). Together, the 2 diagnostic techniques (M + F) detected 33 cases (29.2%). Cases with and without marrow involvement on conventional staging alone (M) had no significant difference in survival (p=NS). However, when bone marrow involvement was defined as positivity on morphology and/ or flow cytometry (M + F), the median survival of patients with involvement was significantly worse than patients without involvement (p=0.02). Conclusions: Flow cytometry positive cases should be included in a summative model to define bone marrow involvement in DLBCL as it may have a potential impact on outcome.

PP3-179

IMMUNOHISTOCHEMICAL EXPRESSION OF CD34 IN LYMPH NODE AND BONE MARROW OF PATIENTS WITH HODGKIN'S LYMPHOMA

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Background: Bone marrow infiltration is present in 5-15% of patients with Hodgkin's lymphoma (HL). It is usually associated with prominent fibrosis and proliferation of blood vessels. Limited information exists about the significance of neoangiogenesis in HL. In this study we analyzed immunohistochemical expression of CD34 in lymph node and bone marrow of patients with HL. Methods: We identified 39 patients with HL (25 male and 14 female, median age 43 years), 25 patients with bone marrow infiltration by HL and 14 patients without bone marrow infiltration. Slides from paraffin embedded lymph node and bone marrow biopsy were stained with hematoxylin&eosin and reticulin fibers (Gordon-Sweet). Microvessels were visualized by immunohistochemical staining for CD34. We counted the number of vessels per high power field (x500) in two the most vascularized areas. Results: Microvessels density (MVD) in lymph node with HL was lower than in reactive lymph node (37±1,73). There was statistically significant difference ($p<0,05$) between MVD in lymph node of HL patients with bone marrow infiltration (14,75± 5,23) and MVD in lymph node of HL patients without bone marrow infiltration (20±3,95). In patients with HL, but without bone marrow lymphoma infiltration, we found increase of MVD in bone marrow (9,15±5,06), compared with control bone marrow (3,50±0,71), but correlation was not statistically significant (p=0,205). We observed that MVD inside the bone marrow lymphoma infiltrate (17,63±9,27) in HL patients was higher than MVD in HL patients without bone marrow infiltration (9,15±5,06). This correlation was statistically significant (p=0,03). Conclusion: Lower MVD in lymph node of HL patients than MVD in reactive lymph node suggests that lymphoma proliferation leads to the destruction of the existing vessels rather than neoangiogenesis within lymph node. But, our findings suggest that neoangiogenesis in bone marrow infiltration by HL was extensively increased. Therefore, the density of microvessels may provide useful prognostic information and basis for the therapeutic investigation of anti-angiogenic agents in the cases of HL with bone marrow infiltration.

PP3-180

THE DIFFERENTIAL DIAGNOSIS OF MAST CELL PROLIFERATIONS ON FIVE REPRESENTATIVE CASES

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The term mastocytosis denotes a heterogenous group of disorders characterized by abnormal growth and accumulation of mast cells (MC) in one or more organ systems. It subdivides into the indolent systemic mastocytosis (ISM), Systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease (AHNMD), aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL). It is important to differentiate the mast cell diseases from the cytokine related mast cell hyperplasia in the bone marrow underlying other inflammatory processes or tumors without typical skin lesions or organ involvement. Demostrating the missense mutations on c-Kit gene is becoming important for astimating result of targeted drugs for treatment modalities. Our aim in this study is to discuss the diagnosis of different types of mast cell involvements in the bone marrow of 5 cases with their clinical and pathological presentations. Three of these cases were fulfulling the criteria of ASM with multiorgan involvement. One of these cases couldn't be diagnosed for 5 years although she had splenectomy 3 years ago.Differing from the other SM cases the skin involvement could not be demonstrated in this case and her skin complaints were accepted as secondary symptom related to histamine effect. Two of the cases were fulfilling the criteria of AHNMD. One of these cases had cured Hodgkin's Lymphoma history diagnosed 13 years ago and cured secondary acute myeloid leukemia (AML) history diagnosed 2 years ago. The bone marrow was in remission for AML but there were focal paratrabecular infiltration of spindle shaped mast cells fulfilling the criteria of atypical type 1 mast cell infiltration. The mast cells were not recognised on the first two bone marrow biopsies taken for the remission control of AML. The second AHNMD case was Waldernstroms macroglobulinemia developed under the treatment of rheumatoid artritis. The bone marrow was interstitionally infltrated by monoclonal lymphoplasmocytic B cells and multifocal paratrabecular groups of mast cells. The mast cell infiltration became more clear and increased on the second biopsy taken for her disease follow up. The mast cell population were confirmed by immunohistochemistry for every case by their positive staining with CD117, CD68 and mast cell tryptase. Conclusion: Neoplastic mast cell proliferations should be diagnosed and classified correctly for performing the appropriate treatment modality. And should be differentiated from secondery reactive mast cell proliferations in order to avoid unnecessary toxic treatment.

PP3-181
THE RELEVANCE OF T CELLS AND FOLLICULAR DENDRITIC RETICULUM CELLS ON PREDICTING THE CLINICAL BEHAVIOUR OF FOLLICULAR LYMPHOMAS

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Follicular lymphomas (FL) are most commonly seen in western populations (22-35 %) whereas their incidence is around 8-10% in Turkish population. Recent studies including large series of FL cases revealed that the well known histopathological prognostic criteria may not be enough for estimating their clinical behaviour. This situation has brought the idea of focusing on the characteristics of microenvironment besides neoplastic cells. Our aim in this study is to compare the microenvironment and proliferation status of follicular lymphomas with known prognostic histopathological criteria. Methods: 33 follicular lymphoma patients (16 female, 17 male) with a mean age of 54,5 (28-75), diagnosed between the years 2002-2007, were included in this study. All cases were reviewed by examining their histological and immunohistocemical features. The WHO criteria was used for histological grading. As well as the known prognostic histopathological parameters, the presence of residual reactive follicules, the preservation of follicular dendritic reticulum cell (FDRC) network, the density of T lymphocytes and histiocytes within the follicules and the proliferation status of the neoplastic follicules by Ki-67 staining were evaluated. Chi square, Mann Whitney U, Kruskal Wallis tests were used for statistical analysis. Results: Eight cases were graded as grade 1, 12 cases as grade 2, 13 cases as grade 3. There was statistically significant correlation between histological grade and Ki-67 index ($p<0.05$), while such correlation was not present between the histological grade and the bcl-2 expression, the density of CD3 positivity and the ratio of diffuse areas. The cases having higher proliferation index (>49%) showed significantly decreased density of reactive T cells ($p=0,01$). No correlation was found between Ki-67 index, the preservation of FDRC network and the expression of bcl-2. Conclusion: Our results suggested that the proportion of reactive T lymphocytes within the neoplastic follicules may predict the biological behaviour of FL. In according to our data, preservation of FDRC network is not associated with histological grade, proliferation status or T cell population in FL.

PP3-182
FIVE YEARS STATITICAL ANALYSIS OF HENOCH SCHOENLEIN PURPURA AND IT'S RELATION WITH SEASONS

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Abstract: Introduction and purposes: HS purpura is the most distinctive subgroup of hypersensitivity vasculitis that is characterized by purpura in buttocks and lower extremities, involvement of kidney, joint and abdomen. The main aims of this study include: Determination of the correlation between HS purpura frequency and seasons. We also determined the distribution of age and sex and frequency of HS purpura. Methods: This descriptive study was done between1993 to1998 in patients referred to dermatology clinic and pediatric emergency of Ghaem hospital. The diagnosis was based on clinical and pathological findings. Results: There were 40 patients, with M/F ratio 0.8. 13 patients (32.5%) presented in spring, 7 (17.5%) in summer, 11 in autumn (27.5 %) and, (22.5%) in winter. All of the patients had typical skin lesions, 57.5 % in lower extremity 22.5% in upper and lower extremity, 17.59 in upper, lower extremity and trunk. And 2.5% only in upper. The most frequent non cutaneous lesion belongs to abdominal and joint involvements (39%). Conclusion: The most frequent seasonal distribution was in spring and the least frequent was in summer and there was a significant differences between the two seasons ($p=0.05$). But there was not such significant difference among all seasons. The most frequent site of skin lesions was the lower limbs (97.5%).

PP3-183
FEED FORWARD NEURAL NETWORK AS A DIAGNOSTIC HELP IN IMMUNOFLOURESCEIN PATTERNS OF SKIN DISORDERS

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BACKGROUND: Examination of the tissues by microscopy still depend upon the visual cognitive abilities of the pathologists. Although automatisation seems to be far away, image analysis methods are in progress. Measurements of area, volume and stained area percentage methods by image analysis may be applied especially for determining prognostic factors. Neural networks are mathematical algorithms that depend upon the functionality of small neural clusters. The study is designed in order to find if Feed Forward Neural Network (FFNN) depending upon automatic image analysis measurements in DIF images from skin disorders might help the pathologist. METHOD: Images from anti IgG, IgA, IgM, C3 and fibrinogen DIF stained sections from the skin biopsies from patients with immunodeposition were collected to a computer from immunoflourescence microscope. The patterns of deposition were grouped as: basement membrane linear (BML), basement membrane granular (BMG), epidermal intercellular (IC), vascular (V), negative and nonspecific background staining. Variables that might be helpful in the differential diagnosis were evaluated and total size, node, link, end point and branch were selected. The training set included 193 images from 32 cases with positive DIF results. An experienced pathologist suggested a possibility range for each image about the 6 categories. After the training process of FFNN the values for each variable were analyzed statistically for comparing the groups. For determining the automatic self made decision process based on learning, 100 images from 40

cases were analyzed by FFNN. The decisions of the pathologist and the category with highest possibility level selected by FFNN were compared. RESULTS: All the variables were significantly different when all the groups were considered (One Way Anova p<<0,000), when groups were compared separately, all values for all variables for IC pattern was significantly different from other groups, but the others gave mixed results. Of the 100 images FFNN gave the highest possibility in 83% images, in concordance with the pathologist, but gave the right result as the second possibility in 14 cases. For the IC pattern best results were obtained (89% first possibility) followed by V (85%), BML (84%) and BMG (76%). CONCLUSIONS: DIF image FFNN may help inexperienced pathologists in future. Selection of total size, node, link, end point and branch as variable seems valuable. The FFNN may provide better results with long term training.

PP3-184

EVALUATION OF MUTANT p53 GENE FREQUENCY IN PATHOLOGIC SAMPLES OF PATIENTS WITH NON-MELANOTIC SKIN CANCERS AND ITS CORRELATION WITH CLINICOPATHOLOGIC ASPECTS

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Introduction: We aimed to investigate frequency and immunoreactivity of mutant p53 gene in non-melanotic skin cancers (NMSC) and to correlate its Over-expression with clinicopathologic aspects. Methods: Sixty paraffin-embedded pathology blocks with NMSC registered Diagnosis, including 39 BCC and 21 SCC were analyzed immunohistochemically To assess over expression of p53 protein related to sun-exposure ,age and gender Not only have we divided the samples to positive mutation samples and negative Mutation ones ,we sorted samples in the quality method to 0(negative), +1(mild), +2(moderate) and +3(severe) for p53 mutation based on visual impression and severity of staining. We also quantified proportion of the cells expressing p53 within a tumor; obtaining the percentage of the stained nuclei. Result: The frequency of mutant p53 gene in BCC and SCC was 82.1% and 71.4% respectively. Age and gender did not affect over expression of p53 significantly Frequency of mutant p53 gene was 89.1% among sun-exposed sites and 42.9% among unexposed sites to UV radiation (p<0.001). When sun-exposed effect was studied in BCC and SCC separately; 87.1% of sun-exposed BCC showed p53 mutation (P=0.137)while 93.3% of sun-exposed SCC significantly showed p53 mutation (P=0.002). however ,sun exposure of the tumor site increases the severity of p53 staining-when mutation existed-in SCC and BCC significantly (P=0.015 & 0.005 respectively). Also, exposure to ultra violet radiation increases the mean of percentages of stained nuclei-when mutation existed- in SCC and BCC significantly (P=0.04 & 0.008 respectively). Conclusion: Chronic UV radiation can\\\'t induce p53 mutation in BCC but when the mutation occurs due to any other factor, in increases the quality of the activity of mutated p53 gene. In SCC chronic exposure to UV induces mutation in TP53 and also it increases the activity of mutated gene in both quality and quantity.

PP3-185

THE EFFECT OF POST-BURN LOCAL HYPERTHERMIA ON THE REDUCING BURN INJURY: THE POSSIBLE ROLE OF OPIOIDS

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Purpose: This paper studied the effect of post-burn local hyperthermia on burn induced injury. Methods: A second-degree burn injury was induced on the right and left flanks of Balb/c mice.Thirty-two burn wounds were divided into four groups. Opioid receptor blocking was done for groups 3 and 4 by intra-peritoneal administration of Naloxone (NLX) 30 min before the thermal injury. Local hyperthermia (45°C, 30 s) was applied only for the peripheral area of burn wounds of groups 2 and 4. Twenty-four hours after burn injury, the burned wounds were assessed for the level of iNOS (by immunohistochemistry) and the number of hair follicles (as an indicator of tissue injury). Results: The wounds that received hyperthermia (group 2) had significantly more hair follicles ($p < 0.001$) compared to the control wounds (group 1). There was no significant difference between the number of hair follicles and acute inflammation of group 1 and group 3 (NLX + burn). Group 4 (NLX + burn + hyperthermia) had significantly fewer hair follicles compared to group 1 ($p < 0.001$), group 2 ($p < 0.001$) and group 3 ($p < 0.001$). The level of iNOS in groups 1, 3 and 4 was not significantly different but significantly more than group 2 ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively). Conclusions: The results showed that local hyperthermia after second degree burn decreased the tissue injury and iNOS expression. It is also concluded that endogenous opioid response may have a key role in the above mentioned effects of post-burn local hyperthermia.

PP3-186

CASE OF THE BROOKE-SPIEGLER SYNDROME

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Case Report : The patient is a 26-year old woman, presented with cutaneous lesions on her scalp and face. These lesions had first appeared when she was 14 years old and primarily on her scalp which later developed on the face and had gradually increased in size and number over the years. In the patient's family, her father and brother, who was 13 years old had similar lesions on their faces. On examination, a group of round to oval –shape skin –coloured papules with a smooth pearly surface measuring 2-6 mm in diameter were seen in the mid-face, particularly in the nasolabial folds, the upper lip and less commonly on the forehead and the periocular areas.In addition to this, a variety of tumoral and nodular lesions was also seen on the scalp. The tumors of the scalp were pinkish –red, dome-shaped to some extent peduncular with surface telangiectasia and induration on palpation. They were measured 0.5-1.5 cm in diameter, the larger lesions were hairless and the smaller ones had less hair than normalAlso there was a subcutaneous firm nodule with a diameter of 0.5 ×0.5 cm on the patient´s right forearm. Her general condition was good; she had no known underlying disease and no previous history of taking any kind of medications. The histopathology examination of the scalp nodule revealed a dermal lesion with defined borders composed of islands of basaloid cells arranged in a jigsaw puzzle-like pattern and separated by hyaline basement membrane material. Two populations of basaloid cells (with a large nucleus centrally and smaller nuclei in a palisading pattern of the periphery) were observed, which can represent Cylindroma. In the facial papules basaloid inclusions with immature (basic) follicular papillae and horncysts were seen which were surrounded by fibrous stroma and are typical of trichoepithelioma .The patient's forearm nodule composed of sheets of small dark staining basaloid cells peripherally and larger cells with paler nuclei centrally, consistent with spiradenoma According to the clinical feature and the histopathology findings, the diagnosis of was Brooke _Spiegler syndrome made.

PP3-187
HISTOLOGICAL ANALYSIS OF HUMAN SKIN FROM EGYPTIAN MUMMIES
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Background. Four Egyptian mummies of the Hellenistic/Roman period (332 B.C.-395 A.D.), an adult man, an adult woman and two young male children, are included in the ethnologic collections of the Museum of Anthropology, University of Athens, Greece. The biological age of the mummies, based on anthropometric and dental studies, is estimated at 25-35, 40-50, 7-8 and 2.5-3 years, respectively. Anatomical and imaging (x-Ray, computed tomography scan) studies combined with 3-D virtual reconstruction of scull and bones were undertaken. The aims of the present study, which to our knowledge is the first histological study on Egyptian mummies in Greece, were to optimize the conditions of mummified tissue specimen preparation for histological analysis using light microscopy and to evaluate the preservation state of tissue components. Method. Samples of skin tissue from all four mummies were taken without damaging the remains. Four different methods of tissue rehydration were applied for specimens from each mummy using a) 10% sodium bicarbonate in distilled water (dH_2O), b) fabric softener, c) 5% glucose in dH_2O and d) 3% dimethylsulfoxide in dH_2O, followed by fixation in 10% neutral formalin and embedding in paraffin. Serial 5 m- thick tissue sections were cut from each paraffin block and the histochemical stains hematoxylin-eosin and Masson Trichrome were performed. Results. Immersion of the specimens in 10% sodium bicarbonate solution for 8 hours was considered the optimal (most rapid and with best tissue preservation) rehydration method for the desiccated mummy skin. Histological analysis using light microscopy showed absence of epidermis in all tissue sections examined, while the dermis appeared well preserved with identifiable collagen fibers in the dense, irregular fibrous tissue which stained lightly with Masson Trichrome stain. No visible cells or cell remnants were present. Conclusions. We performed a, unique in Greece, histological analysis of 1700-2300-year-old human skin from Egyptian mummies. Rehydration in sodium bicarbonate solution followed by routine fixation and embedding was considered the best method for morphological and histochemical analysis of mummified human tissues. Collagen fibres and possible blood vessels were identified in the dermis of the mummified skin.

PP3-188
A STUDY OF MONOCYTIC AND DENDRITIC CELL MARKERS IN BENIGN CUTANEOUS FIBROUS HISTIOCYTOMA (DERMATOFIBROMA)
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Background: Controversies have existed regarding the histogenesis of fibrous histiocytomas (FHs) as well as the issue regarding whether they represent a neoplastic or reactive process. Based on the expression of factor XIIIa (fXIIIa), the proliferating cells of these lesions have been considered to be dermal dendrocyte by some investigators. We report on the expression of various novel monocytic and dendritic cell markers on dermatofibromas (DFs) and, for comparison, giant cell tumor of tendon sheath (GCTTS). Design: A total of 38 cases of DF and 10 cases of GCTTS were stained with CD1a, CD11c, CD68, CD83, CD123, CD205 (DEC-205), CD207 (Langerin), CD208 (DC-LAMP), CD209 (DC-SIGN), fXIIIa, muscle specific actin (HHF-35), smooth muscle actin (SMA), and calponin. Results: CD163, CD68 and fXIIIa were diffusely expressed in approximately two-thirds of the DFs, with the remaining cases being focally positive; myeloid dendritic cell markers including CD83, CD205, and CD208, and Langerhans cell markers such as CD1a and Langerin were negative; plasmacytoid dendritic cell marker, CD123, was completely negative; SMA and calponin were variably expressed in half of the cases; and HHF-35 was focally stained in only two cases. CD11c and CD209, which can be expressed in both myeloid dendritic cells and macrophages, were focally positive. Both CD68 and CD163 were diffusely stained in GCTTSs; fXIIIa was variably expressed in 8/10 cases; dendritic cell markers were negative; and smooth muscle markers were also negative except for focal staining of SMA in one case. CD11c and CD209 were also focally expressed. Conclusion: DFs are composed predominantly of cells with macrophage markers with a variable amount of myofibroblastic elements, without evidence of specific dendritic cell phenotype. GCTTSs are composed of cells of macrophage derivation. Our results support the notion that monocyte/macrophage represent the potential histogenesis of DFs.

PP3-189
HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ASPECTS IN FEW TYPES OF CUTANEOUS SARCOMAS
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The present study describes the histopathological and immunohistochemical aspect in different cases of cutaneous sarcomas which are representative and also rare in the medical practice (dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, leyomiosarcoma, rhabdomyosarcoma, hemangiopericytoma, malignant hemangioendothelioma). Material and method: We have done a retrospective study within a period of two years concerning on a group of 35 patients who presented cutaneous sarcomas. We used surgical excision pieces, which had been fixed in buffered 10% formalin, paraffin embedded and sectioned at 3µm, then stained routinely by Hematoxylin - Eosin was used in the majority of cases and special stains (Gomory - silver impregnation) in several others, than examined by light microscopy. For the immunohistochemical (IHC) staining the following antibodies were used: CD34, CD31, Actin, Myoglobin, Desmin, Cytokeratin, Vimentin. Results:The primary cutaneous sarcomas have a various neoplastic forms; can occur at any age; these neoplasms to children are rare. Both sexes are affected. As an essential and distinctive feature of these tumours there is a risen tendency of recur. Clinically,the most sarcomas appear solitary and have a profound invasion; the usual locations are the extremities and the retroperitoneal region. The lesions are present as nodules or plaques. Frequently, in ultimate stages, the tumour may necrosise and ulcerate. Histopathologically, they are characterized by invazivity, being not encapsulated and unpreciselly delimitate by the neighbouring tissues. The cellular and nuclear atypia of size, form and colour, as well as atypical mitoses are frequently present. The tumours prouve high cellularity with the tendency of desposing the cells in different oriented fascicles. The vascular tumours show irregular vascular lumens surrounded by neoplastical proliferations. Immunohistochemical staining showed a diffuse positivity for vimentin; CD34 and CD31 was positive in vascular structures; cytokeratin was negative and actin and myoglobin were positive in tumour cells and in vassels walls. The cutaneous sarcomas represents a heterogeneous group of tumours with many histopathological aspects. However, a large group of them consists in problems of positive and differential diagnosis. The

immunophenotyping of such a tumour is important because in correlation to the anatomo-clinical features permits a good differential diagnosis and rules out a large number of tumoral lesions.

PP3-190
LANGERHANS CELLS AS A CLUE FOR TUMOROUS NATURE OF DERMATOFIBROMA
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Background. Dermatofibroma is a common benign lesion usually found on extremities and trunk of adults. Despite its frequency and benign nature it is still unknown whether this is an inflammatory lesion or a true neoplasm. In most inflammatory lesions of the skin the number of epidermal Langerhans cells is higher than in normal epidermis. The number of epidermal Langerhans cells in the dermatofibroma could be a useful, although indirect, clue for defining whether this entity is closer to the inflammatory lesions or to the neoplasms. Method. In our study we included 10 patients with dermatofibroma, 10 with dermatofibrosarcoma protuberans and 10 with postoperative scar. All lesions were immunochistochemicaly stained with CD1a (Clone O10, dilution 1:50, Dako Cytomation, Glostrup, Denmark) to highlight Langerhans cells. We counted the number of Langerhans cells in the epidermis above the lesion. The intraepidermal CD1a positive cells were enumerated as a percentage of 100 keratinocytes. Two concomitant areas of high power field were counted and mean +/-SD percentage was calculated in each specimen. For comparing the differences between mean number of epidermal Langerhans cell in scar, dermatofibroma and dermatofibrosarcoma protuberans we used Student t-test. $p < 0.05$ was regarded as statistically significant. Results. By comparing the number of Langerhans cells (mean+/-SD) in epidermis above the scar (7.62+/-1.38), dermatofibroma (2.05+/-0.61) and dermatofibrosarcoma (2.76+/-1.31), we found that there is no statistically significant difference between mean number of epidermal Langerhans cells above the dermatofibroma and dermatofibrosarcoma ($p=0.160$). The difference between means of epidermal Langerhans cells number above dermatofibroma and above scar is found to be statistically significant ($p<0.0001$). Conclusion. This was an indirect way of showing the neoplastic nature of dermatofibroma. If dermatofibroma was an inflammatory lesion, we expected dermatofibroma to have higher number of epidermal LC, just like we found in scars.

PP3-191
DOES MERKEL CELL CARCINOMA REPRESENT AN EPITHELIAL TUMOUR WITH NEUROENDOCRINE DIFFERENTIATION
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Background: Merkel cell carcinoma (MCC) is uncommon malignant skin tumour with a poor prognosis and rapid progression. Most MCCs occur in elderly individuals, on sun-exposed areas of the body, with the head and neck being the most common sites, followed by the extremitaties and trunk. However, the origin of the MCC is still controversial, because its cells have both epithelial and neuroendocrine elements. To answer on this dilemma we have investigated an expression of neuroendocrine and epithelial markers in MCC. Aims: The origin of the Merkel cell carcinoma is still controversial because its cells have both epithelial and neuroendocrine elements. To answer on this dilemma we have investigated an expression of neuroendocrine and epithelial markers in Merkel cell carcinoma. Methods: The study group was composed of 6 cases, with the mean age of 68 years. Multiple subcutaneous masses and lymph node metastases were surgically ressected.Paraffine blocks of the formaldehyde-fixed tumorous tissue were cut and stained with H&E, PAS, HID-AB pH=2,5 and LSAB2 techniques.The following antibodies (Dacopatt) were used: Chromogranine A, Neurone specific enolase (NSE), panCytokeratin, Melan A, CD20 and CD45Ro. Results: The tumours involved the dermis, with sparing of the epidermis. A variety of histological subtypes were recognized, including intermediate, small cell, mixed intermediate and small-cell variantsMitoses were tipically numerous, and atypical forms were frequently seen. Lymphovascular invasion was extrmely common finding. MCCs expressed both neuroendocrine and epithelial differentiation. They showed positive labeling with epithelial membrane antigen, dot paranuclear panCytokeratin, as well as positive labeling with Chromogranine A and NSE, markers for neuroendocrine differentiation. Conclusion: Merkel cell skin carcinoma is immunohistochemically epithelial malignant tumour with neuroendocrine differentiation.

PP3-192
TUMOUR REGRESSION, A VARIABLE TO BE AWARE OF IN EVOLUTION OF CUTANEOUS MELANOMA
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BACKGROUND: Prognostic variables of melanoma in TNM classification (AJC 2001) are Breslow thickness, ulceration of epidermis and Clark level. Besides, some other variables have been considered such as regression and mitotic index. Malignant melanoma is one of the tumours with a highest rate of spontaneous regression, around 15% of all cases. Regression has been associated with an increase of angiogenesis and a higher risk of developing metastases. METHODS: We have studied 116 cutaneous melanomas, of which we had all clinical and outcome data along 3 years at least. We have considered histological variables such as Breslow thickness, Clark level, presence of ulceration, histologic type, mitotic index, lymphoid response and tumour regression. We have also performed an immunological study of beta-catenin, Ki-67, Bcl2, p16, EGFR, c-Kit and cyclines (B1, D1 and D3). RESULTS: Descriptive study shows regression in 15 of the 116 studied patients (12,9%). In univariate analysis there are differences in survival-without-recurrence rate, being shorter the rate of patients with tumour regression ($p=0,0073$). Multivariate analysis shows that recurrence rate is 5,6 times higher in patients with tumour regression, and risk of death is 3,51 times higher for these patients. Presence of regression is associated with a higher risk of recurrence and a shorter specific survival for melanoma. CONCLUSIONS: Most bibliographic data seem to indicate that regression is not a significant prognostic factor, but it is related to an increase of angiogenesis, and therefore with a higher capacity of distant dissemination. In many studies regression does no seem to be a significant prognostic factor, probably because it is difficult to be defined and assessed. Other studies affirm that it can be an important factor, especially when Breslow thickness is less than 1 mm, and most of all when regression is extensive. In our case series, 33,33% of the patients with regression areas had a Breslow of 1 mm or below, and presence of regression correlates with an increase of the risk of recurrence and death.

PP3-193
ASSESSMENT OF THE DEMODEX FOLLICULORUM IN HUMAN CUTANEOUS LESIONS
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Introduction Since the epizootic conditions of the appearance of dermatoses are common and the clinical signs often lead to confusions, a skin smear, correctly executed and interpreted, is helpful in the diagnosis of demodicosis and in the differential diagnosis between skin lesions. Cited by many authors as a saprophyte of the human skin, Demodex Folliculorum is the cause of many skin lesions, such as folliculitis, desquamations, prurit, hyperseborrhea of the facial region. Material and method 14 patients (3 men and 11 women) diagnosed with different dermopathies (endocrine, allergic, bacterial) which were non – responsible to the usual therapy were examined. The patients had erythema, folliculitis, desquamations, hyperseborrhea of the facial region. The skin smears were clarified in lactic phenol and paraffin oil and were microscopically examined. From the positive smears we have made measurements of 31 adult acarians. Results The microscopic exam of the skin smears showed the adult form of Demodex Folliculorum in all the patients. No evolutive stages of the parasite (egg, larva, nymph) were found. Medium sizes of the female and male parasites were 238/47 μm and 212/46 μm, respectively. The explanation for the absence of the intermediate stages of the parasite is that the samples were taken from the superficial skin, careful not to produce supplementary lesions or to leave scars (especially in women). Conclusions The microscopic exam of the skin smears of 14 patients diagnosed with dermatoses with other aetiology than parasitic and non responsible to treatment has shown the presence of the adult form of Demodex Folliculorum. The morphologic description and the dimensions of the adult Demodex Folliculorum were in correlation with bibliographic data.

PP3-194
EXPRESSION OF HPV, p53, Bcl-2 AND PCNA IN PREMALIGNANT AND MALIGNANT EPIDERMAL TUMORS

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Background. Bowen's disease is an intraepithelial squamous cell carcinoma which has been related to human papillomavirus (HPV) infection. Non-melanoma malignant skin tumors such as invasive squamous cell carcinoma and basal cell carcinoma have also been associated with HPV. The interaction between ultraviolet light and HPV infection has been suggested to be a major mechanism for skin carcinogenesis. The purpose of our study was to assess the immunohistochemical characteristics of Bowen`s disease and invasive epidermal tumors. Methods. We examined immunocompetent patients with Bowen`s disease and malignant epidermal tumors such as invasive squamous cell carcinoma and basal cell carcinoma. Ten patients were involved in each group. Biopsy specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. The paraffin sections were stained by hematoxylin-eosin and von Gieson. Immunohistochemistry was performed using monoclonal antibodies against HPV, oncoprotein p53, anti-apoptotic protein Bcl-2, and proliferation marker PCNA. The percentage of positive cells was determined as strongly, moderately or weakly positive. Results. Premalignant and malignant epidermal lesions failed to show specific clinical features suggesting that they are unlikely to be reliably distinguished on clinical signs alone and morphological examination seems to be mandatory. Immunohistochemistry revealed that HPV infection was 1.5 times more frequent in Bowen`s disease, than in invasive skin tumors. HPV+/p53+ types of Bowen`s disease showed higher PCNA expression in comparison to HPV-/p53+ types. Bcl-2 expression was associated with HPV-negativity. In HPV+ and HPV- types of basal cell carcinoma and squamous cell carcinoma, the incidence of p53 and PCNA expression was similar. However, the strong expression of both markers (p53, PCNA) was related to the lower grade of squamous cell carcinoma. Conclusion. HPV infection appears to play a role in development of Bowen`s disease and malignant epidermal tumors. Independently of p53 expression, HPV-positive cases of Bowen`s disease exhibit higher proliferation activity, whereas HPV-negative cases are also Bcl-2-positive. Independently of the presence of HPV infection, basal cell carcinomas and squamous cell carcinomas show similar incidence of p53 expression and proliferation activity, and lower grade squamous cell carcinomas are associated with stronger expression of p53 and PCNA.

PP3-195
EVALUATION OF CORRELATION BETWEEN CLINICAL AND PARACLINICAL DIAGNOSIS IN FEMALE PATIENTS WITH DIFFUSE NON-CICATRICIAL ALOPECIA

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Background: Alopecia in all its patterns is a common symptom in patients who referred to dermatology clinics. It may cause anxiety and stress in these cases. There are four main diagnosis for a woman presenting with the chief complaint of diffuse, nonscaring hair loss: 1) Female pattern hair loss (androgenetic alopecia) 2) Acute and chronic telogen effluvium 3) Diffuse alopecia areata 4) Loose anagen hair syndrome. Management of hair diseases is very difficult, so we aimed to evaluate the correlation between paraclinical diagnosis (hormone studies, scalp skin biopsy/ histopathology) and clinical diagnosis (history and physical examination) in female patients with diffuse non-cicatricial alopecia refer to clinic of dermatology of Ghaem Hospital. Method: in this prospective study, we filled out the questionnaires for 41 cases and their history, physical examination and hormonal and histopathological studies were recorded and final diagnosis was assessed. Then the correlation between clinical and paraclinical diagnosis were evaluated with Chi square test. Results: Most of patients had androgenetic alopecia. Clinical and paraclinical diagnoses were the same in 94.1 % of these cases. Diffuse alopecia areata was diagnosed by pathologic studies in all cases. Clinical diagnosis and hormone studies were the same in most cases of androgenetic alopecia and telogen effluvium. Conclusion: Pertinent history, physical examination and biopsy are suggested in all patients with diffuse alopecia. Hormone studies are not needed unless they have been other signs of hyperandrogenemia.

PP3-196
EXPRESSION AND LOCALIZATION OF THYMIDINE PHOSPHORYLASE/ PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR IN CUTANEUS MALIGNANT MELANOMAS

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Backgraund: Angiogenesis is a significant prognostic factor in melanoma, but the angiogenetic factors controlling the neovascularization are not well defined. Platelet-Derived Endothelial Cell Growth Factor (PD-ECGF), also known as thymidine phosphorylase (TP) stimulates endothelial cell mitogenesis and chemotaxis in vitro and is strongly angiogenic in vivo. TP is overexpressed in various tumors and plays an important role in angiogenesis, tumor growth, invasion and metastasis. The aim of the study was to examine the expression

of TP in melanomas. Method: In this study the expression of thymidine phosphorylase was examined immunohistochemically in 14 cases with melanomas. Result: TP was strongly expressed not only in macrophages in and around tumors but also in fibroblasts and keratinocytes. Immunoreactivity of TP was rarely seen in melanoma cells. TP positive reaction was also observed in ecrine gland epithelium. Conclusion: These results suggest that TP expressing infiltrative macrophages and stromal cells in and around tumors may contribute to angiogenesis and involved in the growth and metastasis of malignant melanomas.

PP3-197
L1 EXPRESSION IS NOT RELATED WITH SENTINEL LYMPH NODES METASTASIS IN MELANOMA PATIENTS

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Background: Recently, the expression of the cell adhesion molecule L1 has been associated with the metastatic phenotype in both human and murine model of malignant melanoma. Thies et al showed a positive association between L1 expression and metastasis in a 10-year retrospective study. L1 is a transmembrane protein and consists of an extracellular region, containing six immunglobulin-like domains followed by five fibronectin III-like repeats, a transmembrane domain and a short, phylogenetically-conserved cytoplasmic domain. Although originally described as a neuronal cell adhesion molecule, L1 expression is not restricted to nervous tissues, but has also been detected in lymphocytes, granulocytes, in epithelial cells of the intestinal and urogenital tract, and in the epidermis. In addition to expression in normal tissues, L1 has also been detected in several highly malignant tumours, including osteogenic sarcoma, squamous cell carcinoma of the lung, rhabdomyosarcoma and retinoblastoma. Therefore we decided to study L1 expression by immunohistochemistry in 52 non-consecutive patients with cutaneous malignant melanoma which underwent sentinel lymph node biopsy (SNB). Methods: L1 immunostaining was evaluated in melanoma cells by a semiquantitative scoring system. The cut-off for the overexpression was set at 20% of positive cells, irrespective of staining intensity. Results: Breslow Score Index (BSI) in all cases ranged from 0.44 mm to 8 mm. Out of the 52 melanoma patients, 23 (44%) had lymph nodes metastasis and 17 of them had the Breslow Index Score (BSI) available, ranging from 0.9 mm to 8 mm (12 cases with BSI >1,5mm). The 29 melanoma without lymph nodes metastasis had a BSI ranging from 0.44 to 4.85 mm (16 cases with BSI >1.5 mm). L1 overexpression in primary tumors was found in 10/23 (43%) cases with sentinel lymph node metastasis, and 16/29 (55%) cases with no evidence of sentinel lymph node metastasis When L1 expression was evaluated in combination with a BSI < 1.5 mm, it was found overexpressed in 3/5 cases with lymph node metastasis and in 4/13 without lymph node metastasis. When cases were stratified for a BSI > 1.5 mm, L1 overexpression was found in 6/12 cases with lymph node metastasis, and in 12/16 without lymph node metastasis. Conclusion: Our data suggest that, in the melanoma settings, L1 plays an important role in predicting long term survival but is not statistically significant in predicting sentinel lymph node metastasis.

PP3-198
LYMPHOEPITHELIOMA LIKE CARCINOMA OF THE SKIN FOLLOWING 5-FLUOROURACIL TREATMENT

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Background: Primary lymphoepithelioma like carcinoma (LELC) of the skin is a cutaneous malignancy with microscopic smilarities to undifferentiated nasopharyngeal carcinoma. It is a very rare neoplasm and just over 30 cases have been reported in the medical literature to date. Relation with previous actinic keratosis was not observed in the reported cases. Methods: We report a case of LELC on the right cheek of a 79 years old female who was treated with 5-fluorouracil for breast carcinoma five years before the onset of LELC. 5-fluorouracil is known to increase sensitivity to sun light. Patient had multiple actinic keratosis on her face three years after the 5-Fluorouracil therapy. All but one of the lesions regressed following topical diclofenac therapy and avoidance of direct sun light. The only remaining lesion was surgically removed with safe margins. Histologically, there was no connection between the tumor and the epidermis. There was no displasia in the neighbouring skin. The entire dermis and superficial subcutaneous tissue were occupied by atypical epithelial cell nests with a syncytial pattern of growth. Tumor cells were positive for cytokeratine and S-100 positive clear cells resembling Langerhans cells were scattered between tumor nests. Conclusions: None of the previously reported cases have been announced to be associated with neighbouring dysplasia or previous actinic keratosis in the vicinity of tumor location. This is the first LELC case which can be connected with sun light exposure and actinic keratosis as the precursor lesion of skin carcinoma of squamous type.

PP3-199
A NEW CONCEPT OF MELANOCYTIC NEOPLASIA PATHOGENESIS BASED ON THE PHENOTYPE OF COMMON ACQUIRED NEVI

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Background: To date, there are no known molecular hallmarks for nevus development but much progress has been made on the classification of melanocytic nevi. Common acquired nevi are classified into junctional, compound and dermal, based on the topography of nevus cells. The aim of this study was to examine whether common acquired nevi excised from the same individual share similarities in their microscopic appearance and in their molecular profile. Method: We retrieved from our files all the reports on excised nevi during the period 2/2005-2/2006. From these we selected only those that were more than one per patient and were diagnosed as common acquired nevi, compound or dermal. In total, our material consisted of 84 nevi from 31 individuals (♀:♂=25:6, mean age: 31 years). Three patients had four excised nevi, 16 had three and 12 had two excised nevi. The histological slides were reviewed and all phenotypical characteristics were recorded. Furthermore, the immunohistochemical expression of E-cadherin (E-cad) was evaluated, using the monoclonal antibody (CM170B, Biocare Medical, Menarini Hellas). Results: Multiple nevi from the same individual share similar morphology, including secondary changes, some of which are not common. Such features include exophytic, papillomatous, acrochordon-like or endophytic architecture, cytologic characteristics, such as nuclear inclusions, predominant type A, B or C cells, multinuclear nevus cells, fatty metaplasia and “active” nevus cells. Regarding the immunohistochemical expression of E-cadherin in these nevi similar changes in expression in nevi of each individual were noted. Conclusion: On the basis of these observations, we hypothesize that melanocytes of the whole body, although

dispersed in the entire epidermis, are all genetically similar in the same individual and changes predisposing to neoplasia are possibly a global melanocytic event characteristic for each person.

PP3-200
LOSS OF BMI-1 EXPRESSION IS ASSOCIATED WITH THE CLINICAL PROGRESS OF MALIGNANT MELANOMA

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Background The incidence of cutaneous melanoma is increasing, and there is a continuous search for improved prognostic markers and novel therapeutic approaches. BMI-1, a candidate stem cell marker is part of the Polycomb group of genes (PcG), which are important in embryonic gene regulation and tumor development. It has been suggested that BMI-1 protein is important in cell cycle regulation since both p16/INK4a and p14/ARF are downstream BMI-1 targets. Further, BMI-1 has been linked to the development of human malignancies of the haematopoietic and lymphatic systems, but its role in melanocytic tumors of the skin is unknown. Method BMI-1 staining in tumor cells was studied by immunohistochemistry of TMA sections made from 178 benign and malignant melanocytic lesions. We used two different monoclonal antibodies (clone F6, Upstate, Lake Placid, NY and the non-commercial clone 6C9). The arrays were scored blindly and there was good correlation between the two investigated antibodies (Spearman's rho correlation coefficient 0.62, $p<0.0001$). The staining was recorded using a semi-quantitative and subjective grading, considering both the intensity of staining and the proportion of tumor cells showing unequivocal positive reaction. A staining index (SI) was calculated as a product of staining intensity (0-3) and area of positive tumor cell nuclei (1<10 %, 2=10 %-50 %, 3>50 %). Results The immunohistochemical staining of BMI-1 showed a predominately nuclear staining pattern. BMI-1 expression was found to be significantly weaker in melanomas than in benign nevi. In established melanomas, loss of BMI-1 expression was associated with features of aggressive tumors, like increased tumor cell proliferation, presence of necrosis, and increased expression of both N-cadherin and β3-integrin. Low levels of BMI-1 expression were significantly associated with decreased patient survival. Conclusions In conclusion, we demonstrate for the first time that loss of the candidate stem cell marker BMI-1 expression is associated with increased proliferative capacity, features of aggressive melanoma subgroups and reduced patient survival in established vertical growth phase melanomas. We also demonstrate that expression of the BMI-1 protein is reduced in melanomas when compared with benign nevi.

PP3-201
A ZONAL COMPARISON OF MIB-1, CYCLIN D1, ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION IN NEVI FROM WOMEN IN PREGNANCY, PUBERTY OR NONE OF THE TWO

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Background: Pregnancy and puberty influence the clinical appearance of common acquired melanocytic nevi, however the histologic changes are controversial and the possible factors implicated are still under investigation. The aim of the present study is to investigate the possible differences in MIB-1 immunoreactivity (a cycle-associated nuclear antigen), in expression of cyclin D1 (a nuclear protein synthesized in early G1) and in expression of estrogen (ER) and progesterone receptors (PR) in common nevi from women in pregnancy, puberty, or none of the two. Method: Our material consisted of 43 common acquired compound or dermal nevi: 11 from pregnant women, 20 from age-matched women that were not pregnant and did not take contraceptive pills, and 12 from girls in puberty. For immunohistochemical staining the antibodies against MIB-1 (DAKO), Cyclin-D1 (Diagnostic Biosystem, Menarini Hellas), ER (Novocastra) and PR (Novocastra), and the EnVision system (DAKO) were used. Positive and negative nevus nuclei were counted in at least six randomly selected fields (X400) in each of the upper (superficial zone), middle (middle zone) and lower (deep zone) third of the dermal part of the nevus. Results: The nevi from pregnant women, exhibited a statistically significant increase in MIB-1 immunoreactivity, more pronounced in the deep zone, compared to controls. In nevi from girls in puberty the counts were in between the other two categories. Cyclin D1 expression was weak and detected in <5% of nevus cells in nevi from all categories, but was more pronounced in nevi from pregnant women. ER expression was rarely detected, only in the pregnant women nevi. The majority of nevi in all three categories expressed PR. Conclusion: Nevi from pregnant women differ from those of non-pregnant women regarding MIB-1 immunoreactivity, cyclin D1 and ER expression. In girls in puberty, nevi exhibit an increase in the MIB-1 index.

PP3-202
THE IMPORTANCE OF TENASCIN AND UBIQUITIN IN ESTIMATION OF WOUND AGE

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Background: Human wound age determination is of prime importance in forensic sciences. The different reactions of tissues to different natures of wounds require modern diagnostic methods to determine wound age. Ubiquitin is a small protein required for ATP-dependent, nonlysosomal intracellular protein degradation, which eliminates most intracellular defective proteins with a rapid turnover. It is expressed in nuclei of neutrophil leucocytes, macrophages and fibroblasts in wound area. Tenascin is an extracellular matrix glycoprotein. It is expression is restricted in adult tissues although it is widely distributed in embryonic tissues. The aim of this study is to determine the importance of ubiquitin and tenascin in wound age. Method: This study included 170 wound biopsies prospectively obtained from 89 forensic autopsy cases with known wound age. Ubiquitin and tenascin were immunohistochemically applied on formalin-fixed paraffin-embedded tissues. Pearson correlation analysis was performed. Results: Seventy four cases (83.15%) were male, while 15 cases (16.85%) were female. The mean age was 39.44 years. Among 170 wound samples 74 (43.5%) were gunshot wound, 20 (11.8%) were blunt injury and 76 (44.7%) were sharp weapon injury or surgical excision. Tenascin was negative in 123 cases (72.4%) in all series; it was negative in 98.3% in cases with wound age under 24 hours. It was positive in 91.8% in cases with wound age over 24 hours. Mean number of cells that express ubiquitin was 10.56%, while it was 4.25% in cases less than 24 hour wound age and, it was 26.14% in cases over 24 hours wound age. In correlation analysis both tenascin and ubiquitin were positively correlated with wound age. But in

cases with wound age over 40 days tenascin becomes negative and ubiquitin is still expressed in fibroblasts. Conclusion: We conclude that tenascin and ubiquitin together is useful in determining wound age semi quantitatively.

PP3-203
MERKEL CELL CARCINOMA – TWO CASE REPORTS

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BACKROUND: Merkel cell carcinoma (MCC) is a distinctive cutaneous malignancy also known as neuroendocrine carcinoma of the skin. It is a rare disease in which malignant cells are located on or gust beneath the skin and in hair follicles. MCC has a malignant potential and thus prompt aggressive treatment is indicated. METHODS & RESULTS: We report two cases, a 28 years old male with a subcutaneous tumor located in the upper left arm measuring 2 cm and a 66 years old female with a subcutaneous tumor located in the buttock measuring 4 cm. The tumors were completely excised and submitted for histopathological examination. The latter showed dediffentiated malignant neoplasms consisting of small and medium sized cells with scanty cytoplasm, oval, hyperchromatic nuclei and numerous mitotic figures. The tumor cells were arranged in a diffused pattern or formed tiny nodules. The tumor extended deeply into the subcutaneous fat. The diagnosis of MCC was confirmed by histological and immunohistochemical analysis. The tumor cells stained positively for neurospecific- enolase, synaptophysin , chromogranin A, cytokeratin 20 and cytokeratin AE1/AE3. CONCLUSION: The differential diagnosis of MCC contains a vast variety of entities among which primary or metastatic malignant tumors are included. It is therefore important to combine physical examination detailed historical data and thorough histological immunohistochemical, ultrastructural even cytogenetic study in order to establish a prompt diagnosis

PP3-204
UNCOMMON METASTASES OF MELANOMA WITHOUT KNOWN PRIMARY LESIONS

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BACKGROUND: Clinically and histologically melanomas exhibit variant patterns of presentation. Melanoma is one of the malignant neoplasms that can regress, partially or completelly. The incidence of complete regression is 2,4 - 8,7 %. This means that many tumors considered to be primary could be metastases after the true primary lesion has regressed. METHOD AND RESULTS: We report four cases of male patients, 69 – 81 years old with unusual metastases, two with gastric melanoma, one at the inguinocrural area and one of the oral mucosa. None of them had a history of primary dermal melanoma. Two of the patients presented with symptoms of the gastrointestinal tract, one patient for treating incarcerated inguinal hernia and the fourth for ulcerative lesion of the oral cavity. The first two patients underwent gastroscopy and a biopsy was taken from an ulcerative neoplastic lesion of the stomach, the third one had a mass the size of tangerine surgically excised from his inguinocrural area and the fourth had un ulcerative lesion also excised. According to the morphological and immunohistochemical findings (positivity for melanoma antigen, HMB-45, S-100 protein and vimentin and negativity for CEA, EMA, cytokeratin AE1 & AE3 and low molecular weight keratin) we diagnosed malignant melanoma in all cases. CONCLUSION: The cases we present are rare, especially in a general hospital. If there is no history of a prior melanoma, problems as far as the identification of the neoplasm can be set. Independently of the primary site or secondary tumor, we must keep in mind that melanomas must be included in differential diagnosis of a neoplasm with poor differentiation.

PP3-205
MERKEL CELL CARCINOMA IN INGUINAL LYMPH NODE IN THE ABSENCE OF A PRIMARY SKIN TUMOR - A CASE REPORT

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Introduction: Merkel cell carcinoma (MCC) is an aggressive primary neuroendocrine neoplasm of the skin, probably originating from precursor cells which give rise to keratinocytes and Merkel cells. It occurs mainly in elderly individuals, slightly more commonly in women, the head, neck and extremities being the most common location. Purpose: We report a rare case of MCC confined within an inguinal lymph node at the time of presentation. Case report: A 56 year old man presented with a large deep-seated mass in the right inguinal region, measuring 6 cm in maximum diameter, which was surgically resected. Histological examination revealed a lymph node extensively involved by malignant neoplasm with histological and immunohistochemical features of Merkel cell carcinoma. After thorough investigation of the patient, no primary skin tumour was found. Discussion: Merkel cell carcinoma is a highly malignant primary tumour of the skin, known for its propensity to metastasize early. MCCs within lymph nodes in the absence of a primary tumour have been sporadically reported. These rare malignant neoplasms might repesent metastases from an occult or totally regressed primary carcinoma. Other proposed explanations for their occurrence are malignant transformation of preexisting intranodal epithelial inclusions or anomalous carcinomatous differentiation of stem cells of the lymphoreticular system.

PP3-206
MALIGNANT GLOMUS TUMOR ARISING FROM A BENIGN GLOMUS TUMOR: A CASE REPORT

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Glomus Tumours (GT) are benign tumours originatin from modified smooth muscle cells of the normal glomus body. GT are solitary, small size, painful lesions usually localized in the upper extremities, with predilection of subungueal regions. Rarely, GT may display unusual features as large size, infiltrative growth, mitotic activity, nuclear pleomorphism and necrosis.A 46 year old man presented with a painful, rapidly enlarging, dermal-ipodermal nodule noted three months before on the medial face of the right leg. The lesion was surgically removed without problems and grossly was cm 2,2 in diameter, solid, non necrotic

and well circumscribed from the surrounding adipose tissues. Microscopically, the lesion appeared multinodular, circumscribed by a fibrous capsule, and composed by a population of round and fusiform cells with round nuclei and eosinophilic cytoplasms. The neoplastic glomic cells were organized in monotonous nests and sheets and formed collars around many blood vessels with prominent endothelial cells. Near the margins of the surgical excision, there were two accessory highly cellular nodules, with marked nuclear atypia, elevated mitotic index (> 30 mitoses / 50 HPF) with atypical mitotic figures, and with rare vascular channels.Immunohistochemical staining for alpha-smooth muscle actin and type IV collagen were suggestive for the glomic origin of the lesion, while MIB-1 reactivity demonstrated a strong difference between the two accessory nodules (> 17%) and the larger neoplastic nodule (< 2%). We concluded for a final diagnosis of malignant glomus tumour (MGT) arising in a benign glomus tumour. The differential diagnosis of MGT is broad. In the skin it can be confused with skin adnexal neoplasms including nodular hidradenoma, hidradenocarcinoma and eccrine spiradenoma. Demonstration of S-100 protein expression and HMB-45 positivity permits the distinction of malignant melanoma and clear cell sarcoma from MGT. Also small round blue cells tumour can be confused with MGT (cytokeratin 20 negative) including Merkel cell carcinoma (cytokeratin 20 positive), Ewing sarcoma and PNET (both muscle actins negative). In the deep soft tissues, MGT (CD 34 negative) should be distinguished from hemangiopericytoma (CD 34 positive), leyomiosarcoma and rhabdomyosarcoma. MGT is a low-grade malignant tumour, locally aggressive but rarely metastatizing, although more than 25% of the reported cases developped metastasis. A wide local excision with histopathologically free margins and close follow up is considered the treatment of choice.

PP3-207
A DERMATOFIBROSARCOMA PROTUBERANS (DFSP) OCCURRING IN A SITE OF MULTIPLE CORTICOID INJECTIONS

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Background: DFSP is a mesenchymal sub-cutaneous tumor characterized by a strong tendency to recur, rarely metastasizing, which grows very slowly. In some instances, inflammatory conditions like trauma, sites of injections, vaccinal scars, burns have preceded the apparition of the DFSP. Method: We report one case concerning a woman of 53 years old .She has been affected 8 years ago by a DFSP on the left shoulder. This one occurred on the site of multiple corticoïd injections for a severe rheumatoid polyarthritis. The clinical exam reveals a round mass, firm covered by a very thin skin, badly limited in the hypodermis with a pink section. Many tumor specimens are realized and stained by hematoxylin-eosine. Results: The microscopic analysis shows a dermal tumor with a Grenz zone.It is composed of spindle cells arranged in a classical storiform pattern (cartwheel pattern).An immunostain for CD34 gives a diffusely and intensively positivity confirming the diagnosis of DFSP. Conclusion: Some patients relate their DFSP to a prior trauma. Only few authors report factors or conditions which precede the apparition of the lesion while others consider these factors just like a coincidence. This aspect must be known in order to control every imperfectly cicatrisation after a vaccination or repeated injections. This imperfectly scar must be analysed because the recommended surgical resection of a DFSP is a wide deeply excision with margins as large as possible.

PP3-208
SILVER STAINED ORGANIZER REGIONS AND IMMUNOGLOBULINS IN CUTANEOUS KERATOACANTHOMAS AND SQUAMOUS CELL CARCINOMAS

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Background: The aim of the study was to investigate the epidermal cells biological activity in Keratoacanthomas (KAs) and Squamous Cell Carcinomas (SCCs) by counting the number of silver stained Nucleolar Organizer Regions (AgNORs), to estimate the quantity of Ig-producing cells and the inflammatory cellular infiltrate (ICI) in these entities and to achieve a comparative evaluation of them. Method: Thirty KAs (10 in growth stage, 10 in mature stage and 10 in involution stage) and 28 SCCs (9 well differentiated-G1, 7 moderately differentiated-G2, 5 poorly differentiated-G3 and 7 pseudoadenoid) were investigated. Results: The KAs examined had a mean number of 1.727 AgNORs (S.D. 0.232) and IgG predominated in most cases. IgG and IgE increase at the involution stage, IgA remains at almost the same level in the three stages and IgM decreases during the maturity stage. The SCCs examined had a mean number of 2.105 AgNORs (S.D. 0.446). IgG predominated and gradually increases in proportion to the degree of malignancy. Conclusion: There is a significant difference in the number of AgNORs among the three stages of KA with an increase in the growth and involution stage and a drop during the maturity stage. The proportion of Ig subclasses was different in the three tumor stages in contrast to the cellular infiltrate. In SCCs, the number of AgNORs and the percentage of Igs and ICI increased gradually in proportion to the degree of malignancy.

PP3-209
ECCRINE SPIRADENOMA MIMICKING THYMOMA

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Eccrine spiradenomas are well demarcated, lobular, often painful adenomas that can be encountered almost anywhere on the body. Occasionally, eccrine spiradenomas show a heavy lymphocytic infiltrate with a resulting appearance reminiscent of thymoma. Here we present such a case. Our case was investigated immunohistochemically with a wide range of antibodies, including lymphocytic and epithelial markers. A comparative study of histological and immunohistochemical characteristics of eccrine spiradenomas in contrast to thymomas was carried out. After a comprehensive study of both morphological and immunohistochemical parameters, we reached a diagnosis of eccrine spiradenoma. The lymphocytic markers CD1a and CD99 which are markers of an immature T-cell phenotype, were found to be negative in our case, verifying the diagnosis of spiradenoma. Despite overlapping histomorphological features of eccrine spiradenoma and thymoma, the immunophenotype of the lymphocytes is helpful in the differential diagnosis of the two entities. In thymomas, the T-cell population has an immature immunophenotype and shows positive staining for CD1a and CD99, while in spiradenomas, the T-cell population has a mature immunophenotype and is hence negative for the above mentioned markers.

PP3-210

THE SIGNIFICIANCE OF HISTOLOGICAL PATERNS IN POSITIVE PPD TEST SITE

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BACKGROUND: The histologic features of tuberculin skin test site is not uniform. It may be related to status of tuberculosis. The aim of this study was to compare the histological patterns of tuberculin skin test reaction of the patients with active and latent pulmonary tuberculosis and to investigate the histopathological differences between them. METHODS: Forty-eight PPD positive cases were chosen for the study among the patients admitted to Gulhane Medical Academy, Haydarpasa Training Hospital, Department of Chest Disease in the year 2006. Thirty of the subjects had active tuberculosis. A 3 mm punch-biopsy specimen was taken from the central portion of the skin reaction after obtaining informed consent from these PPD positive subjects. They were processed in a usual way. Sections were stained with hematoxylin-eosin and examined under light microscope. The histological patterns of reactions were classified into three types: (a) Perivascular (PV) type, (b) Basal spongiotic dermatitis (BSD) type, (c) erythema multiforme (EM) type. The histological patterns in active tuberculosis and latent tuberculosis were comparatively evaluated by statistical analysis. RESULTS: There were 17 (56.7%) EM type, 9 (30%) BSD-type and 4 (%13.3) PV-type inflammatory reactions among 30 active tuberculosis patients. There were 2 (11.1%) EM-type, 7(38.8%) BSD-type and 9 (50%) PV-type inflammatory reactions among 18 latent tuberculosis patients. The EM type inflammations were frequently seen in active tuberculosis group than in latent tuberculosis (56.7% vs. 11.1%) ($p<0.05$). However, the PM type inflammations were less frequently seen in active tuberculosis group than in latent tuberculosis (%13.3 vs. 50%) ($p<0.05$). The BSD type inflammation frequencies between both groups were not significant. Interestingly, the bullae formations were only seen in 7 subjects with active tuberculosis. CONCLUSION: The histological pattern of PPD reaction sites may be an important sign reflecting the nature of the tuberculosis, which may be either latent or active. The bullae formation, another histological finding in our study, seems to be a specific sign for active pulmonary tuberculosis. Further detailed immunohistopathological studies of PPD biopsies including large number of cases might give important clues about tuberculosis immunology.

PP3-211

CHARACTERISTICS OF PRIMARY CUTANEOUS B-CELL LYMPHOMAS: CLINICOPATHOLOGIC FEATURES, IMMUNOPHENOTYPIC PROFILE AND PROGNOSTIC FACTORS

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Background: In the recent WHO-EORTC classification for cutaneous lymphomas, primary cutaneous B-cell lymphoma (PCBL) include cutaneous follicle center lymphoma (PCFCL), cutaneous marginal zone B-cell lymphoma (PCMZL), diffuse large B-cell lymphoma, “leg type” (PCLBCL-LT) and diffuse large B-cell lymphoma, “other type” (PCLBCL-O). Both PCFCL and PCMZL are indolent lymphomas with good prognosis, while PCLBCL has an intermediate-level prognosis. Method and results: In our study, 40 patients with PCBL were reviewed and reclassified according to the new classification scheme. All of the patients, diagnosed during 11-year period (1996-2007), were analyzed for clinicopathologic features, expression of several markers including CD20, Bcl-2, Bcl-6, MUM-1 and Ki-67, in situ hybridization for Epstein-Barr virus, and of Borrelia burgdorferi. The histologic features and the immunophenotypic profiles of each lymphoma group were stated. The patients were classified into the following categories: PCFCL, 16 patients, PCMZL, 16 patients, PCLBCL-LT, 6 patients and PCLBCL-O, 2 patients. The associations between histology, skin site, the number of lesions and survival were determined. Conclusion: The results underlined the clinical significance of the new classification, the importance of the localization and the number of lesions.

PP3-212

PRIMARY CUTANEOUS CD30(+) LYMPHOPROLIFERATIVE DISORDERS: LYMPHOMATOID PAPULOSIS AND PRIMARY CUTANEOUS ANAPLASTIC LARGE CELL LYMPHOMA

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Background: Primary cutaneous CD30(+) lymphoproliferative disorders (LPD) are considered as the second most common group among primary cutaneous T-cell lymphomas (CTCL). Method and results: We reviewed 74 cases displaying atypical CD30(+) large cells in their biopsies. All of the patients, diagnosed during 12-year period (1995-2007), were analyzed for clinicopathologic features, expression of several markers including CD3, CD5, CD4, CD8, CD56, CD30, ALK, EMA, cytotoxic proteins such as granzyme B and in situ hybridization for Epstein-Barr virus (EBV). The patients were categorized as: Lymphomatoid papulosis (LyP), 34 patients, primary cutaneous anaplastic large cell lymphoma (ALCL), 16 cases, borderline CD30(+) LPD, 5 patients, ALCL, B-cell type, 1 patient, MF transformed into CD30(+) large cell lymphoma, 9 patients. One case of LyP and one case of CD30(+) borderline LPD suffered also from mycosis fungoides (MF). ALCL, B-cell type was associated with EBV. Other than these, there were 2 cases of scabies and 6 cases of arthropod bite, showing numerous CD30(+) large T-cells. Conclusion: The results underlined the importance of clinicopathologic correlation. Sometimes the course should be used as decisive criteria for the definite diagnosis. The prognosis of primary cutaneous CD30(+) LPD were excellent, however, MF, transformed into CD30(+) large cell lymphoma had an aggressive course.

PP3-213

PRIMARY CUTANEOUS T-CELL LYMPHOMAS OTHER THAN MYCOSIS FUNGOIDES AND PRIMARY CUTANEOUS CD30 (+) LYMPHOPROLIFERATIVE DISORDERS

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Background: Among primary cutaneous T-cell lymphoma (CTCL) Mycosis fungoides (MF), together with its variants and subtypes, is the most common type of CTCL. The second most common type is primary cutaneous CD30(+) lymphoproliferative disorders (LPD), accounting for approximately 30% of CTCL. In the recent WHO-EORTC classification for cutaneous lymphomas, the rest is classified as subcutaneous panniculitis-like T-cell lymphoma, extranodal NK/T-cell lymphoma, nasal type, primary cutaneous aggressive epidermotropic CD8(+) T-cell lymphoma (provisional), cutaneous γ/δ T-cell lymphoma (provisional), primary cutaneous CD4(+) small/medium-sized pleomorphic T-cell lymphoma (provisional) and , primary cutaneous peripheral T-cell lymphoma, unspecified. Method and results: In our study, 17 patients with CTCL other than MFand primary cutaneous CD30(+) LPD, were reviewed and reclassified according to the new classification scheme. All of the patients, diagnosed during 10-year period (1996-2006), were analyzed for clinicopathologic features, expression of several markers including CD3, CD5, CD4, CD8, CD56, CD30, granzyme B and in situ hybridization for Epstein-Barr virus. The patients were classified into the following categories: Subcutaneous panniculitis-like T-cell lymphoma, 1 patient, extranodal NK/T-cell lymphoma, nasal type, 4 patients, primary cutaneous CD4(+) small/medium-sized pleomorphic T-cell lymphoma, 3 patients, primary cutaneous peripheral T-cell lymphoma, unspecified, 5 patients. There were 3 MF and 1 pagetoid reticulosis patients, who were transformed into CD30(-) large T-cell lymphoma. Two patients were diagnosed as CD4(+)/CD56(+) hematodermic neoplasm (blastic NK cell lymphoma). Conclusion: The clinical and histologic features, the immunophenotypic profiles and their association with prognosis were analyzed.

PP3-214

CLEAR CELL HIDRADENOCARCINOMA ARISING IN A NEVUS SEBACEUS

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The nevus sebaceus of Jadassohn (SNJ) is a hamartomatous proliferation of the skin and adnexa arising in the face and scalp of adults, with frequent association with several neoplasms, most commonly basal cell carcinoma, trichoblastoma, tichoepitelioma and syringocystadenoma papilliferum. We report the case of a 46 year-old female with hidro-adenocarcinoma demonstrating both apocrine and eccrine differentiation arising in a long standing nevus sebaceus on the scalp. Microscopically, the epidermis showed focal benign basaloid hyperplasia and irregular follicles. A small dermal collection of basaloid sebocytes was suggestive of a sebaceoma. The large part of the lesion was composed of lobules of epithelium with focal cystic spaces. The cells showed high mitotic activity and nuclear pleomorphism, with cytoplasmic vacuoles containing abundant glycogen. Cells with eosinophilic cytoplasm were evident in areas adjacent to groups of basaloid cells with no vacuolation very similar to basal cell carcinoma differentiation. Ductal differentiation with well-formed papillae and focal cribriform structures were also present. Other portions demonstrated a high-grade neoplasm with prominent nuclear atypia and a solid pattern of growth resembling ductal breast carcinoma. EMA strongly stained tumor cells and highlighted the ductal origin of these structures. Less than 4% of cells stained for progesterone and estrogen receptors. Her2/neu reactivity was focally present showing 1+ membranous reactivity in 10% of cells. Anti-p63 labelled the basaloid cells surrounding the tumor lobules. A breast primary was ruled out by clinical and radiological examination. Other tumors showing conspicuous cytoplasmatic vacuolation enter the differential diagnosis, including clear cells squamous carcinoma, trichilemmal carcinoma and metastatic clear renal cell carcinoma, lung, liver and female genital tract. This report illustrates an extraordinary adnexal neoplasm with multiple lines of differentiation arising "de novo" in a SNJ.

PP3-215

FOLLICULO-SEBACEOUS CYSTIC HAMARTOMA: REPORT OF 16 CASES

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BACKGROUND Folliculo-sebaceous cystic hamartoma (FSCH), described by Kimura et al. in 1991, is an uncommon malformation composed of epithelial and nonepithelial elements that grow into an infundibular cyst-like structure, with sebaceous glands and ducts radiating from it, and surrounded by a fibrous stroma with variable amounts of vascular or adipose tissue. Some authors have reported neural elements or myxoid change in the stroma. METHODS We performed a retrospective search from 3 institutions between 1999 and 2006 and identified 16 cases. Specimens from all 16 cases had been fixed in formalin and routinely processed. Five □m sections were obtained and stained with hematoxylin and eosin. Alcian blue stain was also done in some cases to highlight myxoid change in the stroma. RESULTS We report 16 cases of FSCH including 11 women and 5 men, ranging from 24 to 87 years-old. Six lesions occurred on the nose, 5 on the trunk, 3 on the scalp, 1 on the cheek and 1 on the labia minora. Epidermal induction, similar to that encountered in dermatofibroma, was present in 10 cases. Reactive melanocytic hyperplasia was present in 5 cases, including 1 which was prominent, similar to junctional nevus. Myxoid stromal change occurred in 4 cases. Hair shafts in the infundibulum were present in 4 cases. One case that presented as several papules around a scar was a recurrence of a hamartoma removed 7 years earlier. CONCLUSIONS FSCH is a rare benign skin lesion, probably underdiagnosed due to its wide range of histological appearances. We report here 16 cases of FSCH, the largest series of this lesion to date. Some rarely described features such as myxoid stromal change, presence of hair shafts in the infundibulum, epidermal induction and melanocytic hyperplasia appear to be fairly common. We also report a previously undescribed mucosal location (labia minora), and the first local recurrence. Histological recognition of FSCH is crucial to distinguish it from other mucinous or hamartomatous lesions.

PP3-216

ROLE OF MISMATCH REPAIR PROTEINS AND TELOMERASE IN CELL KINETICS OF SUPERFICIAL SQUAMOUS CELL CARCINOMA OF THE SKIN

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Background: The cell kinetic of superficial squamous cell carcinoma (SCC) has revealed controversial results due to the heterogeneity of lesions considered, making more difficult to assess key elements in squamous cell tumor progression. This study analyzes kinetic, cell survival and mismatch repair in a series of high-grade intraepithelial and microinvasive SCC. Design: We selected bowenoid actinic keratosis (HG-AK, 22 cases), SCC in-situ (37 cases) and microinvasive SCC (<3mm depth, 36 cases) that have appropriate archival material. Representative samples were evaluated by standard immunohistochemistry for MCM2 (minichromosome maintenance-2), telomerase, mlh1, msh2 and TP53. The immunoexpression was assessed in the whole lesion and the positive cells as percentage of tumor cells. Appropriate controls were run in each sample. The results were statistically compared using analysis of variance and Student t-test, and considered significant if $P<0.05$. Results: MCM2 inmunoreactivity was observed in 9% of cellularity of AK-HG, 19.17% of SCC IN-SITU and 28.58% of SCC MICRO. TELOMERASE was expressed in 55.75% (AK-HG), 48.08% (SCC IN-SITU) and 72.39% (SCC MICRO). MLH1/MSH2: in 7.73% (AK-HG), 19.04% (SCC IN-SITU) and 20.15% (SCC MICRO) (NULL: 58% AK-HG, 12.7% SCC IN-SITU and 10.86% SCC MICRO) and finally, TP53 inmunoreactivity in 30.5% (AK-HG), 32.78% (SCC IN-SITU) and 36.63% (SCC MICRO). Both MCM2 and TP53 expression increased from HG-AK to microinvasive SCC, revealing no statistically significant differences. Telomerase showed significantly upregulated expression in microinvasive SCC when compared with intraepidermal lesions ($P=0.0037$) and mlh1 expression was significantly down-regulated in HG-AK ($P=0.0022$). Conclusions: 1) The invasion capacity in superficial SCC is marked by up-regulation of telomerase in lesions preferentially expressing abnormal TP53. 2) The differential growth pattern of intraepidermal SCC is kinetically related with down-regulation of mismatch repair proteins.

PP3-217

A COLLISION TUMOR OF THE SKIN ASSOCIATING BASAL CELL CARCINOMA AND NODULAR MALIGNANT MELANOMA (MALIGNANT BASOMELANOCYTIC TUMOR)

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Background: The coexistence of two malignant tumors in the same histological specimen is rare. A collision tumor consisting in basal cell carcinoma and malignant melanoma (malignant basomelanocytic tumor) has been rarely reported. Most of these case reports presented in situ malignant melanoma component. Material and method: We report the case of an 86 years old woman with a whitish, ulcerated tumor of the left cheek, macroscopically measuring 3/1.5/0.8 cm. Results: Histological examination revealed basal cell carcinoma solid type with adenoid areas that invades the reticular dermis admixed with ulcerated and amelanotic, nodular malignant melanoma, Clark level IV and Breslow index 5.9 mm (maximum thickness of the tumor). In some areas the distinction of the two lesions was difficult. Cords and islands of basal cell carcinoma invade malignant melanoma nodule with intimate association of these two cell populations. Both lesions have been closely attached to the epidermis, data supporting primary cutaneous origin of both of the tumoral proliferations. Immunohistochemical studies showed that the malignant melanoma component was positive for S100 protein, HMB45 and CD63 and negative for CK34betaE12 and BerEp4, while the basal cell carcinoma component of the tumor was negative for S100 protein, HMB45 and CD63 and positive for CK34betaE12 and BerEp4. Conclusion: This collision tumor could be interpreted as a tumor with bidirectional keratinocytic and melanocytic differentiation or a coincidence, an extremely rare event.

PP3-218

PROGNOSTIC FACTORS FOR RECCURRENCES IN BASAL CELL CARCINOMA

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Background: Basal cell carcinoma (BCC) is the most common cutaneous malignant tumor that occurs at any age with increased incidence after the 4-th decade. BCC rarely metastasize (reported incidence of 0.0028-0.5%), but it causes a considerable morbidity by invading and destroying the surrounding tissue and location especially involving the head and neck region. Method: We studied 366 cases diagnosed in our department with BCC in two consecutive years. Among these, 335 cases were primary BCC (group A) and 31 were recurrences of a previously resected BCC (group B). We analyzed clinical informations, location, histological types and the integrality of excision. Results: Insignificant differences were recorded in respect of sex or age between the two groups, but the location of the tumors was different between the two groups. All the group B cases occurred in the head area (nose 32.25%, zygomatic 16.12%, perioral 12.90%, temporal 6.45%, orbital area 6.45%, frontal 3.22%, laterocervical 3.22%) while group A included 206 cases (61.49%) in the head area (orbital area 21.94%, nose 12%, zygomatic 6.56%, temporal 6.26%, frontal 4.18%, perioral 1.19%, laterocervical 0.89%) and 129 cases (38.50%) with other locations. There was a preponderance of adenoid, keratotic and metatipic types in both group A and B. We noticed that the infiltrative type of BCC was quite rare: 23 cases in group A (5.67%) and 4 in group B (1.09%). Incomplete excision was found in 93 cases (27.76%) of the group A and 13 cases (41.93%) of the group B. Conclusion: The aim of entire excision of BCC is difficult to obtain because of both macroscopic indistinct margins of tumors and the need of maximal skin preservation that appear in specific areas as eyelid or nose. There is even more difficult to entirely remove the recurrent BCC because of the preexisting scars that interfere with complete excision. Despite our expectations, we found a low correlation between the infiltrative histological subtype of BCC and recurrence. The location and the completeness of the excision have a stronger influence on the recurrence rate than the histological type. The adequate excision of the primary tumor is the key of surgical treatment in BCC.

PP3-219

LYMPHOEPITHELIAL CARCINOMA OF THE LARYNX IN A 60-YEAR-OLD TUNISIAN MAN

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Background: Lymphoepithelial carcinoma of the larynx is extremely rare, representing less of 0,5 % of larynx carcinomas, it is uncommonly associated with Epstein Barr Virus. Method: We report a case of a 60 year-old Tunisian man, who presented with hoarseness, dyspnea. Laryngoscopy showed a tumor of the left larynx extending to the pharynx. Biopsy and the resection specimen were both submitted for histopathological study. In-situ hybridization was performed in the latter to detect the presence of Epstein-Barr virus . Results : Histopathological examination of the specimen biopsy showed an undifferentiated carcinoma with a prominent lympho-plasmocytic infiltrate. A pharyngolaryngectomy with left neck dissection was performed and the final diagnosis retained was a lymphoepithelial carcinoma non EBV induced. A post-operative radiotherapy was indicated. Clinical outcome was favourable one year later without any recurrence nor metastasis. Conclusion: Through this case and literature review, the diagnosis and therapeutic problems raised by this very rare tumor are discussed.

PP3-220

CHRONIC SINO-NASAL PROGRESSIVE DESTRUCTIVE DISEASE IN COCAINE ABUSERS: A MORPHOLOGICAL STUDY AND ELABORATION OF A GRADING

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Background In the last decade cocaine consumption as a recreational drug by snorting or sniffing has worlwide increased. Cocaine can cause tissue damage virtually in every organ system. Cocaine's adverse effect on the nasal cavity was recognized and described first by Owens in 1910 and cause heterogeneous spectrum of lesions from ulceration and nasal septum perforation to idiopathic central facial destructive granuloma. Mehod A population of 16 cocaine abusers patients was carefully investigated by the standard clinical, laboratory, radiological methods and multiple biopsies of the nose. Results In all cases the erythrocyte sedimentation rate was elevated, but the complete blood count, antinuclear, antimithocondrial, anti-DNA, antineutrophil cytoplasmatic antibodies (c-Anca) and bacteriological cultures were normal. Computed tomography showed destruction of nasal septum in 11 cases, of inferior and middle turbinate in 3 cases, of medial wall of maxillary sinus and of hard palate in one patient. Biopsy specimens showed no evidence of malignancy, vasculitis or granulomatosis. In nasal mucosa microscopy found an acute, chronic and partly necrotising inflammation process. Interesting in this condition there are "onion skin" deposits of perivascular fibrosis with numerous eosiniphils. Plasma cells and lymphocytes mainly of T cell type were present in early stage. Conclusion Cocaine damage nose by a combination of intense vasoconstriction and irritation. The ischemia and irritation of the nasal mucosa lead initially to an acute inflammation clinically visualize as a dystrophic lesions (initial stage or grade 1 of cocaine lesion), then a chronic inflammation characterized by ulceration and crusting (intermediate stage or grade 2) and finally various and deeply necrosis process that cause a destruction of normal anatomic strctures such as septum, turbinate and palate (advance stage or grade 3). The altered muco-ciliary clearance and air flow together came up infection cause a continued expansion and progression of the clinico-pathologic process.

PP3-221

SURVIVIN AND INDUCIBLE NITRIC OXIDE SYNTHASE PRODUCTION DURING 4NQO-INDUCED RAT TONGUE CARCINOGENESIS: A POSSIBLE RELATIONSHIP

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Background. This study was undertaken to investigate, by immunohistochemistry, the expression of survivin and inducible nitric oxide synthase during 4NQO-induced rat tongue carcinogenesis. Methods. Male Wistar rats were distributed into three groups of 10 animals each and treated with 50 ppm 4NQO solution through their drinking water for 4, 12, and 20 weeks. Ten animals were used as negative control. Results. Although no histopathological abnormalities were induced in the epithelium after 4 weeks of carcinogen exposure, survivin and iNOS were expresssed ($p<0.05$) in some cells of the 'normal' oral epithelium. In pre-neoplastic lesions at 12 weeks following carcinogen exposure, the levels of survivin and iNOS were increased ($p<0.05$) when compared to negative control, being the strongest effect observed to iNOS. In well-differentiated squamous cell carcinoma induced after 20 weeks of treatment with 4NQO, survivin and iNOS were expressed in some tumor cells. Lack of immunoreactivity for both markers was observed in the negative control group. Conclusion. Taken together, our results support the belief that expression of survivin and iNOS are early events during malignant transformation and conversion of the oral mucosa.

PP3-222

PLACENTAL GLUTATHIONE S-TRANSFERASE CORRELATES WITH CELLULAR PROLIFERATION DURING RAT TONGUE CARCINOGENESIS INDUCED BY 4-NITROQUINOLINE 1-OXIDE

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Background. Taking into consideration that glutatione S-transferase (GST) and cellular proliferation play a crucial role during carcinogenesis, the goal of this study was to investigate the expression of placental GST, called GST-P, and proliferating cellular nuclear antigen (PCNA) by means of immunohistochemistry during rat tongue carcinogenesis induced by 4-nitroquinoline 1-oxide (4NQO). This is a useful model for studying oral squamous cell carcinoma phase by phase. Method. Male Wistar rats were distributed into 3 groups of 10 animals each and treated with 50 ppm 4NQO solution by drinking water for 4, 12 or 20 weeks. Ten animals were used as negative control. Results. GST-P positive foci were detected in non-neoplastic oral cells at 4 weeks of 4NQO administration. In the same way, GST-P positive cells were detected in pre-neoplastic lesions and squamous cell carcinomas induced after 12 and 20 weeks-treatment, respectively. None of the control animals expressed GST-P positive cells. Regarding cellular proliferation, PCNA positive nuclei were higher at 12 and 20 weeks following 4NQO exposure ($p<0.05$) when compared to negative control. Conclusion. These results suggest that the expression of GST-P is correlated with cellular proliferation, in which GST-P is associated with risk and progression of oral cancer, whereas PCNA is closely involved during neoplastic conversion.

PP3-223
DIAGNOSTIC VALUE OF CYTOKERATIN MARKERS IN DIFFERENTIAL DIAGNOSIS OF SALIVARY GLAND TUMORS
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Inroduction: Salivary gland tumors have one of the most complex histopathologic features of any organ/tissue. In this study we want to evaluate diagnostic value of various cytokeratin markers and smooth muscle actin in differential diagnosis of pleomorphic adenoma (PA) and adenoid cystic carcinoma (ACC). All of the cases had both typical and atypical areas and we suppose that pattern of positivity of cytokeratin markers with SMA in atypical areas can help in proper diagnosis of poorly differentiated tumors. Material and Methods: We selected 35 cases of salivary gland tumors from the pathology files of Khalili Hospital between 2002-2006. These cases were 19 PA, 16 ACC .We performed immunohistochemical staining with six cytokeratin markers (CK7, CK8, CK13, CK14, CK17 and CK18) and SMA. Positivity was defined if 10% or more of the cells were immunostained. Result: In PA group CK7 and CK8 showed 100% positivity in both typical and atypical area. Positivity of CK13 in typical area was 0% but in atypical area was 10.5%. SMA immunoreactivity in typical and atypical areas was 36.8% and 21% respectively (P-value 0.003). 100% of cases showed CK18 positivity in typical area whereas 42% of cases in atypical area were positive.57.9% of cases showed CK17 positivity in typical area and 26.3% in atypical area . In ACC group 87.5% of cases showed SMA positivity in typical areas and 62.5% in atypical areas . CK7 positivity was present in 93.5% of cases in typical areas and 93.8% in atypical areas (p-value = 0.00). 100% of cases revealed CK8 positivity both in typical and atypical areas. None of the cases showed immunoreactivity for CK13 in typical or atypical areas. 93.8% of cases showed immunoreactivity for CK14 in typical areas and 81.3% in atypical areas. 31.3% of cases revealed positivity for CK17 in typical areas whereas none of the cases showed positivity in atypical areas. Conclusion: The following conclusions were obtained: 1- Five markers, SMA, CK7, CK8, CK13, CK14 have significant correlation between typical and atypical areas of both pleomorphic adenoma and adenoid cystic tumors. 2- Positivity of SMA and CK8 is more infavor of ACC rather than pleomorphic adenoma. 3- Coexpression of CK7 and CK14 and simultaneous negativity of CK13 and CK18 are significantly more infavor of pleomorphic adenoma than adenoid cystic carcinoma.

PP3-224
METACHRONOUS DEVELOPMENT OF NASAL KAPOSI'S SARCOMA AND MALIGNANT MELANOMA IN A NON AIDS-RELATED PATIENT
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Primary malignant neoplams of the nasal cavities is believed to comprise about 1% of all human malignancies. Moreover, nonsquamous cell malignant neoplasms are very infrequent, comprising about 0.5% of all malignant tumors in this site. Among the few reported cases of Kaposi's sarcoma (KS) involving the head and neck region, only 6 had primary presentation in the nasal cavity and 5 of them were related to the acquired immune deficiency syndrome (AIDS). Nasal malignant melanomas (MM) are rare, accounting for less than 1% of all MM. Metachronous development of nasal KS and MM in a non-AIDS related patient has not been reported. We report the case of a 59-year-old woman who was admitted in our hospital due to rhinorragia. On physical examination it was found a purplish tumor with maximum diameter 0,8 cm mostly obstructing the left nasal cavity and arising from the nasal septum. There was no peripheral lymphadenopathy, hepatosplenomegaly or skin lesions. Hematological examination and chest x-ray was normal. All laboratory tests for bacteria, viruses, HIV, fungi, parasites as well as complete immunological studies for autoimmune diseases were negative. Histological examination of the excised tumor revealed features of KS. Immunohistochemical examination showed positivity of the neoplastic cells for CD34 whereas they were negative for S100, HMB45, Melan A, EMA, SMA and CD45. The patient received no further treatment and 6 years later she was readmitted due to difficulty in breathing. On rhinoscopy a polypoid tumor was found in the left nasal cavity which was considered to be a relapse. The tumor was excised within free surgical margins, it had 3,2 cm maximum diameter and a dark colored cut surface. Histological examination showed features of MM. Immunohistochemicaly, the neoplastic cells were positive for S100, HMB45 and Melan A whereas they were negative for CD34, CD45, SMA and EMA. The patient received no further treatment and 1 year later she is in an excellent condition with no evidence of local recurrence or metastasis, both clinically and radiologically. In the present study we report a unique case of primary KS of the nasal cavity with metachronous development of MM in the same location, in a patient with adequate immune system.

PP3-225
A CASE OF CERVICAL ECTOPIC MENINGIOMA: CHALLENGING IN FROZEN SECTION
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Background Primary extracranial and extraspinal meningiomas are rare.they are usually limited to the head and neck region or to the paravertebral soft tissues. histologic diagnosis (particularly of fresh specimens) is often difficult. Case report A 16-year-old girl presented with a cervical mass.It was a sizable mass with an approximate diameter of 5 cm.It was diagnosed in fine needle aspiration as a pleomorphic adenoma. The diagnose of surgon,during the operation was paraganglioma and frozen section suggested an infiltrative tumor. Histologically, the lesion showed uniform spindle cell proliferation separated by hyalinized collagen bundles . The spindle cells were often arranged in sweeping fascicles and concentrically wrapped in tight whorls. Immunohistochemically, tumor cells were positive for EMA and vimentin,while showed negativity for cytokeratin, chromogranin,NSE and thyroglobuline. A diagnose of ectopic meningioma was established based on microscopic appearance and immunohistochemical profile of tumor. Conclusion Although rare,ectopic meningioma should be considered in differential diagnosis of any mass lesion in head and neck region,comosed of spindle cells.

PP3-226
THE Ets-1 TRANSCRIPTION FACTOR IS INVOLVED IN PTERYGIAL ANGIOGENESIS
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Introduction : Pterygial pathology is characterized by abnormal corneal epithelial proliferation, stromal modulation, matrix degradation, and a strong tendency for otherwise absent corneal vascularization. As the proto-oncogene c-ets1 is known to play a key role in angiogenesis and matrix degradation in other tissues. If its involvement in corneal vascularization was to be checked. Method: 25 pterygia in two Groups. Group 1 consisted of five

clinically active, group 2 consisted of 20 samples clinical non active pterygia. were investigated with 35 S-labeled ets-1 antisense and sense in-situ hybridization of c-ets-1 transcription factor. Results: The cytoplasm of blood vessel endothelial cells showed strong expression of c-ets-1 mRNA in all group 1 pterygia. In contrast, no expression of c-Ets-1 or c-ets-1 was found in the pterygia of group 2. Conclusion: Proto-oncogene c-ets 1 expression has been shown for the first time in the metaplastic pterygium, an eye tissue of unknown pathogenesis.

PP3-227
ERDHEIM-CHESTER DISEASE, A CASE REPORT AND REVIEW OF LITERATURE
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Erdheim-Chester disease (ECD) is a rare form of non-Langerhans histiocytosis of unknown origin, characterized by adult onset with multi-systemic involvement, in which diffuse sclerosis of the dia-metaphysis of the long bones, especially in the lower limbs, is the most consistent finding. Orbital involvement is a rare feature seen in less then a third of cases. we are presenting a case of a 42 year old lady, who presented initially to the ophthalmology clinic with a 2 year history of progressive marked proptosis of the left eye, complicated by complete loss of vision and pain of 2 weeks duration. There was mild proptosis of the right eye, the vision however was preserved. enucleatin of the left eye revealed the presence of histiocytosis of non Langerhans cell type. Discussion of this rare form of presentation of ECD as well as review of the literature is presented.

PP3-228
DERMATOFIBROSARCOMA OF HEAD AND NECK. PATHOLOGIST POINT OF VIEW
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Background : Dermatofibrosarcoma protuberans (DFSP) is a rare locally aggressive malignant neoplasm. It is most commonly found on the trunk and rarely on the head and neck. In fact only 12% to 15% of the cases are localized in the head and neck region. It represents 1.4% of the soft tissue sarcomas of this area, and its main feature is the high rate of recurrence. This study defines the clinical behavior of DFSP evaluates the role of pathologist in surgical margins analysis, and identifies factors that predict local control. Methods: Hospital records and pathological slides were reviewed for 5 patients with pathologically confirmed head and neck (DFSP) treated at Charles Nicolle Hospital between 1999-2006. Results: This study included 5 cases (3 men and 2 women; mean age 38 years, (range 28–48 years) of DFSP. 4 cases were primary tumors and 1 was recurrences after surgery excision. The tumors were located in the following areas: the scalp in two cases, the cheek in three cases. In all cases clinical and instrumental examinations showed absence of regional and distant metastases. Tumor size ranged from 4 cm to 10 cm. All 5 subjects were treated by surgical excision of the tumor. 2 our 5 subjects cases were with positive margin. These 2 patients had a recurrence of the disease, after 12 months of follow up. The new lesion was treated by resection with a 5-cm margin. Conclusions: Wide margin resection of head and neck DFSP predicts negative histological margins and impacts favorably on local recurrence-free survival.

PP3-229
OSTEOPLASTIC TRACHEOPATHY
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Background: Osteoplastic tracheopathy is a very rare disease that presents itself as multiple submucosal nodules composed of mature bone and cartilage. Method: We report the case of a 35-year-old male diagnosed with schizophrenia 12 years ago and tried to attempt suicide by sedative ingestion. These induced comatose (GCS = 5), which required oro-tracheal intubation. The patient recovered from comatose and stabilized within 4 days. 4 months later the patient presents respiratory distress that accentuates progressively. He's diagnosed with hypercapnic acute respiratory failure (PCO2 = 120 mmHg) and subglotic tracheal stenosis. The fiberoptic bronhoscopic procedure reveals a 2.5 cm in depth / 1 cm in width subglotic tracheal stenosis, caused by multiple partially necrotic granulomas, developed mainly on the left lateral wall. After a moderate hemoptoic episode and the aggravation of respiratory troubles surgery is considered necessary: tracheal subcricoid segmentary resection with termino-terminal anastomosis. The method used for diagnose was paraffin-embedded tissue and stained with hematoxylin and eosin (H&E). Results: Histopathological out findings revealed the presence of bone and cartilage nodules and plaques mainly in subepithelial tissues, underline tracheal epithelium acanthosis and papilomatosis with richly vascularised stromal repairing tissue. Only isolated epithelial ulcerations are seen. Conclusions: In this case the lesion's etiology seems to be inflammatory, as a consequence of the oro-tracheal intubation.

PP3-230
HISTOPATHOLOGY OF FLOOR OF MOUTH LEUKOPLAKIA: A MIMIC OF ORAL LICHEN PLANUS?
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Background: Oral lichen planus (OLP) is reputed to have characteristic clinical and histopathological appearances that should allow confident diagnosis. What is not well recognised is that the histopathological features of OLP may overlap with changes due to tobacco use. The floor of mouth (FOM) is rarely affected by OLP but can develop leukoplakia in smokers. Defining the spectrum of histopathological findings of FOM leukoplakia biopsies will highlight the proportion of tobacco-associated lesions that exhibit changes similar to those seen in OLP. Method: Laboratory records were trawled for FOM biopsies from August 2000 to December 2003. When cases of carcinoma, dysplasia and other "non-leukoplakia" diagnoses were excluded, 59 suitable cases were identified. Original slides of these 59 patients were reviewed by an oral pathologist blinded to the initial diagnosis and the following features recorded semiquantitatively: degree and type of keratosis, altered epithelial thickness, intraepithelial lymphocytes, apoptotic cells, lymphoid follicles and melanin pigmentation. The findings were compared to the clinical summary and smoking history. Results: A tobacco history was recorded for 53 (90%) of patients: 50 were current smokers, 3 were "non-smokers". No smoking history was available for 6 (10%) patients. All cases exhibited hyperkeratosis and alterations of epithelial thickness, most often a mixture of ortho- and parakeratin with adjacent areas of atrophy and hyperplasia. Intraepithelial lymphoctyosis was seen in 49 (83%) cases, of which 44 (75%) were known to be smokers. Apoptotic cells were present in 35 (59%) cases and were numerous in 9 (15%) cases, of which 33 (56%) were known to be smokers. Abundant melanin pigment accumulation was seen in only 20

(34%) cases, of which 19 (32%) known to be smokers. Conclusions: The presence of clusters of intraepithelial lymphocytes, apoptotic cells and/or melanin pigment in FOM mucosa is strongly associated with a history of smoking, particularly when abundant. These "interface mucositis"-like changes mimic oral lichen planus and question the validity of such a diagnosis in patients who smoke.

PP3-231
IMMUNOEXPRESSION OF PDGF AND PDGFR IN DIFFERENT DEGREES OF CARCINOMA EX-PLEOMORPHIC ADENOMA INVASION

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Background: Immunoexpression of PDGF and PDGFR was investigated along the progression of pleomorphic adenoma to carcinoma ex pleomorphic adenoma (CXPA) using sixteen cases of CXPA in different degrees of invasion –intracapsular, minimally and frankly invasive carcinoma. Method: To this aim, we counted positive cells in different areas including reminiscent pleomorphic adenoma, in situ and peripheral areas of both intracapsular and minimally invasive tumours as well as in the frankly invasive carcinoma with only epithelial component and/or myoepithelial component. A minimum of 500 cells at 400X in the most marked areas (hotspots) was counted for each site by the computer-assisted image analysis. Results were presented as % of positive stained cells. Results: PDGF and PDGFR were observed in the cytoplasm of the cells but PDGFR was also present in some nuclei. Reminiscent pleomorphic adenoma showed focal stained of PDGF and PDGFR in luminal and plasmacytoid cells and in the cells of myxoid and condroid areas. In situ areas of intracapsular and minimally invasive types showed only strong expression in malignant luminal cells (100%). Both PDGF (71%) and PDGFR (80%) were present in the peripheral areas of intracapsular and minimally invasive with irregular distribution. In frankly invasive ones of the epithelial type, the percentage of positive cells was 90% for PDGF and 80% for PDFGR. In areas with small nests, negative cells were more evident. In frankly invasive type with myoepithelial component, 92% for PDGF and approximately 100% for PDGFR were observed. Conclusion: It seems that the expression of PDGF and PDGFR is involved at the beginning of the malignant transformation since its increase is present in in situ areas when compared with the reminiscent PA. But, their absence in the focal areas in the periphery of the tumours and in some cells of the small groups leads us to the hypothesis that they are not involved in the invasive process what merits further investigation.

PP3-233
ERCC1 IMMUNOHISTOCHEMICAL EXPRESSION IS RELATED TO HISTOLOGICAL DIFFERENTIATION AND PREDICTS OBJECTIVE RESPONSE AND DISEASE-FREE SURVIVAL IN PATIENTS TREATED BY CISPLATIN-BASED CHEMOTHERAPY FOR LOCALLY ADVANCED HEAD AND NECK SQUAMOUS-CELL CARCINOMA

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Background: The excision repair cross-complementation group 1 (ERCC1) enzyme plays a rate-limiting role in the nucleotide excision repair pathway which recognizes and removes cisplatin-induced DNA adducts. The relation between ERCC1mRNA expression and resistance to platinum compounds has been studied in advanced-stage gastric, ovarian, colorectal, esophageal, and non-small-cell lung cancer. ERCC1 immunohistochemistry was predictive of the survival benefit of cisplatin-based chemotherapy in non-small cell lung cancer. Purpose: The goal of this study was to examine whether ERCC1 immunohistochemical expression in locally advanced head and neck squamous-cell carcinoma (HNSCC) was correlated to clinico-pathological characteristics and p53 mutations of patients treated with a cisplatin-based chemotherapy. Design: Pre-therapeutic biopsy samples from 96 patients with a known tumor response were evaluated for ERCC1 immunohistochemistry. The median of the H-score (the proportion of stained tumour nuclei multiplied by the staining intensity of nuclei) was used as cutoff. P53 mutations had been previously studied. Results: Patients' age varied between 51 and 67 years. Tumors were classified as T1/T2 (18) or T3/T4 (78), and nodal involvement was classified as N0 (46), N1 (13), N2 (22) or N3 (15). Of 96 patients, 68 (71%) had tumors that expressed ERCC1 intensively and diffusely, at high levels. Tumors with low ERCC1 were less differentiated than those with high levels (p=0.03). There was no difference for any other characteristics including age, gender, T, N, M, tumor localization or p53 mutation. Among 28 patients with tumors with low ERCC1, 22 (79%) had an objective response as compared to 38 of 68 (56%) patients with tumors with high ERCC1 (p=0.04). Using the logistic regression method, the 28 patients (29 %) with low ERCC1 had a four time greater odds of benefiting from an objective response to chemotherapy (OR 4.3, 95% CI: 1.4–13.4; p=0.01) as compared to the group of 68 patients with high ERCC1. ERCC1 and p53, but not their interaction, were independent predictors of tumor response. In a Cox model adjusted on age, T, N, M, tumor differentiation, and tumor localization, low ERCC1 was associated with a lower risk of cancer death (RR 0.42, 95% CI: 0.20-0.90; p=0.04), while p53 status had no prognostic value. Conclusion: The results of our study suggest that: 1) tumours with low ERCC1 were less differentiated and that, 2) those patients having HNSCC with low ERCC1 are more likely to benefit from cisplatin chemotherapy as compared to patients with high ERCC1.

PP3-234
PATHOLOGIC ESTIMATION OF ORAL MUCOSA LESIONS

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Background In periodontal destruction, bacterial substances mainly interact with inflammatory cells and stimulate epithelial cells either towards proliferation or death. Actinomycosis is an infectious disease that frequently has chronic granulomatous and suppurative lesions; however, intraorally and periodontally types of actinomyces infection occur rarely. Heat shock proteins (HSPs) are produced by cells as a protection against stress and may play a role in course of infections. CD43 is a cell surface glycoprotein with an intracellular domain implicated in signal transduction and consequent activation of phagocytes. Material and methods Thirty one oral mucosa specimens infected by

actinomyces were studied with regard to the immunohistochemical expression of HSP70, histiocytic marker CD68 and CD43. Gram stain was performed in all cases. Results The inflammatory reaction consisted mainly of lymphocytes and plasma cells with a focally increased presence of neutrophils. The percentage of macrophages, as assessed by CD68 immunoreactivity, did not exceed 10% of the inflammatory infiltrates. HSP70 was overexpressed in inflammatory cells as well as on actinomyces' surface and epithelial cells of the basal layer with a concomitant heavy inflammatory reaction. When actinomyces expressed HSP70 strongly, the adjacent epithelium above the basal layer was denuded; on the contrary, when HSP70 expression on actinomyces was low, the neighboring epithelium was hyperplastic. CD43 was expressed mainly in mononuclear inflammatory cells and it was practically negative in neutophils; the latter is probably linked with the increased presence of actinomyces in the examined samples. Conclusions HSP70, when expressed on actinomyces' surface is likely to interact with adjacent epithelium. The absence of CD43 on neutophils seems to favor the colonization of oral mucosa by actinomyces and the progress of the infection-induced lesions.

PP3-235
p53 EXPRESSION IN SINONASAL MUCOSA OF LEATHER WORKERS

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Background. The association between sinonasal intestinal-type adenocarcinoma and the exposure to leather dusts has been widely documented, but the identification of precursor lesions and early alterations of p53 expression in the exposed nasal mucosa has so far received little attention. Methods. Biopsies of the mucosa of the middle turbinate were obtained from 40 subjects who had been employed in leather factories for 9-48 years (median 27 years). Thirty-three subjects had been employed in leather tanning activities, 21 in shoe making activities, and 14 had been exposed to both. Tissue samples were routinely processed for histologic examination and immunostaining for p53 was performed using the monoclonal antibody DO7 (Dako Co., Glostrup, Denmark). For statistical analysis, cases showing >20% of positive cells were considered positive. Results. In 22 biopsies (55%) we observed nuclear positivity for p53 in surface nasal epithelium. Immunoreactivity was present in basal and supra-basal epithelial cells in squamous metaplastic epithelium, while native schneiderian epithelium and goblet cells were negative in most cases. Concerning glandular epithelia, positivity was detected in 35 samples, 8 of which showed >20% of positive cells. Overall, p53 expression in surface epithelia was more frequently found in subjects employed in leather tanning than in subjects employed in shoe making or in both activities (p=0.06, Fisher's exact test). p53 expression in surface epithelia was significantly associated with longer exposure to leather tanning activities (p=0.02, Mann Whitney U test), while no correlation was found with shoe making activities, or with global work exposure. Similarly, no correlation was found between p53 expression in glandular epithelia and work exposure parameters. Conclusions. In nasal mucosa, a site at high risk of developing intestinal type adenocarcinoma in subjects exposed to leather dust, epithelial cells frequently overexpress p53 protein, and this appears to be related particularly to leather tanning activities. Further molecular studies are needed to clarify the role of p53 overexpression in the pathway leading to the development of these tumours in exposed subjects.

PP3-236
THE ENAMEL MATRIX DERIVATIVE (EMDOGAIN®) ENHANCES HUMAN TONGUE CARCINOMA CELLS GELATINASE PRODUCTION, MIGRATION AND METASTASES FORMATION

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Background: Enamel matrix derivative Emdogain® (EMD) is widely utilized in periodontal therapy to regenerate lost connective tissue and to improve the attachment of the teeth. Gelatinases, matrix metalloproteinases -2 and -9, are supposed to have an essential role in the progression of oral cancer growth and metastasis formation. The aim of this study was to determine the effects of EMD on human tongue squamous carcinoma (HSC-3) cells in vitro and in vivo. Methods: Subconfluent cultures of HSC-3 cells and spontaneously immortalized oral mucosal keratinocytes (HMK) were incubated for 48 h in serum-free culture media containing 0, 100 or 200 µg/ml of EMD. Gelatinase production was analyzed using gelatine zymography and enzyme-linked immunosorbent assay (ELISA) kits. For transwell cell migration assay both cell lines were pre-incubated with EMD and allowed to migrate overnight. The attached cells on the lower side were stained and quantitated. In a set of experiments, a cyclic anti-gelatinolytic CTT-2 peptide was added to the transmigration assay. Cell proliferation was analyzed using microtiterplates coated with or without EMD. The cells were incubated from 12 to 96 h. The proliferation was determined using Cell Proliferation ELISA BrdU kit. For in vitro wound closure assay BSA pre-coated confluent cell culture plates were wounded and exposed to various concentrations of EMD. After incubation the wound areas were measured. For in vivo metastasis assay HSC-3 cells were injected subcutaneously into the back of athymic nude mice. After three days of inoculation, mice were injected subcutaneosly with or without EMD daily during five days of treatment. After scarification the number of the metastases were counted. Results: EMD at concentrations of 100 µg/ml and 200 µg/ml significantly induced the production of MMP-2 and -9 from HSC-3 cells. EMD also slightly induced the gelatinase production from HMK cells. Furthermore, EMD induced the transmigration of HSC-3 cells, but reduced the migration of HMKs in transwell assays. The in vitro wound closure of HSC-3 cells was significantly accelerated by EMD, whereas it did not effect on the wound closure of HMKs. The migration of both cell lines was inhibited by a specific cyclic anti-gelatinolytic peptide CTT-2. EMD had no effect on HSC-3 cell proliferation. EMD significantly induced metastases formation, but had only a limited effect on mice survival bearing human tongue HSC-3 carcinoma xenografts. Conclusion: We suggest that the use of EMD for patients with oral mucosal malignancies should probably be avoided.

PP3-237
A COMPARISON BETWEEN THE FREQUENCY RATE OF MALIGNANT & PREMALIGNANT WHITE ORAL LESIONS AND THE REST OF WHITE ORAL LESIONS REFERRED TO THE RECORDS OF TEHRAN UNIVERSITY (IRAN) PATHOLOGY CENTER
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BACKGROUND: White lesions of oral mucosa is caused by thickening of keratin layer, epithelial hyperplasia, intraepithelial edema and reduced vascularity in the underlying lamina propria that in some cases transformation into malignancy may occur. The aim of this study is to assess knowledge of potentially malignant white oral lesions indices rates, in order to prevent oral cancer. METHODE: In this descriptive study a total of 4799 documents of patients referred to the records obtained from the pathology section of Tehran University were reviewed from the year 1995 to 2005. Documents of affected patients by white oral lesions were evaluated. Variables (sex, age, lesion type, location of oral involvement, microscopic diagnosis) were assessed by Chi-Square analytical test. RESULTS: Of our samples, 425 patients (9%) were affected by white oral lesions, of them, 7.06% were premalignant and 22.35% were malignant and the rest (70.59%) had no report of malignancy and were benign. Among premalignant white lesions idiopathic leukoplakia with mild dysplasia had maximum percentage. Based on the results of this study, the maximum percentage of premalignant lesion occurrence were reported to be among the males over the age of 50 on the buccal mucosa vs. malignant lesions which were predominantly reported in females over the age of 50 on the tongue. CONCLUSION: Obtained results showed that 9% of oral lesions are white with less prevalence rate compared with that obtained in previous studies. This may arise from deficit consideration and carelessness of dental staff during examination and exclusion of white lesions. However it is found that 22.35% of white lesions are malignant, bigger percentage than that obtained by similar investigations. This may also arise from less consideration in primary diagnostic approaches performed by dentists. Considering prevalence of premalignant and malignant lesions among elderly patients, paying more attention during clinical examination is mandatory particularly for high risk patients.

PP3-238
INTRAMUSCULAR HEMANGIOMA OF THE RETROPHARYNGEAL SPACE. A CASE REPORT
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Intramuscular hemangioma is a distinctive type of benign vascular tumor confined to the skeletal muscle. This tumor is prone to recur and behaves in a locally aggressive manner. The most common site of involvement is the lower limb, followed by the head and neck region. To our knowledge, only one case of intramuscular hemangioma arising in the retropharyngeal space has been reported in the English literature. We, herein, report a further case in a 51-year-old woman. The tumor manifested as a left-sided neck swelling of 15-day duration. The patient was found to have bulging of the posterior oropharyngeal wall on indirect laryngoscopy and an ill-defined left-sided retropharyngeal mass on computed tomography scan. The tumor was excised by a transoral approach and microscopically diagnosed as intramuscular hemangioma, the complex malformation subtype. It was mainly composed of irregularly shaped large- and medium-sized blood vessels, reminiscent of arteries and veins. The second vascular component corresponded to capillaries, lined by prominent endothelial cells. Vascular channels were set in a fibrous-fatty stroma with entrapped striated muscle fibers. In addition, isolated bundles of smooth muscle fibers were present throught the tumor. Some of these structures contained lumens and were attached to vessel walls. We assume that at least some of these smooth muscle bundles could be malformed blood vessels, as well. Intramuscular hemangioma should be considered in the differential diagnosis of any soft tissue mass found in the retropharyngeal space.

PP3-239
THE EXPRESSION OF Ets, E-CADHERIN AND Ki-67 IN HEAD AND NECK SQUAMOUS CELL CARCINOMA. AN IMMUNOHISTOCHEMICAL STUDY
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Background: Despite the development of diagnostic tools and therapy, squamous cell carcinoma of the head and neck (HNSCC) may present challenges to pathologists and clinicians as well. A particularly difficult problem is the treatment of regional metastases. We examined immunohistochemically molecules involved in the metastatic process: ets-1 transcription factor that regulates expression of matrix metalloproteinase-3, E-cadherin that is responsible for homotypic cell-cell adhesion, and cell proliferation marker Ki-67. We studied their possible relations relevant to the clinical practice. Method: 98 patients (87 males and 11 females) with HNSCC were treated only surgically in our institute during a period of 3 years . We divided the primary tumor samples into two groups. Group N(0) (n=49) were selected from patients without synchronous lymph node metastasis or nodal recurrence in a follow-up period of 2 years and Group N(+)(n=49) were from patients with lymph node metastases. Using immunohistochemistry the targets mentioned above were detected in paraffin-embedded specimens representing the primaries and the metastases as well. The following clinicopathological parameters were taken into account: sex, age, tumor localization, grade and stage. The results were statistically analyzed. Statistical significance was defined as $p<0.05$. Results: Correlation between ets-1 expression and clinicopathological setting, metastasis and Ki-67 labeling index (LI) was not significant. Reduced expression of E-cadherin showed significant correlation with metastasis, survival and Ki-67 LI. In addition, we found that the degree of E-cadherin expression in primary tumors may differ from that of the metastases. Conclusion: Ets-1 can be demonstrated mainly in the peritumoral stromal cells and has no direct prognostic significance. However, testing the Ki-67 labeling index and E-cadherin expression may be useful in identifying high-risk patients who need more aggressive therapy. Moreover, these markers may be valuable in a panel of prognostic factors for HNSCC.

PP3-240
ANGIOGENIC SWITCH DURING TUMOR PROGRESSION OF CARCINOMA EX-PLEOMORPHIC ADENOMA (CXPA)
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BACKGROUND: Angiogenesis is considered to be a critical process for tumor growth, invasion and metastasis. CXPA is the principal form of malignancy arising in pleomorphic adenomas (PA) and is usually a high-grade neoplasm, with frequent

metastases and a poor clinical outcome. We investigated the question whether an angiogenic switch would take place during the malignant transformation of PA into carcinoma and during tumor progression. METHOD: In 8 cases of early CXPA (intracapsular and minimally invasive), 8 of advanced CXPA (widely invasive tumors) and 10 cases of PA without malignant transformation, tumor vascularisation was assessed by measuring total microvascular area (TVA) and microvessel density (MVD) in CD34 and CD105 stained sections. In digitalized images the texture of the CD34 stained capillaries was analyzed calculating Shannon´s entropy (as a measure of the CD34 staining heterogeneity) and the fractal dimension, which represents the complexity of the architecture. RESULTS: Whereas the CD34 values did not differ among the tumors, MVD for CD105 increased significantly during tumor progression (PA without malignant transformation: mean 3.3; early CXPA: 9.0; widely invasive CXPA: 33.6) with carcinomas without myoepithelial differentiation revealing higher values (mean 46.5) than carcinomas with such cellular differentiation (mean 16.3). In relation to TVA, in advanced CXPAs the TVA value was higher (mean 179.4) than in early carcinomas (mean 63.8) and tumors with myoepithelial differentiation showed higher values (mean 260.2) than those without (mean 118.9). In widely invasive tumors with myoepithelial differentiation the carcinoma cells often formed large hypovascularized aggregates surrounded by large vessels whereas in those without myoepithelial differentiation the cell aggregates were smaller with thinner vessels around them. Entropy and the fractal dimension raised during carcinogenesis (lower in adenomas and higher in frankly invasive carcinomas), which is equivalent to an increase in heterogeneity of the endothelial CD34 expression and complexity of the vessel architecture. CONCLUSION: During the progression from adenoma to carcinoma there is an increasing complexity of the vessel architecture. The antibody CD105 reflects better the angiogenic switch than CD34. The degree of angiogenesis and the total vascular area have distinctive patterns in CXPA with and without myoepithelial differentiation. Low angiogenesis associated with high TVA value is more characteristic of CXPA with myoepithelial differentiation. FAPESP-04/07960-0

PP3-241
POTENTIAL PROGNOSTIC IMPORTANCE OF THE EXPRESSION OF MATRIX METALLOPROTEINASE MMP9 AND TISSUE INHIBITOR OF METALLOPROTEINASE TIMP2 IN PATIENTS WITH LARYNGEAL SQUAMOUS CELL CARCINOMA

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Matrix metalloproteinases belong to the family of human endopeptidases involved with degrading of the extracellular matrix. MMP9 belongs to the group of matrix proteins called gelatinases and TIMP2 acts as an inhibitor of metalloproteinases and also may promote growth inhibition. The aim of the study was the evaluation of the immunoexpression of matrix metalloproteinase MMP9 and tissue inhibitor of metalloproteinase TIMP2 in patients with laryngeal squamous cell carcinoma. Our material consisted of 67 cases of laryngeal squamous cell carcinoma (LSCC) from the files of the Department of Pathology Medical University of Lodz. For immunohistochemical study we used Novocastra Company antibodies and EnVisio (DAKO) visualisation system. The MultiScanBase CSS Ltd. Company image analysis system was used for scoring the intensity of expression of evaluated proteins. Only tumor cells with distinct cytoplasmic immunoexpression were classified as positive. Our date showed that the significantly higher number of TIMP2 positive cells were observed in grade three LSCCs than in low and intermediate grade tumors. We also noted that the intense MMP9 expression was present more frequently in patients with high grade carcinomas but the results were not significant. We seen the MMP9 positive neoplastic cells mostly at the margins of tumor areas adjacent to the stroma. We also found the positive correlation between MMP9 and TIMP2 expression in evaluated carcinomas. In conclusion we believe that the TIMP2 and MMP9 expression might be useful prognostic markers in patients with oral squamous cell carcinoma.

PP3-242
PLEOMORPHIC ADENOMA (MIXED TUMOR) OF THE MINOR SALIVARY GLANDS

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BACKROUND: Pleomorphic adenoma is an epithelial tumor of the salivary glands. The first prescription was made by Billroth and Von Bruns in 1859 and the initial name "mixed tumor" was given by Minssen in 1874 as a manifestation of a mixed epithelial and mesenchymal origin. Today, the prevalent name is pleomorphic ade-noma because of the proved isolated epithelial origin and the pleomorphic histological picture per location. The frequency is estimated about 60-80% of the salivary gland tumors and a 90-96% of the benign salivary gland tumors with a 85-90% of the cases located in the parotid glands and mainly to the distal caudal section. A pleomorphic adenoma in the minor salivary glands is not often. The purpose of this work is to pre-sent cases of pleomorphic adenomas of the minor salivary glands clinically presented as sebaceous cysts and or lipomas and to discuss about this entity. METHODS-RESULTS: The clinical, surgical and histological pictures in cases with pleomorphic adenomas of the minor salivary glands are presented and the particulari-ties in histological sections and stains are referred. CONCLUSION: Definite diagnosis in the above mentioned pleomorphic adenomas of the minor salivary glands is emphasized by histological evidences. Because of the relative small size of the tumor and the expansion in the cheek and labial subcutane-ous tissue these tumors clinically could be misdiagnosed as sebaceous cysts, pilar cysts and or lipomas.

PP3-243
HUMAN PAPILLOMAVIRUS PRESENCE AND ASSOCIATED RISK WITH LARYNGEAL NEOPLASIAS

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BACKROUND: The purpose of this study was to determine the relationship between human papillomavirus (HPV) infection in patients with invasive squamous cell carcinoma, in situ carcinoma, keratosis and mild-severe dysplasia of the larynx. HPV has been implicated as a risk factor in this carcinogenic process after cigarette smoking and chronic alcohol consumption. MATERIALS & METHODS: Formalin-fixed, paraffin-embedded tissue was obtained from 20 males (aged 60-79) with

laryngeal neoplasias. Extraction of total DNA from sections was performed using the QIAamp DNA mini kit (QIAGEN). Quality and integrity of extracted DNA was tested by PCR amplification of an amplicon of the IFN-γ gene. A nested, multiplex, highly sensitive PCR method (~1fg; 103 viral copies) was used for HPV detection and genotyping. In this assay consensus primers for first-run amplification of a broad spectrum of mucosal HPV genotypes, including all high-risk HPV genotypes, were combined with nested PCR amplifications of type-specific primers. RESULTS: Out of the 20 samples, two of them (10%) were found positive for the high-risk subtype HPV16 (case 3, invasive carcinoma, moderately differentiated) and the low risk subtype HPV43 (case 16, invasive carcinoma, well-moderately differentiated), respectively. CONCLUSION: Both high and low risk HPVs (HPV16 and HPV43, respectively) were detected in patients with invasive laryngeal squamous cell carcinomas. The presence of, especially, high-risk HPV genotypes may identify a subset of patients at risk to develop invasive laryngeal squamous cell carcinoma. Nevertheles, examination of larger numbers of patients are required to expand these findings.

PP3-244
IMMUNOHISTOCHEMICAL DETECTION OF E-CADHERIN AND BETA-CATENIN IN SALIVARY GLAND TUMORS

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BACKGROUND & OBJECTIVE: E-cadherin links to the cytoskeleton via catenins and mediates cell-cell homophilic adhesion. Beta-catenin not only regulates cell-cell adhesion, as a protein interacting with cadherin, but also functions as an important component of the Wnt signaling pathway which has been found to be closely associated with tumor formation. This study was performed to examine the expression of E-cadherin and beta-catenin in salivary gland tumors in order to evaluate their possible roles in the formation of salivary gland tumors. MATERIAL & METHODS: Archival formalin-fixed, paraffin-embedded tissue sections of 48 mixed tumors (pleomorphic adenomas), 17 Warthin tumors, 5 mucoepidermoid carcinomas, total 70 tumors, were studied immunohistochemically using an Envision/horseraddish peroxidase (HRP) technique. Healthy salivary glands were used as controls. RESULTS: Membrane and cytoplasmic associated E-cadherin and beta-catenin expression was present in 54/70 and 68/70 of the tumor types studied, respectively. E-cadherin and beta-catenin showed a similar distribution; however, E-cadherin was less frequently expressed than beta-catenin. Expression of both proteins was stronger in Warthin tumors and in mucoepidermoid carcinomas. Reduction and/or absence of E-cadherin were only observed in mixed tumors. CONCLUSION: This study suggests a direct association of E-cadherin and beta-catenin expression with neoplastic histology phenotype of salivary gland tumors.

PP3-245
EXPRESSION OF METALLOPROTEINASES IN SALIVARY GLAND TUMORS

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Matrix metalloproteinases (MMPs) are involved in extracellular matrix turnover. MMPs belong to a family of zinc-dependent endopeptidases and are classified according to their substrate specificity. Although MMPs are among the potential key mediators of cancer invasion, their involvement in premalignant lesions is not clarified. The aim of our study was to evaluate the expression of gelatinase A (MMP-2) and gelatinase B (MMP-9) in salivary gland tumors. Material and Methods: We examined immunohistochemicaly formalin-fixed, paraffin-embedded tumor samples from 70 patients (48 pleomorphic adenomas,17 warthin tumors and 5 mucoepidermoid carcinomas) for MMP-2 and MMP-9 (Chemicon Laboratories) expression. Results: Cytoplasmic MMP-2 staining was detected in epithelial component of all pleomorphic adenomas and warthin tumors, and also in stoma fibroblasts and endothelial cells. MMP-9 cytoplasmic staining was detected in myoepithelial cells of pleomorphic adenomas, in epithelial cells of warthin tumors and in inflammatory cells in stroma. Nuclear immunostaining for MMP-9 was also detected in epithelial component of pleomorphic adenomas. In mucoepidermoid carcinomas MMP-2 and MMP-9 were also immunoexpressed in squamous malignant component. Conclusions: Our results suggest that MMP-2 and MMP-9 expression is an early event in salivary gland tumorogenesis. Our findings suggest roles of MMPs in tumor initiation in addition to invasion.

PP3-246
DESMOPLASTIC FIBROBLASTOMA OF THE LARYNX

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BACKGROUND Desmoplastic fibroblastoma (DM) is a rare benign soft tissue tumor of fibroblastic origin, first described by Evans in 1995. It affects mainly adult males, most commonly limbs and trunk and has no recurrences. It is characterized by a paucicellular proliferation of stellate shaped fibroblasts in a collagenous or myxoid background. Two cases have been described in the palate and one in the parotid gland. The authors describe the first case in the larynx. METHOD Representative slides were stained by hematoxilin and eosin and immunohistochemistry study was conducted using the Envision Plus detection system (Dako, Carpinteria CA) according to well established technique. Clinical and follow-up information were obtained from the files of the clinician. RESULTS Case presentation: ASA, 51 years old male presented complaining of "sore throat" and a foreign body sensation. Direct laryngoscopy reavealed a two centimeters, deglutation mobile nodule, in the larynx. It was locally ressected. Grossly the nodule was white-grayish, firm, well demarcated, measuring 1,9 X 1,9 X 1,6 cm. Microscopically there was a well demarcated hypocellular nodule, underneath a squamous mucosa, composed of proliferating spindle and stellate fibroblasts. The stroma was collagenous and focally myxoid. The diagnosis was desmoplasic fibroblastoma. Because of the unusual location of the lesion, the case was sent in consulation to Dr. C.D.M. Fletcher who agreed with the diagnosis. He also performed and immunohistochemical study. The lesional cells were focally positive for CD-34, while negative for smooth muscle antigen (SMA) and S-100 protein. DISCUSSION AND CONCLUSION Desmoplastic fibroblastoma is a benign fibroblastic tumor that most frequently affects males between the 5th and 7th decades of life. It presents as an asymptomatic mass usually in the subcutis, but fascial and muscular involvement can occur. It occurs most often in the soft tissues, but two cases have been described in the palate and one in the parotid gland. It is well-demarcated, white-grayish, firm and nodular. It is typically a paucicellular proliferation of spindle and stellate regular fibroblasts over a collagenous stroma that can be focally myxoid and can be SMA, CD-34 and keratin positive. Two cases studied showed a 2;11 (11q12) translocation. To the best of our knowledge, this is the first case described in the larynx. The case is presented to call attention that visceral cases of desmoplastic fibroblastoma can occur.

PP3-247
LYMPHANGIOMATOUS POLYP OF THE PALATINE TONSIL: REPORT OF TWO CASES OF A RARE TONSILAR LESION
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BACKGROUND Benign tumors of the palatine tonsil are rarer than malignant ones. The most common is the squamous papilloma. Lymphangiomatous polyp of the tonsil is a rare lesion typically occurring in the palatine tonsil and considered a hamartoma. Until 2000, 36 cases have been plublished in the literature. We describe two cases to divulge this uncommon lesion. METHOD The two cases were seen recently in the Department of Pathology of the Faculty of Medecine of Marília. Hematoxylin and eosin–stained slides were reviewed. Clinical and follow-up information were obtained from the patients hospital charts. RESULTS Case reports: Clinical findings Case 1: P.M.R., fourteen years old white female with essential leukopenia and palmo-plantar hyperhydrosis presented to the department of othorhynolaryngology complaining of a sensation of a "meat ball" in the throat on deglutition, of two years duration. Examination showed a polyp in the left palatine tonsil. Bilatered tonsilectomy was performed with a diagnosis of left tonsilar polyp. The patient is currently asymptomatic. Case 2: E.W.S., nineteen years old mulatto male presented with a history of a foreign body sensation in the throat and choking, of two years duration. Examination found a polypoid mass in the right palatine tonsil. Bilateral tonsilectomy was performed and the postoperative course was uneventful. Pathologic findings Case 1 and 2: Macroscopy: In both cases a pedunculated tonsilar polyp was found. They were yellow-brownish, firm and measured 1,7 cm and 2,0 cm in greatest dimension in cases 1 and 2 respectively. Microscopy: histological appearances were similar in both cases. The pedunculated polyps were lined by non keratinizing squamous epithelium. The core of the polyps was composed of poorly cellular fibrous tissue containing numerous dilated vessels, mainly lymphatics. Underneath the epithelium, lymphoid tissue composed of small lymphocytes and some follicles, even with germinal centers, was focally found. There was focal epithelial lymphotropism with formation of small lymphocytic aggregates within the epithelium. Mature plasma cells were present in smaller numbers. Case 2 showed more abundant lymphoid tissue and larger clusters of lymphocytes within the epithelium than case 1. Pathologic diagnosis: Case 1 and 2: Lymphangiomatous polyp of palatine tonsil. CONCLUSION: Lymphangiomatous polyp of the tonsil is a rare benign hamartomatous lesion with typical location and histological appearance, here presented so that pathologists can learn to recognize and diagnose it.

PP3-248
INVESTIGATION OF EPSTEIN-BARR VIRUS (EBV) PREVALENCE IN THYROID CARCINOMAS IN TUNISIA
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Background: Epstein-Barr virus (EBV) infection has been linked with several malignancies of lymphoid and epithelial cell origin. It has been recently suggested that EBV may be involved in the progression of thyroid papillary carcinoma to undifferentiated carcinoma in Japanese patients. The purpose of our study was to evaluate the prevalence of EBV in thyroid carcinomas in Tunisia. Methods: To achieve this issue, 101 thyroid specimens from 101 patients, comprising malignant tumors (41 papillary carcinomas, 6 follicular carcinomas, 7 anaplastic carcinomas, 2 medullary carcinomas), and benign lesions (31 follicular adenomas, 10 multinodular goiters, 2 Hashimoto thyroiditis, and 2 Basedow disease), were randomly-selected. EBV infection was investigated by EBER1/2-in situ hybridization on formalin-fixed and paraffin-embedded tissues. Results: EBER1/2 was not detected in epithelial cells in any of the 101 specimens, but in 6 cases, EBV positivity was found in scattered normal lymphocytes. Conclusion: Given the sensitivity of the technique used, theses results do not support a relationship between EBV and thyroid carcinomas, in spite of EBV being high prevalent in our population when other tissue specimens are concerned.

PP3-249
INVESTIGATION OF HUMAN PAPILLOMAVIRUS (HPV) IN BREAST CANCER AMONG WOMEN FROM TUNISIA
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Introduction: Human papillomavirus (HPV) infection has been associated with a variety of benign and malignant epithelial lesions. The association of HPV with a subset of breast carcinomas has been reported but remains controversial. The aim of this study was to evaluate the frequency of HPV infection in breast carcinoma in Tunisia. Methods: 96 randomly selected invasive ductal carcinomas of the breast were investigated for the presence of HPV DNA sequences by polymerase chain reaction (PCR). DNA was extracted from frozen tumor tissues. HPV sequences were investigated by three different methods including the E1350L/E1547 primers amplifying a 180-bp sequence in a highly conserved region of a wide spectrum of anogenital HPV, the pU-1M/PU-2R primers specific for the oncogenic HPV types 16, 18, 31, 33, 52, 58, and the PU-31B/PU-2R primers for the low-risk HPV 6 and 11. Results: PCR for the detection of a 268-bp sequence in the β-globin gene demonstrated the presence of amplifiable DNA in all cases. No HPV-related DNA sequences were identified by PCR in any case of breast carcinomas tested. Discussion: Although our results may reflect a geographic feature, they do not support a relationship between the anogenital HPV tested and breast carcinomas; however, the possibility that other types of HPV contribute in the breast carcinogenesis can not be excluded.

PP3-250
ALLELIC IMBALANCE OF THE SHORT ARM OF CHROMOSOME 3 IN NASOPHARYNGEAL CARCINOMA AMONG PATIENTS FROM TUNISIA
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Background: As part of North Africa, Tunisia is one of the world's intermediate-risk areas for nasopharyngeal carcinoma. Loss of heterozygosity on the short arm of chromosome 3 (3p) is the most frequent genetic change reported in nasopharyngeal carcinoma from endemic areas. Method: We investigated the chromosome 3p for loss of heterozygosity in 47 microdissected primary nasopharyngeal carcinoma specimens and corresponding non-cancerous tissues from Tunisian patients using six microsatellite polymorphic markers. Results: Loss of heterozygosity was identified in 87.2% of cases on at least one of the six markers tested. The highest frequency of loss of heterozygosity was found at D3S1038 (60%), D3S1067 (57%), D3S1568 (52%), and D3S1076 (44%). Nine cases showed coexistence of loss of heterozygosity for three consecutive adjacent markers, suggesting a common deleted region flanked by D3S1568 (3p21.31) and D3S1067 (3p21.1-14.3), centered at D3S1076. Furthermore significant higher frequency of loss of heterozygosity were observed in patients older than 25 years than in patients under 25 years at the locus D3S1076 (57% versus 17%; p=0.02). Conclusion: These findings supports the hypotheses suggesting that loss of heterozygosity on chromosome

3p in nasopharyngeal carcinoma is a common genetic event irrespective of geographic and ethnic origins, and suggests that one or more gene(s) affected by loss of heterozygosity at the locus D3S1076 were specifically involved in the pathogenesis of adult form of this cancer.

PP3-251
CLINICOPATHOLOGIC STUDY OF CARCINOMA ASSOCIATED WITH SINONASAL INVERTED PAPILLOMA: NOT ASSOCIATED WITH EPSTEIN-BARR VIRUS

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Background: Carcinoma associated with sinonasal inverted papilloma is uncommon. Patients with inverted papillomas and carcinomas may occur in three patterns: 1) those who have carcinoma with recurrent inverted papilloma; 2) those whose tumors are predominate typical inverted papilloma with only focal invasive carcinoma or carcinoma in situ; 3) those who have carcinoma with a pattern similar to that of papilloma. Status of Epstein-Barr virus in the pathogenesis of inverted papilloma is controversial. We investigated the role of Epstein-Barr virus in carcinoma associated with sinonasal inverted papilloma. Method: Carcinoma associated with sinonasal inverted papilloma were retrieved from the surgical pathology files. Tumor DNA was extracted from paraffin blocks. Oligonucleotide primers for detecting LMP-1 gene were used to evaluate for the presence of Epstein-Barr virus DNA by a polymerase chain reaction (PCR) amplification. Results: Eleven cases were identified. Seven patients were female and four were male. The mean age was 52 (31-72) years. Two patients had moderately differentiated squamous cell carcinoma 162 and 148 months after excision of inverted papilloma with recurrences in the interim. Three cases presented inverted papilloma with focal invasive squamous cell carcinoma or carcinoma in situ. Six cases had carcinoma with a pattern similar to that of papilloma. Genomic DNA extraction was not workable in four of eleven cases. Epsten-Barr virus was not detected in the seven cases by PCR. Conclusions: The study demonstrates that the pathogenesis in carcinoma associated with sinonasal inverted papilloma is not dependent on concurrence of Epstein-Barr virus infection.

PP3-252
SOLITARY FIBROUS TUMOR OF THE UVULA

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Solitary fibrous tumor (SFT) is an uncommon spindle cell neoplasm rarely located in the oral cavity. We report a very unusual case of SFT located in the úvula.We have not found another one in this location in the literature. CASE REPORT A 80-years-old woman had a polipoid lesium 2 cms located in the úvula. The lesion was removed with a clinical diagnosis of Papiloma.Microscopically we found a spindle cell tumour with atipia , but no necrosis and some mitotic figures (< 4 in 10 HPF). The cells showed a strong immunoreactivity for vimentin and CD34, and variable for bcl-2 and negativity for other immunomarkers. The patient has been follow-up for 7 years (2000-2007)and in spite of a very small recurrence (may 2006) after removal of the new lesion, she is well and free of disease. Diagnosis of SFT extrathoracic is difficult and, although uncommon it should be considered in the differential diagnosis of oral soft tissue tumors.

PP3-253
HUMAN SALIVARY GLAND BRANCHING MORPHOGENESIS: MORPHOLOGICAL LOCALIZATION OF CLAUDINS AND ITS PARALLEL RELATION WITH DEVELOPMENTAL STAGES REVEALED BY EXPRESSION OF CYTOSKELETON AND SECRETION MARKERS

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Background: The development of salivary glands from simple precursor epithelial buds to functional glands is a highly complex and dynamic process termed branching morphogenesis, where tissue generate branched structures that subsequently differentiate into mature glands. Tight junctions (TJ) are thought to be the principal structures that contribute to cell polarity, by acting as an intramembrane barrier to prevent lateral movement of membrane proteins that form specific sites in the apical or basolateral membranes, thereby playing a critical role in the physiological functions of tubular organs. Several lines of evidence have demonstrated that claudins are directly involved in the formation of TJ strands as well as their barrier function. Method: Using immunohistochemistry and immunofluorescence we have mapped the distribution of claudins-1, 2, 3, 4, 5, 7 and 11 and compared it with the expression of differentiation markers in human salivary glands obtained from foetuses ranging from weeks 4 to 24 of gestation. Results: Expression of all claudins, except claudin-2 was detected in the various phases of human salivary gland development, up to fully mature salivary gland. The expression of all claudins increased according to the progression of salivary gland maturation evidenced by the classical markers – cytokeratin 14, cytokeratin low molecular weight, smooth muscle actin and human secretory component. Conclusion: Tight junction proteins – claudins appear to be important in the final shape and physiological functions of human salivary glands and are parallel related with markers of salivary gland differentiation.

PP3-254
IMMUNOHISTOCHEMICAL STUDY OF TYPE I COLLAGEN EXPRESSION IN DIFFERENT DEGREES OF CARCINOMA EX- PLEOMORPHIC ADENOMA INVASION

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Background: Carcinoma ex-pleomorphic adenoma (CXPA), a rare salivary gland tumour, is an important model to study the complex mechanism of tumour malignization and progression.

Our group has already studied various aspects of this tumour concerning cells and stroma. Collagen is the major component of extracellular matrix proteins. Interaction between cells and collagen can regulate cellular gene expression, differentiation and growth. Besides it plays an important role in tumorigenicity. Recently, it was demonstrated that type I collagen induces disruption of E-cadherin-mediated cell-cell contacts and promotes cell proliferation. The aim of this study was to analyze the expression of type I collagen in CXPA. Method: Seventeen cases of CXPA, classified according to the degree of invasion in intracapsular, minimally invasive carcinoma and frankly invasive carcinoma with epithelial and/or myoepithelial component were immunostained for type I collagen. Results: Two patterns of type I collagen were disclosed: fibrilar; and amorphous or dense fibers. The fibrilar was interpreted as newly formed collagen and the amorphous or dense fibers as the mature collagen. The amorphous and dense fibers were observed in reminiscent pleomorphic adenoma stroma, in the capsule of the intracapsular type, in most of the stroma of intracapsular and invasive types of CXPA, and also as a dense line surrounding the glandular structures of the in situ areas. The fibrilar component was found in focal peripheral areas of the intracapsular and minimally invasive types, interpreted as invasion front. In frankly invasive type with only epithelial component the fibrilar pattern was found in focal areas involving small nests of cells and scattered throughout the stroma. When the tumour had myoepithelial cells as component, the fibrilar collagen, if present, was never in direct contact with tumoral cells. Conclusion: Fibrilar and dense fibers of type I collagen was observed in all types of CXPA. In CXPA with only epithelial component, focus of fibrilar collagen in peripheral border of the tumours and its presence surrounding and permeating small nests of cells in invasive type lead us to suggest that it should be an important component of the matrix in the invasion process of CXPA with epithelial component. Nevertheless, the same can not be postulated for the CXPA with myoepitelial component.

PP3-255
HPV IN SITU HYBRIDIZATION ANALYSIS OF JUVENILE LARYNGEAL SQUAMOUS PAPILLOMATOSIS

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Background: Laryngeal squamous papillomas are benign neoplasms, often affecting the true vocal cords of children. They can be multiple with local recurrence, referred to as papillomatosis. Papillomas are thought to be associated with Human papilloma virus (HPV), demonstrated by using polymerase chain reaction (PCR). HPV in situ hybridization (HPV-ISH) is a novel technique that can be applied on paraffin-embedded tissue with well-preserved histological architecture. We analyzed for low and high risk HPV types in the laryngeal papillomas by HPV-ISH. Materials and Methods: Twelve excisional biopsy specimens of the larynx were retrieved from 4 patients over a 4 year period (2003-2007) from Mount Sinai Medical Center. Age ranged from 4months to 9 years (average 5 years). All tissue was formalin-fixed and paraffin embedded. Automated HPV in situ hybridization (ISH) analysis was performed for the hybridization on 4μm thick tissue sections, using a Bench Mark XT (Ventana Medical Systems, Tucson, AZ) with DNA probes for low risk HPV (6,11) and high risk HPV (16,18,31,33,35,39,51,52,56,58, and 66). Positive and negative controls were hybridized alongside the study cases. Positive staining was visualized using the precipitating chromogenic reaction NBT/BCIP with a nuclear localization. Results: Laryngeal papillomas are soft excrescences grossly. Histologically, they are composed of multiple slender, finger-like projections coved by squamous epithelium with central fibrovascular cores. In 1 patient (Case 1), focal mild cellular atypia was seen in a recurrent biopsy. No significant high-grade dysplasia seen in any of the biopsies. HPV-ISH revealed that 4 specimens from 2 patients (Cases 1 and 4) were positive for low risk HPV (50%). Three of these 4 specimens were recurrences from the same patient (Case 1). All twelve specimens of 4 patients were negative for high risk HPV (Table). Conclusions: Localized juvenile laryngeal papillomatosis is a benign condition related to HPV infection, with no evidence of high risk HPV. The identification of low risk HPV by ISH may be useful to predict local recurrence. HPV- ISH is a useful technique that can be easily performed on paraffin embedded tissue.

PP3-256
SIGNET-RING CELL SALIVARY DUCT CARCINOMA - A CASE REPORT

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Background:Salivary duct carcinoma (SDC) was first described by Kleinsesser in 1968 and was classified as an independent entity by the 1991 Revised World Health Organization. It is a rare but highly malignant tumour. Purpose: We report a rare case of primary high-grade ductal carcinoma of salivary gland with signet-ring cells, that was confirmed by autopsy. Case report: A case of a high-grade ductal carcinoma of salivary gland originated “de novo” in parotid gland is presented. A 73 year-old male came to our hospital for a parotid swelling. He was treated by superficial parotidectomy. The tumour was unencapsulated, poorly circumscribed, grayish white, measuring 2,8cm in maximum diameter. The results of gross, microscopic, and immunohistochemical examinations are described. Microscopic examination showed that the tumour was entirely consisted of large signet-ring cells, positive for PAS and PAS-diastase. Lymphatic, vascular and nerve invasion were detected. Immunohistochemically, the tumour cells were positive for Ker7, Pankeratin, CEA, KerLMW and EMA, and negative for S-100 protein, KerHMW, PSAP, PSA, ER, PR, TTF1. Further ENT-investigations including endoscopy and scans (MRT, ultrasound) showed no pathologic findings. In particular, growth of cervical lymph nodes and distant metastases were not revealed. Discussion: Salivary duct carcinoma is one of the most aggressive types of salivary gland carcinoma and is characterized by local invasion, lymphatic and hematogenous metastases and poor prognosis. The occurrence of signet-ring cells within salivary duct carcinoma is an extremely rare finding. The poor prognosis and aggressiveness of salivary duct carcinomas dictates aggressive therapy.

PP3-257
RELATIONSHIP BETWEEN ACTINOMYCES AND CLINICOPATHOLOGIC FINDINGS IN ROUTINE TONSILLECTOMY AND ADENOIDECTOMY SPECIMENS

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BACKGROUND: Tonsillectomy and adenoidectomy are frequently performed operations but there is little information about the histological features. In this study it is aimed to investigate the presence of actinomyces in tonsillectomy and adenoidectomy specimens and analyse the patient’s profile and

main pathological changes. METHOD: The medical records of patients who underwent tonsillectomy and adenoidectomy at Zonguldak Karaelmas University, Faculty of Medicine, Department of Pathology, in the period between January 2001 and December 2006 were included. RESULTS: Out of 387, 179 (46.25%) were female and 208 (53.75%) were male patients. Mean age was 12.69 years ranging from 2 to 67 years. There were 309 (79.85%) in the pediatric age group and 78 (20.15%) were adults. Main surgical indication was concomitant presence of recurrent throat infections and stenotic symptoms of upper airways. In 62 (16.03%) patients an adenoidectomy and tonsillectomy was performed, 248 (64.08%) underwent tonsillectomy alone and 77 (19.89%) adenoidectomy alone. There was actinomyces in 42 (10.85%) of the 387 patients, of whom 17 were adult and 25 were pediatric. That is, actinomyces was present in 17 of the 78 adult patients (21.7%), and 25 of the 309 pediatric patients (8.09%). It was almost always seen in tonsillectomy specimens, but in one of the adenoidectomy specimen. In adult population,; Actinomycosis was present in all of the obstructive patients (17/17), but none in tonsillitis cases (0/17); however in pediatric population actinomycosis ratio was nearly equal; 48% and 52%, in terms of lymphoid hyperplasia cases (12/25) and inflammatory conditions (13/25), subsequently. CONCLUSION: The presence of actinomycetes may indicate an etiologic role for this organism in adenoid and tonsillar inflammatory conditions and hypertrophy. Our results indicate that actinomycetes may play an active role in the etiology of hypertrophy of tonsillar disease in adult populations and as well as tonsillitis in pediatric patients as the causative agent.

PP3-258
EXPRESSION OF α-, β-, AND y-CATENINS IN LARYNGEAL CARCINOMAS: CORRELATION WITH CLINICO-PATHOLOGICAL FEATURES

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BACKGROUND: Carcinoma of the larynx is one of the most frequent tumor in developed countries. Although several genetic and epigenetic alterations are characteristic of laryngeal carcinomas no definitive prognostic markers for advanced tumours have been identified. Catenins are a family of proteins including the α-, ß-, and y-catenins. In the last years, it has been demonstrated that alterations in the cadherin-catenin adhesion complexes are involved in tumor initiation, progression and metastasis. The aim of this study was to evaluate the immunoreactivity of the α-, ß-, and y-catenins in laryngeal squamous cell carcinomas (SCC) and to correlate their expression with clinicopathologic features. PATIENTS AND METHODS:This study involved a series of 279 formalin-fixed, paraffin-embedded laryngeal SCC. We constructed eight tissue microarray blocks where immunohistochemical staining was performed. Monoclonal antibodies against α-catenin (clone α-catenin-1, Dako, 1:35) ß-catenin (BD Biosciences, clone 14, 1:700), and y-catenin (BD Biosciences, clone 15, 1:1000) were applied using the Envision (Dako) method. The membranous expression of the catenins was scored as preserved, reduced or absent. RESULTS: Most of the tumors were located in the supraglottis (39.3%), followed by the glottis (25.5%), and subglottis (3%). The remaining cases were transglottic. Tumors were histologically classified as well (25%), moderately (42.6%) and poorly (32.4% differentiated. Overall, more than 75% of the tumors showed a reduced membranous expression of β- and α-catenin, especially in poorly differentiated carcinomas. In contrast, only 31.1 % of the cases showed a reduced expression of y-catenin expression. Conserved membranous y-catenin expression was associated significantly with tumor size (T1 vs T4), location (glottis vs non-glottis) and differentiation (well differenciated vs poorly differentiated carcinomas) ($p<0.005$. No other associations between catenin expression patterns and recorded clinicopathological variables were detected. DISCUSSION: Several studies have suggested that loss of β-catenin protein is associated with tumour progression, metastasis and poor patient prognosis in many types of cancer, and this may be attributable to its role in cell adhesion. In this study, we have show that loss of expression of α-, and β-catenins is a common abnormality in SCC of the larynx. A preserved membranous pattern of y-catenin was found in glottic, well-differentiated and small tumors. However, expression of α-, ß-, and y-catenins did not correlate with the prognosis of the patients.

PP3-259
SECOND PRIMARY SQUAMOUS CELL CARCINOMA ARISING IN CUTANEOUS FLAP RECONSTRUCTIONS OF HEAD AND NECK CANCER PATIENTS: A RARE LONG-TERM COMPLICATION

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The advances in reconstructive surgery have allowed surgical eradication of tumours which were considered inoperable in the past. Currently, surgical reconstructions by means of various types of skin flaps are commonly performed for advanced primary tumours of the head and neck. These techniques have been shown to increase loco-regional control of the disease and to improve functional rehabilitation. Early complications of regional and distant free flap transfers have been extensively described. However, there is still limited knowledge on the potential long-term complications of these techniques. Here, we report the cases of two patients with antecedents of squamous cell carcinoma of the head and neck (HNSCC), experiencing the development of a second primary SCC on the cutaneous surface of the flaps used for reconstruction. Growth of the second primary tumours occurred within a minimum of three year-interval. Both lesions arose at distance from the original mucosa of the oral cavity and from the deep surgical margins of the flaps. This observation ruled out any putative spread of a second primary tumour from the oral mucosa nearby the flaps. This is a rare complication of skin flaps reconstruction in HNSCC patients. Given the important expansion of these interventions in patients with head and neck cancers, this type of complication may become more frequent in the future and merit to be recognized by pathologists as a distinct entity. Interestingly, it seems that the well-known risk of a second primary HNSCC development in patients with previous head and neck carcinoma also applies to foreign tissues implanted in the area at risk. Therefore, long-term follow-up of patients previously treated for HNSCC, not only require careful evaluation of the normal mucosa of the upper aero-digestive tract but also of the cutaneous surface of the flap used for reconstruction.

PP3-260
TWO DIFFERENT SALIVARY GLAND TUMOURS LOCATED IN PAROTIS GLAND AND PARAPHARYNGEAL REGION SYNCHRONOUSLY: A CASE REPORT

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BACKGROUND: The presence of more than one salivary gland tumour is a rare condition. Cytologic and histologic findings of a patient having two different tumours located in different regions (parotis gland and parapharyngeal area) at the same time are presented. CASE: A 58 years old male patient was admitted to the hospital with a mass in left side of the neck for 3 months. Physical examination revealed a mobile, painless, left jugulary mass (lymph node?) which was 4x3 cm in dimensions and revealed a parapharyngeal mass behind left tonsilla palatina. On the CT examination of cervical region and pharynx, two soft tissue masses are identified. The first mass was in the left parapharyngeal region and it was about 3,5x3x3 cm in dimensions, well-circumscribed and showed heterogeneous contrast uptake. The second one was 4,5x2,5x2 cm in dimensions, sharply delineated and homogeneous. It was located in the caudal region of the left parotid gland and partially embedded in to the gland. Initial clinical diagnosis was metastatic malign tumour. Fine needle aspiration was carried out for the cervical mass. Cytologic findings were consistent with Warthin tumour. The parapharyngeal mass was excised and microscopic examination diagnosis was pleomorphic adenoma. The servical mass was excised as partial parotidectomy 3 months later and microscopic examination diagnosis was Warthin tumour. When these two masses were evaluated with operative and clinical findings, they were determined unrelated anatomically. CONCLUSION: Although cytological examination is difficult in salivary gland lesions because of its complex morphology, fine needle aspiration of salivary gland lesions is a simple and cheap procedure which allows surgeon to decide the appropriate treatment before operation.

PP3-261
SYNOVIAL SARCOMA: A RETROSPECTIVE ANALYSIS OF 12 CASES

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Background: Synovial cell sarcoma is a mesenchymal malignancy that represents 10% of all soft tissues sarcomas. It most often occurs in the lower limbs of young adults. Our study aim is to report clinical and histopathological findings, treatment and outcome of such tumors. Methods: This is a retrospective study of 12 patients diagnosed with synovial sarcoma, retrieved from 1990 to 2005, at the pathology department of F. Hached hospital (Sousse, Tunisia). Results: The median age of patients was 21 years. The sex ratio was of 1. The most common sites of involvement were: The lower limbs (10 cases), the upper limbs (1 case) and the oropharynx (1 case). The revealing symptoms were: limb swelling in 11 cases, pain in 3 cases, dyspnea (1 case), cough (1 case) and a pulmonary metastasis (1 case). Our cases were classified according to the WHO's classification in: monophasic type (8 cases), biphasic type (3 cases) and undifferentiated type (1 case). The tumors were classified in grade 2 (8 cases) and in grade 3 (4 cases), according the FNLCC recommandations. The treatment consisted in surgical removal in 10 cases (consisting in an amputation in 3 cases); adjuvant chemotherapy was indicated in 3 cases, a post-operative combination of chemo-radiotherapy was indicated in 1 case. Therapeutic abstention was indicated in 1 case. Another patient refused treatment. Clinical outcome was unfavourable with death occurrence in 6 cases. Conclusion: Synovial cell sarcoma carries usually a poor prognosis, that is correlated to multiple factors such as: anatomic location, size of tumor, histological type, grade, treatment modalities, especially the quality of surgical resection.

PP3-262
GIANT CELL TUMOR OF BONE: A CLINICOPATHOLOGICAL STUDY 14 CASES

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Background: Giant cell tumor of bone are particular by their morphologic features and the absence of accurate prognostic parameters. Method: This is a retrospective study of 14 cases of giant cell tumor of bones diagnosed at the pathology department of F. Hached hospital in Sousse between 1987 and 2004. Radiographic and histopathological features were reviewed and confronted with both clinical data and patient's follow-up. Results: 5 patients among six with a grade 1 tumor had a local recurrence; 1 patient among 8 with a grade 2 tumor had a local recurrence, another one developed pulmonary metastases after a local recurrence; 7 patients had neither recurrence nor metastasis after treatment. Conclusion: Histopathological parameters cannot be by their own predictive of a local recurrence or metastasis. Clinical and radiological features must be also taken in consideration.

PP3-263
LETHAL COMPLICATIONS OF RHEUMATOID ARTHRITIS - A RETROSPECTIVE CLINICOPATHOLOGIC STUDY OF 161 AUTOPSY PATIENTS

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The aim of this study was to determine: (1) the spectrum and incidence of complications related to RA such as systemic vasculitis (SV) systemic AA amyloidosis (AAa), lethal septic infection (SI), carditis (endo-, myo-, epi-, or pancarditis), interstitial pneumonitis, interstitial lymphoid hyperplasia, polyserositis, glomerulonephritis, atlanto-axial subluxation, etc, (2) the mortality of complications related to RA, and (3) the clinically missed diagnosis of these. Patients and Methods A randomized autopsy population of 161 in-patients with RA was studied. RA was diagnosed clinically according to the ACR criteria. The complications of RA and cause of death were confirmed histologically. Results The lethal complications of RA were: Systemic vasculitis, which was found in 36 (22.4 %) of 161 RA patients, and led to death in 19 (11.8%) of 36 patients. SV was recognized clinically in 7 (19.4 rel%) of 36, and not recognized in 15 (78.95 rel%) of 19 patients with lethal complication of SV. AA amyloidosis complicated RA in 34 (21.1%) of 161 patients. AAa led to death by uremia in 17 (10.56%) of 34, and was clinically recognized in 9 (52.95 rel%) of 17 lethal cases, and not recognized in 8 (47.05 rel%) of 17 patients. Lethal septic infection was detected in 24 (14.9%) of 161 RA patients at autopsy, and was recognized clinically in 11 of 24 (45.8 rel%) cases. Cardiac insufficiency was the direct cause of death in 29 (18 %) of 161 patients, and was always recognized clinically. A rare form of interstitial pneumonitis (characterized by intensive interstitial lymphoid infiltration and secunder lymphoid follicle) led to death in 2 (1.24 %) of 161 cases, and was never diagnosed clinically. Interstitial nephritis in two (1.24 %), glomerulonephritis in one (0.62 %) of 161 RA patients led to death by uremia. The underlying disease leading to uremia was never recognized clinically. In 2 (0.9 %) of 161 patients acute liver necrosis (“yellow atrophy”), subacute liver necrosis (“red atrophy”) were the direct cause of death, clinically labeled as hepatorenal insufficiency but basically not recognized as the underlying disease leading to death. Discussion Two thirds of deaths were related to RA. Systemic vasculitis, AA amyloidosis and lethal septic infection were the most important major complications of RA. They were responsible for the death in 60 (53.1 rel%) of 113 cases related to RA (37.3 % of 161 RA patients). In this study systemic vasculitis was the most likely lethal complication to be missed clinically with high probability.

PP3-264
PATHOANATOMIST'S VIEW ON CERTAIN PROBLEMS IN PRESENT-DAY ONCOLOGY

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Mesothelioma is a comparatively rare neoplasm, more frequently affecting the pleura and peritoneum, less often - the pericardium. The diagnosis is imperfect, being usually delayed, and it is not by chance that this tumour remains an enigma for the oncologist. The accurate diagnosis is made at best while a pathomorphological examination of the removed tumour, at the worst - during a post-mortem examination.The author carried out a retrospective analysis of the pathoanatomical material at the Municipal Institution "Taldom Central Regional Hospital" (NCRH) during 1984-2004. Of the 1387 postmortem examinations of adults, we revealed only one case of malignant diffuse highly differentiated papillary mesothelioma of predominantly the visceral peritoneum.Below is our case report.A 79-year-old woman was admitted to the Surgical Department of the TCRH on 6th February 1994, complaining of abdominal pain, and lack of defecation for two weeks, having considered herself sick during two months when she had developed lack of appetite and less of flesh. At examination by the physician on duty, the following diagnosis was made: partial ileus, predetermined, in all likelihood, by a colonic tumour. Surgery performed on 15th February 1994 was laparotomy: ovarian cancer with metastases into abdominal organs. The cytological study of ascitic fluid: adenocarcinoma. The woman died on day 8 after surgery. The final clinical diagnosis: ovarian cancer, involving abdominal organs;ascites;disseminated atherosclerosis.At post-mortem examination: the dead body of an undernourished elderly woman;the abdominal cavity contains up to 300 ml of reddish, muddy fluid.The greater omentum is deformed, solid, in the form of detached conglomerate. Pathoanatomical diagnosis:malignant diffuse mesothelioma predominantly of the visceral peritoneum with the histological form of papillary adenocarcinoma;acquired mechanical ileus; serous-fibrous peritonitis;generalized progressing atherosclerosis of the aorta, of the ileal arterias stage IV, degree 5 (according to Avtandilov). Hence peritoneal mesothelioma as the primary cause of death amounted to 0.072% amongst 1387 postmortem examinations of adult patients of our general somatic hospital over 21 years.In our opinion,one should reconsider the policy of regional oncologists towards improvement of the material and technical basis,activization of their activities and making courageous decisions aimed at using vitally important emergency chemotherapy in order to increase the survival rate.

PP3-265
SUBCUTANEOUS SOLITARY FIBROUS TUMOUR OF THE BACK

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Background Wagner in 1870 is credited of the first histologic description of solitary fibrous tumour (SFT) an uncommon spindle cell neoplasm recognized as a distinct entity among primary pleural neoplasm by Klemperer in 1931. In the last years has been observed that SFT is anatomically ubiquitous and multiple new extrapleural locations of this rare mesenchymal neoplasm have been reported. SFT may be difficult to diagnose outside the usual locations because of its histologic variability and its back of distinctive features. Method A 42-year-old female presented with a slow-growing and painless subcutaneous mass in her back that was initially clinically suspected of being an epidermal cyst or a lipoma. The patient underwent simple excision under local anaesthesia of the tumour which was observed to be located in the deep subcutis and focally in close proximity of the fascia at tumour base without involvement of the underlying musculature. Results Histologically the excised tumour consisted of a proliferation of spindle cells arranged in a storiform pattern embedded in a fibrous matrix. A prominent vascular pattern, focal myxoid and thick hyalinized collagen fibers were observed. There were few normal mitotic figures, but no abnormal mitoses. The tumour cells were positive for CD34, bcl-2, CD99 and vimentin whereas stains for S-100, EMA, cytocheratin, desmin and alpha-smooth muscle actin were negative. Based on these findings a diagnosis of SFT was made. Conclusion Clinically SFT presents as a slow-growing mass in middle-aged adults with no sex predilection. The histogenesis of this tumour remains a matter of controversy. It has been recently suggested that SFT may represent the neoplastic transformation of bcl-2/CD34 fibroblastic-like dendritic cells involved in antigen presentation within the connective tissue. Grossly SFT are well

demarcated, but partially encapsulated neoplasm. Histologically is characterized by a morphological appearance of alternating hypo- and hypercellular areas of spindle-shaped cells, dense bands of collagen and a vascular pattern. Differential diagnosis is sometimes difficult and is possible a mistaken with a variety of other soft tissue and spindle cell neoplasm including dermatofibrosarcoma protuberans, synovial sarcoma, schwannoma, spindle cell lipoma and cutaneous myofibroma. Immunohistochemistry is particularly important in the identification of rare and atypical soft tissue tumour. Immunohistochemically the diagnosis of SFT has been refined by the availability of immunohistochemical marker such as CD 34, bcl-2 and vimentin.

PP3-266
COMPARISON OF TWO METHODS OF ABDOMINAL FAT PAD ASPIRATION AND BIOPSY IN DIAGNOSIS OF AMYLOID DEPOSITION IN PATIENTS WITH LONG STANDING RHEUMATOID ARTHRITIS

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Objective: To comparing two methods of abdominal fat pad aspiration and needle biopsy in diagnosis of secondary amyloidosis in patients with long standing rheumatoid arthritis (RA). Method: 220 consecutive patients (53 males and 167 females) with a history of at least 5 years of RA in two rheumatology clinics were studied. Abdominal subcutaneous fat pad aspiration (ASFA) and abdominal subcutaneous fat pad biopsy (ASFB) was performed. Specimens were stained by alkaline congo red method. All specimens examined for presence of apple green birefringence under polarized light. For confirmation, all positive biopsy specimens and 40 negative controls with long standing RA (duration>15y) stained for AA protein by IHC method. Results: Amyloid was positive in 16 patients(7.3%). Nine of them were positive in both methods. Amyloid deposition was positive in 11(5%) patients with ASFA and in 15 (6.8%)patients with ASFB. All had 1+ deposits except in 2 of ASFB specimens, which had 2+ deposits. The sensitivity of ASFA and ASFB were 57% and 78.9% respectively. Conclusion: According to this study, the sensitivity of ASFA is lower than ASFB, however because ASFA is technically simple and less aggressive than ASFB, It is recommended that ASFA be used as first step in screening of patients with amyloidosis.

PP3-267
HISTOPATHOLOGICAL ASSESSMENT OF THE EFFECT OF NON- ABSORBABLE PROSTHETIC MESH ON THE SPERMATIC CORD CONTENTS: AN EXPERIMENTAL STUDY

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Background: Previous studies have documented that the use of synthetic prosthesis in repair of inguinal hernia markedly reduces the incidence of recurrence. However, little is known about the effect of these prostheses on the spermatic cord contents. This work was carried out to study the histopathological changes in response to contact with the commonly used prostheses (Prolene & Mersilene) on spermatic cord contents in male dogs. Materials and Methods: Thirty two male dogs were included in this study. The spermatic cords and testes were harvested from 2 dogs as a base line for the normal histology. The remaining 30 dogs were divided into 3 main groups. In the first (control) group (GI,n=10), the spermatic cord was delivered, its coverings were dissected and left intact on one side but on the other side it was skeletonized by removing its coverings. In the second group (GII,n=10), a strip of polypropylene (Prolene) mesh was wrapped snugly without strangulation around the dissected intact cord on one side and the skeletonized cord on the other side. A polyester (Mersilene) mesh was similarly applied in the third group (GIII,n=10). In each group, the spermatic cords and testes were examined histopathologically one week, and three months postoperatively. Results: At one week, the microscopic examination of the spermatic cord contents revealed more acute inflammatory reaction in mesh groups than the control. This reaction considerably subsided after three months with varying degrees and patterns of fibrous tissue deposition. These changes were more noticed in GIII than GII and in skeletonized cords than intact ones. The testes of all animals showed no histopathological changes. Conclusion: This experimental study proved the safety of the used non-absorbable prostheses on the spermatic cord contents and testes. Prolene mesh without skeletonization of the cord revealed more favorable results than Mersilene one.

PP3-268
CYCLOOXYGENASE-2 EXPRESSION IN BENIGN AND MALIGNANT CHONDROID TUMORS

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Background: Recent studies have shown increased levels of cyclooxygenase-2 (COX-2) in various human malignancies to include various bone and soft tissue tumors. However, little is known with regard to COX-2 expression patterns in chondroid tumors. Method: Immunohistochemistry assays were performed for COX-2 in enchondromas (n=10), chondroblastomas (n=11), chondromyxoid fibromas (n=5), conventional chondrosarcomas (n=17), clear cell chondrosarcomas (n=7), and mesenchymal chondrosarcomas (n=6). Results: Among the benign chondroid tumors, chondroblastomas revealed characteristic strong positivity in 6 of 11 cases (54.5%). All enchondromas and chondromyxoid fibromas were negative except in one case. In conventional chondrosarcomas, three cases (17.6%) were strongly reactive with COX-2 and all positive cases represented grade III chondrosarcomas. Clear cell chondrosarcomas were found to be focally positive in two cases (28.5%), while all mesenchymal chondrosarcomas were negative. Conclusion: These findings suggest that COX-2 overexpression in conventional chondrosarcoma may represent both a negative prognostic factor and also an advanced histologic grade. Interestingly, expression of COX-2 in chondroblastomas could be an important factor for inducing peritumoral inflammatory changes in these specific tumors.

PP3-269
EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-C AND ITS RECEPTOR IN OSTEOSARCOMAS

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Background: Vascular endothelial growth factor-C (VEGF-C) and its receptor, vascular endothelial growth factor receptor-3 (VEGFR-3) have been implicated as important factors in the

formation of lymphatic vessels, but its role in osteosarcomas has not yet been fully investigated. This study aims to define the expression of VEGF-C and VEGFR-3 in primary and metastatic osteosarcomas and their relationship to various clinicopathologic parameters. Method: Thirty three primary osteosarcomas and two pulmonary metastatic samples were immunostained for VEGF-C and VEGFR-3. In addition, VEGF-C and VEGF-D mRNA expression levels in three different human osteosarcoma cell lines and control fibroblasts were evaluated by real-time quantitative polymerase chain reaction (PCR). Results: Both VEGF-C and VEGFR-3 were mainly expressed in the cytoplasm of the tumor cells. Of the 35 patients with osteosarcoma, 16 patients (45.7%) showed strong positive reaction with VEGF-C. Four cases (11.4%) were negative and 15 cases (42.9%) showed weak immunoreactivity. For VEGFR-3, 12 patients ((34.3%) showed strong positive reaction. Fifteen cases (42.9%) were negative and 8 cases (22.8%) showed weak immunoreactivity. A significant positive correlation (Rs=0.42, p=0.01) was found between the expression of VEGF-C and VEGFR-3 in osteosarcomas. The expression of VEGF-C was significantly associated with osteoblastic subtype and high histologic grade in osteosarcomas. However, the expression of VEGF-C showed no significant correlation with the presence of metastasis. Expression of VEGFR-3 was not related to any clinicopathologic features analyzed. Two of the three tested osteosarcoma cell lines showed amplification of VEGF-C mRNA compared to control cells. No amplification of VEGF-D was noted in these cell lines. Conclusion: Our data suggest that VEGF-C and its receptor are expressed in osteosarcomas. Although the level of VEGF-C was high, it does not seem to have a direct influence on tumor metastasis in osteosarcomas.

PP3-270
RETROPERITONEAL LYMPHANGIOLEIOMYOMATOSIS ARISING FROM CYSTIC ENDOMETRIOSIS

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Aim and design: A case of retroperitoneal lymphangioleiomyomatosis (LAM) arising from cystic endometriosis was described. Results: A 25-year-old woman with no history of tuberous sclerosis or hormonal therapy presented with a painless, palpable abdominal mass. Computed tomographic and magnetic resonance imaging studies of the abdomen demonstrated a 4 cm cystic mass in the retroperitoneum. Macroscopically, the excised retroperitoneal cyst was multilocular and measured 4.0 X 3.5 X 3.5cm. Histologically, the lesion demonstrated three components. The first were multiple cysts or glands lined by columnar epithelial cells with cilia. The second component was a condensation of endometrial-type small stromal cells immediately subjacent to the cystic epithelium or glands. The third component was a thick exterior wall composed of plump spindle cells with clear to palely eosinophilic cytoplasm in a fascicular pattern, and slit-like vascular spaces, resembling LAM. Immunohistochemically, the epithelium and glands were positive for cytokeratin 7. The endometrial-type stroma cells were positive for vimentin and CD10. The cells of the LMA-like component showed positive staining for HMB45, alpha-smooth muscle actin, muscle actin and h-caldesmon. Conclusions: The lesion, LAM arising from endometriosis, represents a distinctive pathologic entity that should be recognized and studied further. This type of lesion should be included in the differential diagnosis of retroperitoneal cystic lesions.

PP3-271
ONCOGENIC OSTEOMALACIA ASSOCIATED WITH PHOSPHATURIC MESENCHYMAL TUMOR, A CASE REPORT AND REVIEW OF LITERATURE

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Oncogenic osteomalacia is an acquired syndrome characterized by renal phosphate wasting, hypophosphatemia, normal serum calcium levels and low serum 1,25-dihydroxyvitamin D osteomalacia caused by mesenchymal neoplasms that are termed phosphaturic mesenchymal tumors (PMTs). The condition improves or completely disappears after removal of the tumor. This is a case of a 22-year-old male who presented with a 1-year history of increasing bone pain and difficulty in walking. This was associated with generalised muscle weakness. Physical examination was unremarkable. Laboratory investigation revealed the presence of hypophosphatemia, phosphaturia, elevated alkaline phosphatase levels in the presence of normal calcium and parathyroid hormone levels. By MRI a paraspinal heterogenous well circumscribed mass was seen.Complete excision of the mass was performed. By microscopic examination, phosphaturic mesenchymal tumor was diagnosed. Oncogenic osteomalacia as well as phosphaturic mesenchymal tumor are disscused along with review of the literature.

PP3-272
GIANT CELL BIPHASIC SYNOVIAL SARCOMA WITH MASSIVE OSTEOID AND BONE FORMATION SIMULATING OSTEOSARCOMA

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Introduction Synovial sarcoma with giant cells and predominant osteoïd and bone formation is a rare variant difficult to distinguish from osteosarcoma especially in presence of lytic areas. Case report We report a case in a man aged 49 years admitted in Orthopaedic Department for pathologic fracture of the upper right femoral metaphysis. Radiography shows a well-circumscribed and calcified tumour of 37mm diameter with lytic areas in upper metaphysis and diaphysis of the right femur. The surgeon discovers a hard, reddish, rounded mass with focal calcifications occupying the external hillside of the fracture and infiltrating the neighboring bone. Histopathologic examination leads to a diagnosis of giant cell biphasic synovial sarcoma with massive osteoïd and bone formation. The pattern is biphasic with epithelial component expressing intensely CK and EMA, numerous mononuclear and giant cells and extensive osteoid and bone formation. Discussion Diagnosis is difficult because of the existence of lytic areas accompanying the tumor, massive osteoïd and bone formation and the character of the tumour cells associating a mononuclear background and giant multinucleated cells. It is important for the pathologist not to misdiagnosis this rare entity of synovial sarcoma because of its different course from osteosarcoma. The main argument which plead in favour of synovial sarcoma is essentially biphasic pattern with glandular and squamous component expressing CK and EMA.

PP3-273
ALVEOLAR SOFT PART SARCOMA OF BONE, A CASE REPORT
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Background:Alveolar soft part sarcoma (ASPS) is a distinct sarcoma that was defined by Christopherson et al in 1952.The incidence of ASPS has been reported to be less than 1% of all the primary soft tissue malignancies.Primary involvement of bone by ASPS is extremely rare. ASPS usually grows as a painless mass without functional impairement.Grossly,the tumor is well circumscribed,usually firm,grayish- yellow.Microscopically, the tumor cells are separated by fibrovascular tissue into nests.The cells are large and have vesicular, prominent nuclei and a granular cytoplasm.Mitoses are exceptional.Intracytoplasmic PAS positive diastase resistant crystalline material is diagnostic of the tumor. Case presentation:We report a 20 year old man with swelling of left heel.Radiography showed a well- defined large lytic lesion of calcaneous bone.Microscopic examination of curettage derived sample showed proliferation of large polygonal cells with vesicular nuclei and eosinophilic cytoplasm arranged in nests.Intracytoplasmic needle like PAS positive structures were seen.Immunohistochemical staining for S-100 protein was negative.Staining for desmin and vimentin was focally positive. Results:The mentioned microscopic findings confirmed the diagnosis of ASPS..Three months after curettage of the lesion metastatic foci were revealed in lung. Conclusion:Primary involvement of bone by ASPS is extremely rare,however it should be considered in differential diagnosis of primary lytic bone lesions with distant metastases,in particular metastatic hypernephroma in a young patient.

PP3-274
CLEAR CELL ADENOCARCINOMA OF ABDOMINAL WALL
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Background Endometriosis is a frequent benign disorder. Several observations of the coexistence of endometriosis and cancer have been published. Malignancy arising in extra-ovarian endometriosis is a rare event. Method Through a new case of clear cell adenocarcinoma associated with endometriosis foci occurring in the abdominal wall of a 49-year-old woman, we highlist the possibility of endometriosis malignant transformation and we review recommended criteria for this diagnosis. Results The patient was operated 20-years before for uterus leiomyoma. Actually, she presented with a large painful abdominal mass. After radiologic exploration, she was operated with a diagnosis of desmoid tumor. Histological examination showed a clear cell adenocarcinoma associated to endometriosis foci. Exploration of uterus and ovary did not show any malignancy or endometriosis. Conclusion When confronted to an extraovarian tumor with endometrial appearances, pathologists should concider a primary tumor and an origin in endometriosis. The demonstration of endometriosis might require the examination of multiple sections. Confirmation that a tumor is of endometrial type is of clinical importance.

PP3-275
MALIGNANT PERIPHERAL NERVE SHEATH TUMOUR ARISING IN ASSOCIATION WITH ECTOMESENCHYMOMA: DOES IT REALLY EXIST?
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BACKGROUND: Two situations have been described in which a MPNST is associated with ganglioneuromatous or ganglioneuroblastomatous tissue; the first, when one of the tissues transforms into a MPNST; the second, when the ganglioneuromatous portion of an ectomesenchymoma does the same. Ectomesenchymoma is a tumour that usually appears during paediatric age, and it is composed by rabdommyoblastic elements, mature ganglion cells and neuroma-like structures. MATERIAL AND METHODS: Newborn male that presents a right paratesticular mass of 4x3x1,5 cm, wich is removed. RESULTS: Histological study reveals three well differentiated areas; one has a nodular pattern, and it is composed by small round blue cells with Homer-Wright rosettes, reactive for neuroendocrine and skeletal muscle immunohistochemical markers. Other areas are fusiform and loose, and present abundant scattered mature ganglion cells. It is also seen densely cellular areas consisting of spindle atypical cells with a high mitotic index, reactive for S-100 protein. It is diagnosed as malignant ectomesenchymoma with areas of high grade MPNST, originated from the schwannian constituent of ganglioneuroblastoma. CONCLUSION: Does the possibility of a MPNST developing from the ganglioneuromatous portion of an ectomesenchymoma actually exist? Two cases of complex tumours which contain neuroblasts or ganglion cells and also mesenchymal elements, usually rhabdomyosarcoma have been described, although there are authors who are uncertain about them based on the fact that, in spite having MPNST, neither of them reports clear images of it. Besides, Conzutto et al. believe that an ectomesenchymoma must not have features of MPNST. Our case shows evident areas of rhabdomyosarcoma and ganglioneuroblastoma, with focus of MPNST, which leads us to consider it an ectomesenchymoma that undergoes transformation to MPNST.

PP3-276
PRIMARY OSSEOUS RHABDOMYOSARCOMA WITH FOCAL MATRIX FORMATION MIMICKING OSTEOSARCOMA: A CASE REPORT
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We present an unusual case of primary osseous pleomorphic rhabdomyosarcoma with focal matrix formation mimicking osteosarcoma. The patient was a 21-year-old man who suffered for two months with pain and slight enlargement of his left calf. The plain film demonstrates large, predominantly osteolytic mass in the region of proximal fibula with typical features of malignant primary tumour. On open surgical biopsy, the tumour was consisted of atypical cells, some presenting spindle morphology. Between them, there were bands of densely hyalinized matrix with osteoid appearance, but without definite lacunae or calcifications, and the diagnosis of osteosarcoma was established. Consequently, the tumor was removed. The tissue presented more pleomorphic cells with some definite rhabdomyoblasts. Desmin, actin, Myf4 and MyoD1 were positive in tumor cells and the diagnosis of rhabdomyosarcoma was eventually established. A few cases of primary pure rhabdomyosarcoma of bone were reported, and there were also reported cases of other bone tumours with rhabdomyosarcomatous differentiation: dedifferentiated chondrosarcoma, fibrosarcoma and osteosarcoma. Our case does nor meet criteria for sclerosing rhabdomyosarcoma as matrix formation is focal and cells are spindle and pleomorphic, but it shows the another possiblity for mistake with primary osseous tumour.

PP3-277
CORRELATION BETWEEN MICROVASCULAR DENSITY AND DIFFERENTIATION GRADE IN SOFT TISSUE TUMOURS

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Introduction Although there are thousands of publications on the subject of tumour angiogenesis, their great majority refer to carcinomas, and the publications on the subject in soft tissue tumours are very rare and, until present, with discordant results. According to this perspective, we have made the assessment of microvascular density in soft tissue tumours. Materials and methods 54 cases of soft tissue tumours (43 malignant and 11 benign tumours) were studied according to the following protocol: surgical samples were fixed in buffered formalin, embedded in paraffin, sectioned at 5 μ, then stained for morphological diagnosis (H&E), and for immunohistochemical detection of microvessels (smooth muscle actin – SMA, CD31 and CD34). Weidnet method of microvessel counting was used. Results All the tumours included in the study have presented rich blood vessel network, the majority (>80%) of the blood vessels from within the tumour area being of immature and intermediate type (positive for the endothelial markers and negative for SMA). This aspect was more obvious in the case of liposarcoma and malignant fibrous histiocytoma, but with no correlation with the differentiation degree. The highest values of microvascular density (MVD) were recorded in liposarcoma and malignant fibrous histiocytoma, in straight correlation with the high number of immature and intermediate blood vessels. We have noticed major differences in the blood vessel number between benign and malignant tumours, which were statistically significant ($p<0.02$). We have also noticed significant differences between the group composed of leiomyosarcomas and fibrosarcomas on one side, and liposarcoma and malignant fibrous histiocytoma on the other side ($p<0.05$). The results of microvascular density assessments were concordant for either endothelial marker used (table). Microvascular density was significantly higher in the malignant tumours than in the benign ones. Tumour MVD/CD34 MVD/CD31 Benign tumours 10.3 9.4 Fibrosarcoma 34.6 32.2 Liposarcoma 61.2 58.3 Malignant fibrous histiocytoma 58.9 55.7 Leiomyosarcoma 21.5 22.4 Conclusions Malignant soft tissue tumours have a rich blood vessel network, especially the liposarcoma and malignant fibrous histiocytoma. The majority of the blood vessels from within the tumour area are of immature and intermediate type. MVD does not correlate with the differentiation degree, but have significantly higher values in malignant tumours then in the benign ones.

PP3-278
FLOW CYTOMETRIC ANALYSIS OF DNA PLOIDY AND S-PHASE FRACTION IN PRIMARY LOCALIZED MYXOFIBROSARCOMA: CORRELATIONS WITH CLINICOPATHOLOGICAL FACTORS, Skp2 EXPRESSION, AND PATIENT SURVIVAL

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Background: Histological assessment for prognostication of myxofibrosarcomas remains challenging, for which we recently identified Skp2, an oncoprotein involved in S-phase progression, as an independent prognosticator . Methods: We assessed S-phase fraction (SPF) and DNA ploidy of myxofibrosarcomas and the association between SPF and Skp2. Flow cytometric findings were analyzed by CellQuest software for 75 primary myxofibrosarcomas and correlated with clinicopathological factors, Skp2 labeling index (LI), metastasis-free survival (MeFS), and overall survival (OS). Results: Forty-eight and 27 cases were classified as diploid and non-diploid, respectively. High SPF (≧20%) was detected in 32 of 61 interpretable cases. Skp2 overexpression (LI ≧ 10%) was seen in 36 of 72 cases with successful scoring. Non-diploidy was associated with higher FNCLCC grades ($p=0.006$), remarkable necrosis ($p=0.010$), and Skp2 overexpression ($p=0.018$). Noticeably, SPF was significantly related to Skp2 LI ($p<0.001$, $r=0.458$) and other clinicopathological factors, including FNCLCC grade, AJCC stage, and mitotic rate, etc. Non-diploidy predicted shorter OS ($p=0.0045$) and MeFS ($p=0.0489$), whereas SPF ≧ 20% was associated with inferior MeFS ($p=0.0252$) alone. In multivariate analyses, non-diploidy remained as an independent predictor for both OS ($p=0.020$, RR=3.337) and MeFS ($p=0.013$, RR=5.780), together with Skp2 overexpression ($p=0.014$ for OS; $p=0.017$ for MeFS) and positive margins ($p=0.004$ for OS; $p=0.002$ for MeFS). Conclusion: Skp2 overexpression promotes S-phase progression in myxofibrosarcomas, with a strong association between Skp2 LI and SPF. However, SPF, probably overshadowed by Skp2, provides no independent prognostic utility. Non-diploidy, along with Skp2 overexpression and positive margins, is independently predictive of adverse outcomes.

PP3-279
INTRAMUSCULAR HEMANGIOMA. REPORT OF 41 CASES

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Intramuscular hemangiomas are uncommon soft tissue tumors with potential for recurrence. There are two large series in the literature (Allen and Enzinger: 89 cases; and Beham and Fletcher: 74 cases). Several single case reports or series of few cases are reported. We report herein a series of 41 cases. During 30-year period (1974-2003), a total of 41 cases of intramuscular hemangiomas were diagnosed at our laboratory. There were 16 males and 25 females. The mean age at presentation was 20 years with a range from 1 to 62 years. 15 patients (38%) were less than 15 years, and 33 (80%) were less than 30 years of age. The tumors were located in the upper limbs (10 cases), lower limbs (13 cases), trunk (8 cases) and head and neck (10 cases). The tumors were classified according to the type of blood vessels dominating the lesion. In some instances, a mixture of histologic patterns was observed in the same case. The commonest variant was the cavernous type (22 cases). Other variants included capillary (6), venous (6) and mixed (7). Several tumors showed the presence of thrombi (9) or phleboliths (10). Occasional tumors showed Masson lesion (Intravascular papillary endothelial hyperplasia). Most lesions showed variable amount of fatty tissue. Four tumors recurred locally, most probably due to incomplete excision on initial surgery.

PP3-280
CANINE OSTEOSARCOMA: HISTOGENETIC AND CELL PROLIFERATION CHARACTERISTICS
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Osteosarcoma is the most common of all malignant canine bone tumors and has a high aggressiveness potential. From the histological point of view it is a tumor with a heterogeneous cell pattern that is characterized by the production of bone matrix and/or immature bone by atypical osteoblasts. Systematic studies with a objective to evaluate histogenetic aspects and the cellular proliferation index in canine osteosarcomas have not yet been described. The objective of this study was to determine the differentiation of the cellular elements and evaluate the cell proliferation index of these tumors correlating them with their different morphological subtypes, using markers such as: vimentin, osteocalcin, S-100 protein, 1A4, HHF53, factor VIII, AE1/AE3, CD31, CD34 and Ki-67. A retrospective study was performed using 65 cases of canine osteosarcoma, clinically evaluated according to sex, breed, age and topography of the lesions. Tumors were classified according to their histological subtype in: nonproductive, moderately productive and productive osteoblastic tumors, chondroblastic, fibroblastic, giant cell type, telangiectatic and undifferentiated subtypes. Males from the Rottweiler breed with an average age of 7,4 years were more frequently affected by osteosarcoma. In 77,4% of cases, the lesions were on the appendicular skeleton (mainly on femur). The identified histological subtypes were: osteoblastic (47,7%), fibroblastic (23,1%), chondroblastic (21,5 %), giant cell type (4,7%), telangiectatic (1,5%) and undifferentiated(1,5%).In 98,2% of all tumors there was vimentin reactivity. Immunoreactivity to osteocalcin occurred in osteoblastic, chondroblastic and fibroblastic cells of 91% of all osteosarcomas. The S-100 protein was expressed in 79,7% of the cases (100% of the chondroblastic subtype). A significant proportion of osteosarcomas expressed positively to 1A4 and HHF35 antibodies. All osteosarcomas were negative for AE1/AE3 and CD31 and CD34 testing. Ki-67 analysis revealed a high cell proliferation index in all cases nonproductive osteoblastic osteossarcoma subtype. Canine osteosarcomas are constituted by a diverse cell population, composed of osteoblastic, chondroblastic, myofibroblastic and mioblastic elements, which are probably originated from a single pluripotent mesenchymal cell or from immature osteoblasts that go thru a differentiation process. Nonproductive osteoblastic osteosarcoma presented a considerably high cell proliferation index that may relate to a more aggressive behavior.

PP3-281
FIBROMATOSIS-PRIMARY AND FIRST RECURRENT TUMORS. A FLOW CYTOMETRIC AND CLINICAL ANALYSIS IN SEARCH TO PREDICTORS OF OUTCOME
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Fibromatosis is a benign fibrous tissue tumor that has a significant propensity to locally recur. To help identify features predictive of aggressive behavior, we analyzed retrospectively, a large series (68 cases) of deep seated and plantar fibromatosis. We determined the phases of cell cycle and DNA concentration, recorded clinical information, and evaluated a variety of histological features (degree of cellularity, mitotic index, stroma composition, margin of growth, and status of resection margin) of primary (50 cases) and first recurrent (18 cases) tumors. We correlated these findings with the patient's outcome to ascertain prognostic parameters. The patients included thirty-eighty males and 30 females who ranged in age from 5-79 (mean 34) years. All tumors were resected and nineteen patients also received radiotherapy. Recurrences developed in 38 patients. Tumor size ranged from 1 to 16 (mean= 6.7) cm. Flow cytometric analysis was performed on 51 tumors (35 primary and 16 first recurrent tumors). All tumors were diploid and greater than 93% of the analyzed cells were in the G0/G1 (resting) phase. The morphologic and flow cytometric findings in fibromatosis were similar in the primary and first recurrent tumors and were not associated with any prognostic features. However patient age, involvement of surgical margins and tumor size were predictive of local recurrence.

PP3-282
AGGRESIVE ANGIOMYXOMA. A REPORT OF TWO CASES IN MEN
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These are the first two cases of aggresive angiomyxoma diagnosed and treated in men in the General Hospital of Mexico City. The first case was a 69-years-old man, presented with an inguinal mass, at first diagnosed as an hernia. The second case was a 32-year-old man with an increased volume in the scrotum diagnosed as lymphedema. The aggresive angiomyxoma (AA) is a soft tissue neoplasm found in pelvis or perineum, locally aggressive but not metastatic, it is frequently found in women, but it is very rare in men. However, it seems to have a better prognosis in men. The tumors in both men and women must be differentiated from intramuscular myxoma, myxoid neurofibroma and angiomyofibroblastoma, all of them benign neoplasms without any risk of relapse or local invasion. In several cases, the AA can be mistaken with a malignancy, because of the size and infiltration of surrounding tissues. So it is important to recognize it to select the correct treatment and give a prognosis.

PP3-283
REACTIVE ANGIOENDOTHELIOMATOSIS IN A PATIENT WITH CHRONIC RENAL FAILURE
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BACKGROUND: Reactive angioendotheliomatosis (RAE) is a rare condition of unknown pathogenesis characterized by a reactive cutaneous vascular proliferation that often occurs in the setting of coexistent systemic disease. Different terms have been used for this lesion, including systemic proliferating angioendotheliomatosis, diffuse dermal angiomatosis and glomeruloid angiomatosis. METHODS: We describe a case of RAE in a patient with chronic renal failure. RESULTS: A 66-year-old woman presented with a dermal swelling of the left thigh. No previous trauma was recorded. The patient had several concomitant diseases, including diabetic chronic renal failure, anaemia, hypertension, coronary heart disease, mitral and aortic valve disease. Calcium serum level was normal. Histologically, the lesion was characterized by a diffuse proliferation of anastomosing capillaries within the dermis and subcutis. Endothelial cells showed focal hobnailing, but lacked significant atypia or significant mitotic activity. Media calcinosis was observed in some dermal arteries as well as foci of subcutaneous fat necrosis. Immunhistochemically, endothelial cells expressed CD31 and CD34 and were negative for HHV8. Smooth muscle actin stained pericytes. CONCLUSION: RAE is a rare, probably under-recognized benign lesion, which can easily be confused with other benign and malignant vascular neoplasia. RAE is

commonly associated with underlying systemic diseases, including renal or hepatic failure, chronic infections, rheumatoid arthritis, valvular heart disease, and cryoglobulinemia. Immunoreactivity for HHV8 has been detected in up to 40% of the cases.

PP3-284
DISTRIBUTION OF CHONDROCYTES CONTAINING ALPHA-SMOOTH MUSCLE ACTIN IN HUMAN NORMAL, OSTEOARTHROTIC AND TRANSPLANTATED ARTICULAR CARTILAGE

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Background: Relatively little is known about the expression of a contractile actin isoform, alpha smooth muscle actin, in the normal human articular chondrocytes and in chondrocytes during certain pathological conditions. The aim of our work was to evaluate the occurence of alpha-smooth muscle actin in normal, osteoarthrotic and transplantated articular cartilage by means of immunohistochemistry. Method: Histology and immunohistochemistry were performed on paraffin embedded cartilage specimens obtained during total hip replacement for femoral neck fracture (15cases) and or osteoarthrosis (15 cases). Moreover, tibial plateau were retrieved from seven males with haemophilic arthropathy undergoing total knee arthroplasty. We also used samples from 9 patients with autologous chondrocytes transplantastion in the region of the traumatic defect in the distal femur 10 months after operation. Results: Approximately 20% of the chondrocytes in the superficial region of normal cartilage expressed alpha-smooth muscle actin as demonstrated by immunohistochemistry. It was readily apparent that fever cells in deep region contained alpha-smooth muscle actin. In the osteoarthrotic cartilage and cartilage from haemophilic patients occurred predominantly cells comprising clonal grouping stained for alpha-smooth muscle actin. In regions with formation of fibrocartilagineous repair tissue the majority of cells stained also positive. In the case of transplantated autologous chondrocytes the samples obtained 10 months after operations new cartilage had partly hyaline character and the majority of cartilage cells contained alpha-smooth muscle actin. Conclusion: Our study showed that significant percentage of articular chondrocytes express alpha-smooth muscle actin in health, disease , response to injury, defect healing and after autologous transplantation. They may utilize the contractile actin isoform in manipulating the extracellular matrix of articular cartilage.

PP3-285
CUTANEOUS LEIOMYOSARCOMA

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BACKGROUND: Superficial leiomyosarcomas are rare soft tissue tumors that account for less than 3% of all cutaneous soft tissue neoplasms. They further divided into cutaneous and subcutaneous and they usually occur in the extremities. METHOD AND RESULTS: We report a case of an 80-year-old female patient who came for excision of a firm protruding nodule at the temporal area, measuring 3,5 cm. Histological examination of the specimens of the resected nodule showed a spindle-cell tumor with morphologic and immunohistochemical features compatible with leiomyosarcoma; the tumor was positive for smooth-muscle actin, caldesmon, desmin and vimentin but negative for S-100 protein, HMB-45 and pankeratin. CONCLUSION: Immunohistochemical study seems necessary due to the difficulties in the differential diagnosis, which includes spindle-cell dermal carcinoma, spindle-cell melanoma and malignant fibrous histiocytoma. Furthermore the location of this particular neoplasm is considered very rare.

PP3-286
GLOMANGIOMA OF THE THIGH: CASE REPORT. CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS

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Background: Glomus tumors are usually benign perivascular neoplasms composed of cells resembling modified smooth muscle cells of the normal glomus body with three variants: the solid type, glomangioma and glomangiomyoma. They are sporadic but autosomal dominant inheritance with linkage to chromosome 1p21-22 (locus VGLOM), leading to mutation of the glomulin gene has been reported. The thigh is an exceptionally rare site with less than 10 published cases. Methods: A 41-year-old female presented with a disturbing, painless mass at the inner side of her right thigh. An MRI scan revealed a mass fed by a branch of the deep femoral artery situated in between the adductor muscles of the right thigh. The tumor was excised and it was an encapsulated, 3.5 cm, brown, friable mass with recognisable transversing fibrous bands. The tissue was formalin fixed and examined with H+E, PAS, PAS-diastase and with the immunohistochemical method of Envision-HRP for detection of vimentin, α-SMA, desmin, CD 34, factor VIII, CD 99, Bcl 2, c-kit, chromogranin, mixed cytokeratins, ki 67, S-100 and p 53. Results: The tumor was an encapsulated, non invasive mass with a complete fibrous vascular capsule, surrounded by fibrofatty tissue with scattered striated muscle fibers. The bulk of the tumor mass comprised large vascular channels with thin or hyalinised wall and in between the cellular areas were composed of oval cells with uniform hyperchromatic nuclei (ki 67 <1%,) and eosinophilic cytoplasm arranged in compact, trabecular masses, single cells or as perivascular wreath like. The neoplastic cells were vimentin, α-SMA, CD 34, Bcl 2 positive with focal strong expression of desmin. With PAS-diastase a distinct perivascular linear positivity was noted and a histological diagnosis of glomangioma was made. Conclusion: This is a case of a well circumscribed glomangioma of the right thigh, with an aberrant immunophenotype in an unusual site which required differential diagnosis from hemangioma, cellular leiomyoma and neuroendocrine tumors.

PP3-287
SOFT TISSUE SARCOMAS: REVIEW OF 614 CASES

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BACKGROUND: Even with the reform in molecular genetics , soft tissue sarcomas (STS) are still among the main problems of diagnostic pathology and the pathogenesis of most soft tissue tumors is still unknown. We need larger epidemiologic studies to define the pathogenesis and to find out the most effective therapy options. METHOD: We reviewed 614 cases diagnosed in our department between the years 2000- 2007 according to the latest WHO classification; and analyzed them considering age, sex, etiology, localization, recurrence, metastasis, and therapy. RESULTS: Fifty-five percent of the cases were between 21-60 years; and the median age was 46 years. In the first two decades of life trunk and abdomen and then lower extremities were the most popular localizations. There were no significant relation between age and sex. Immunhistochemistry was used for the tumors in early ages (<40), but histological analyses were enough in adult tumors. Tumors of uncertain differantiation group were mostly diagnosed type (21%). Highest recurrence rate was seen in abdominal tumors and especially in liposarcomas. Lung was the first site for metastasis and the highest metastasis rate was seen in synovial sarcoma. There were only 2 postradiotherapy sarcomas; a synovial and a pleomorphic sarcoma. The prefered therapy option was mostly surgery; but chemotherapy and radiotherapy were also used for the aproppriate tumors. CONCLUSION: The median age in our material is younger then in the English literature. Distribution of localization is the same; but there is no correlation between gender and other variables. Tumors of uncertain differantiation constitute the largest part of our review. As we cannot obtain the origin of many sarcomas even with immunhistochemistry, histopathologic examination is still the gold standart in sarcoma diagnosis.

PP3-288
REPORT OF THREE CASES OF MESENTERIC FIBROMATOSIS. IS IMMUNOHISTOCHEMISTRY NECESSARY FOR THE DIAGNOSIS?

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Mesenteric fibromatosis is a form of deep fibromatosis. According to the 2002 WHO classification fibromatoses belong to the category of intermediate , locally aggressive tumors which are characterized by infiltrative growth and local recurrences but no metastatic potential. Both sporadic cases and cases associated with familial adenomatous polyposis have been described. The purpose of this study is to report the histologic and immunohistochemical findings in 3 cases of mesenteric fibromatosis and discuss the role of immunohistochemistry in the diagnosis of these tumors. Two of our patients were female, aged 64 and 29 years and one male, aged 33. One gave a history of previous abdominal surgery, one presented with abdominal discomfort six months postpartum and the third presented with acute abdomen. None had familial adenomatous polyposis. In the first case the tumor extended from the mesentery to the pancreas infiltrating large vessels and was only partially excised. In the other two cases the tumors involved the mesentery, infiltrated the wall of the small and the large bowel respectively and were excised together with a segment of the involved bowel. All three cases exhibited a proliferation of spindle cells with myofibroblastic features forming long fascicles and associated with collagenous and focally loose stroma. Despite the variable cellularity and the infiltrative border, mitoses were absent to rare and there was no atypia or pleomorphism. Inflammatory infiltrates were distinctively absent. The immunohistochemical evaluation of the tumors included staining for Keratin AE1/AE3, CD34, S100 protein, smooth muscle actin, desmin, CD117 which were all negative and for β-catenin which was positive with characteristic nuclear staining. The diagnosis of mesenteric fibromatosis usually relies on careful observation of the histologic features in H+E sections. The immunohistochemical evaluation is a helpful adjunct in cases in which there is doubt regarding its distinction from sclerosing mesenteritis and gastrointestinal stromal tumor(GIST) or as part of protocol immunohistochemistry in spindle cell lesions of the mesentery. Our finding of nuclear β-catenin positivity in the three cases studied is consistent with previous studies that have shown β-catenin positivity in mesenteric fibromatosis as compared to the negativity in GIST and in sclerosing mesenteritis.

PP3-289
MORPHOLOGICAL AND CLINICAL CHARACTERISTICS OF THE PERIODONTAL TISSUES INFLAMMATION RESULTED FROM THE JAW FRACTURES

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Numerous epidemiological data suggested that periodontal tissues inflammation resulted from the conservative therapy of jaw fractures have high incidence rate in Ukraine. The aim of present study was to find out the morphological and clinical markers of the effectiveness of the improved design of a dental splint device combined with the ‘Uimisbol’ gel &Bolus Alba treatment in patients with jaw fractures. Methods. 25 volunteers were enrolled while receiving dental care for the jaw fractures. Each patient had a fixing of the jaw by the improved Tigershtedtt’s splints. The gel “Uymisbol” &Bolus Alba treatment was used locally on the fracture area in the form of the oral applications. The main active substance in “Uymisbol” was a plant extract of the Comarum palustre. 10 patients were treated in routine way without ‘Uimisbol’ gel &Bolus Alba application (group of comparison). Histological slides of gum tissue biopcies stained with H&E, Van Gison, and then stereological evaluation by point count method performed. The volume fractions of inflammatory infiltrates, vascularity, areas of dystrophy and necrosis of connecting tissue and others were determined. The results showed in the group of comparison the increase of volume fractions of inflammatory infiltrates, vessels, foci of periodontal connective tissue dystrophy and necrosis. The conservative therapy with the gel “Uymisbol” resulted in the reduction of hyperemia and vessels number. Inflammatory infiltrates and areas of connecting tissue dystrophy revealed occasionally. The improved device did not damage periodontal tissues in aesthetically important areas of oral cavity Conclusion These findings suggested the jaw fractions without improved treatment stimulate persistent inflammation in the gum of patients. On this background there is braking of repair processes, which can unfavorably alter bone repair processes. The use of gel “Uymisbol” &Bolus Alba treatment, possessing antioxidant, osteotropic, anti inflammatory and immune stimulating properties allows normalizing gum tissue’s metabolism, to recover the normal parameters of gum tissues and accelerate bone repair.

PP3-290
CYTOGENETIC STUDY OF 108 ADIPOSE TISSUE TUMORS

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Background. Cytogenetic investigation of soft tissue tumours, revealing clonal chromosome alterations associated to specific gene sites, allows better comprehension of pathogenesis and more

precise classification of soft tissue neoplasms. We studied 108 adipose tissue tumours from 105 patients, 63 males, 42 females, mean age 52 (range 23-79). Tumour sites were mainly shoulder, forehead, dorsum, arm and cervical region. Size of tumours was ranging between 0.5 cm and 10.5 cm. Methods. All the 108 adipose tissue tumours were cytogenetically analysed. Chromosome preparations were performed after short-term culture, using standard cytogenetic technique and karyotype were established according to ISCN nomenclature. Hystopathologic features and karyotype of tumours were investigated and reported independently. Results. According to pathological diagnosis, 73 tumours were conventional lipoma, 12 fibrolipoma, 10 angiolipoma, 7 spindle cell lipoma, 1 lipomatosis, 4 atypical lipomatous tumours/well differentiated liposarcoma (ALT/WDL), 1 de-differentiated liposarcoma. Cytogenetically, 51,4% of lipomas showed abnormal karyotype involving 12q13-15 (58,3%), other sequences of chromosome 12, mainly 12p11-12 and 12q24 (22,2%) and chromosome 6 (16,7%). Angiolipomas showed a normal diploid karyotype in 90% of cases. Spindle cell/pleomorphic lipoma showed monosomy of chromosome 13 and 16 in 42,9% of cases, translocation of 6p21-23 with Xp21.1, 1p32 and 11q13 in 42,9% of cases and balanced translocation of chromosome 12 in 28,6% of cases. Five liposarcomas, 4 ALT/WDL and 1 de-differentiated liposarcoma, showed a complex karyotype, characterized by ring- and giant-supernumerary chromosomes. One case of ALT/WDL was a recurrence and the morphology was deceptively benign in despite of the presence of ring- and giant-chromosome. Conclusion. In the 108 cases of adipose tissue tumours analised cytogenetic studies confirm differences between lipoma and liposarcoma. Although the same chromosome can be involved in benign and malignant neoplasms, aberrations are quite different. The present case-study shows that in conventional lipoma chromosome 12 frequently translocates with 3q27-28, while in liposarcoma chromosome 12 gives rise to ring- and giant- chromosomes. So far even when lipomatous tumour has minimal atypical morphology, cytogenetic analysis still shows it's sarcomatous nature. Although histopathological examination is the gold standard in lipomatous tumour diagnosis, cytogenetic analysis can help in difficult cases.

PP3-291

PRIMARY EMBRYONAL RHABDOMYOSARCOMA OF LONG BONE IN A CHILD 5 YEARS OLD. A CASE REPORT AND REVIEW OF THE LITERATURE

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Background: RMS is a well documented soft tissue sarcoma. The majority of RMS that involve bone in children are metastatic or represent secondary involvement by contiguous growth of a soft tissue lesion. This case is the sixth one involving the femur in childhood. Design: We present the Clinico-Radio-Pathological findings in a boy 5 years old, who submitted with a painful left knee's joint due to a left limb swelling. Results: The x-ray, CT-scan and MRI of the left femur revealed a diaphyseal large lytic lesion with cortical destruction and reactive periostic "onion skin-like" proliferation, compatible with a Ewing's Sarcoma. The histological and immunohistochemical investigation evaluated a high grade spindle cell sarcoma, with a herringbone pattern of proliferation, enlarged, bizarre or polygonal cells and abundant mitosis without any producing of malignant osteoid. The diagnosis of RMS has been prevailed over the diagnosis of Ewing's Sarcoma and supported by the positive reaction in Desmin, Vimentin, Myf-4 and MyO-D1 abs. The patient has been treated according to the RMS protocol and five months later the bone tumor has been completely resected showing histologically 95% tumor necrosis and two nodules of live RMS without any evidence of malignant osteoid production. Conclusion: We ordinarily do not make the diagnosis of a RMS in a bone tumor. Although the clinical and radiological data are compatible with those of a Ewing's Sarcoma, the immunohistochemical results support the diagnosis of a RMS involving the periosteum and the adjacent skeletal mass. RMS described in children's bones have not specific radiographic features and show bone destruction by a lytic process. Histologically they do not differ from RMS of soft tissue and any type of them can occur in bone. The presence of other component, such as cartilage, bone or undifferentiated sarcomatous elements in bones raise the suspicion that the lesion in question may represent dedifferentiation phenomenon rather than a primary RMS of bone or may be a part of a malignant mesenchymoma.

PP3-292
SYSTEMIC AND CORONARY VASCULITIS IN RHEUMATOID ARTHRITIS - A RETROSPECTIVE CLINICOPATHOLOGIC STUDY OF 161 AUTOPSY PATIENTS

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Systemic vasculitis (SV) is one of the main, and the most likely lethal complication to be missed clinically with high probability of rheumatoid arthritis (RA). The aim of this study was to determine: (a) the prevalence, the mortality, and the clinically missed diagnosis of SV at autopsy in RA, (b) the prevalence, the mortality, and the clinically missed diagnosis of coronary arteritis and arteriolitis (CAa). Patients and Methods A non-selected autopsy population of 161 in-patients with rheumatoid arthritis was studied. RA was confirmed clinically according to the criteria of the ACR. Vasculitis was diagnosed histologically. Results (a) SV was found in 36 (22.4%) of 161 RA patients. SV directly led to death in 19 (11.8%) of 36 patients (due to coronary arteritis and/or arteriolitis in 12, pulmonary and bronchial arteriolitis with disseminated lobular-sublobular pneumonia in 3, cerebral vasculitis and multifocal brain necrosis in 2, thrombovasculitis of renal artery and renal necrosis in 1, or thrombovasculitis of mesenteric artery and hemorrhagic intestinal necrosis in 1 patient). SV was recognized clinically in 7 of 19 with the lethal complication of SV. (b) Coronary arteritis and/or arteriolitis were associated with SV in 25 of 36 cases (15.5 %). CAa led directly to death in 12 of 25 patients (due to coronary arteritis and thrombosis of the main coronary artery with a large myocardial infarct in 1, coronary arteriolitis and multiple focal microinfarctions of the myocardium (myocardiocytolysis) in 11 cases). Coronary arteritis and/or arteriolitis was detected clinically only in 3 of 25 patients. Discussion In RA various organs are involved by vasculitis with different incidence. The leading and most endangered target is the heart, which was involved in this study in two thirds of 36 RA patients with SV. In most cases the arterioles and the small arteries are affected by vasculitis. Three types of vasculitis (non-specific, fibrinoid necrotic, granulomatous) may be present simultaneously in different vessels or combined in one. Different stages of inflammation can be found simultaneously, reflecting histologically the relapsing nature of this type of vasculitis. In most of the cases the clinical diagnosis of vasculitis is based on the visible skin involvement. Vasculitis of the skin does not necessarily run parallel with the heart involvement. Coronary vasculitis should be kept in mind, and it is very important to look for minor, transient, and recurrent clinical signs of cardiac disease, especially if there is a history of vasculitis.

PP3-293
PATHOGENESIS SILENT MYOCARDIAL INFARCTION IN RHEUMATOID ARTHRITIS - A RETROSPECTIVE CLINICOPATHOLOGIC STUDY OF 161 AUTOPSY PATIENTS

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Coronary vasculitis is the main cause of silent myocardial infarction in rheumatoid arthritis (RA), and the most likely lethal complication to be missed clinically with high probability. The aim of this study was to outline the pathogenesis of silent myocardial infarction due to coronary arteritis and/or coronary arteriolitis (CAa) in RA. Methods A non-selected autopsy population of 161 in-patients with RA was studied. RA was confirmed clinically and CAa was diagnosed histologically. Results 1. SV was found in 36 (22.36%) of 161 RA patients. CAa was associated with SV in 25 of 36 cases (15.5 %, 69.44 rel%). Subepicardial (main) coronary arteritis with, or without intramural coronary arteriolitis were present in 14 (56 rel%) of 25 patients. Coronary arteritis and thrombosis of the main coronary artery with a large myocardial infarct led directly to death in 1 (4 rel%) of 25 patients Coronary arteriolitis with multiple focal microinfarctions of the myocardium (myocardiocytolysis) was the direct cause of death in 11 (44 rel%) of 25 cases. 2. Three types of vasculitis (non-specific, fibrinoid necrotic, granulomatous) were present simultaneously in different vessels or combined in one. Different (acut-subacute-subchronic-chronic) stages of inflammation can be found simultaneously. Discussion All types of autoimmune vasculitis are of a relapsing (recurrent) nature. Histologically different stages of inflammation can be found simultaneously, reflecting histologically the relapsing nature of vasculitis. Vasculitis of the main coronary arteries with or without thrombosis may result in ischemia and may lead to a large myocardial infarct, macroscopically similar to myocardial necrosis due to coronary atherosclerosis and/or thrombosis. Vasculitis of the arterioles and small arteries can lead to local ischaemia and to regressive changes. This process is more or less widespread and multifocal, depending on the number of involved vessels. The necrotic foci are small and they are present in different stages of necrobiosis; small (1-2 mm of diameter) homogeneous areas alternating with small lytic foci of myocardium, and scars of a similar size simultaneously side by side. Because of the recurrent nature of immune vasculitis the regressive changes accumulate with time in the myocardium and may lead to unexpected sudden death. Clinically it is difficult to recognize the small accumulating myocardial necrosis (myocardiocytolysis). The history of vasculitis, transient cardiac complaints, low voltage electrocardiogram (ECG) may help in the diagnosis.

PP3-294
PATHOBIOLOGY OF ATHEROGENESIS

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Background. We have studied the role of the immune inflammation (IINF) in the set-up and development of atherosclerosis, both in experimental material and in samples of human coronary arteries, obtained during surgical operations, from 250 patients. Methods. The material has been studied by ultrastructural and immunohistochemical methods. Results. The role of the lymphocytes in the atherogenesis (AG) is viewed from the standpoint of their input into the expression of the lymphocytes and monokines, into the regulation of the production of chemokines and in situ antibody synthesis. The input of the smooth muscle cells (SMC) into the AG is analyzed not only from the standpoint of the proliferation of the synthetic phenotype of the SMC and the synthesis of connective tissue matrix proteins, but also taking into account their involvement into IINF reactions, expression of cytokines and class II antigens. During initial AG the depositing and/or formation into the subendothelial intima layer of modified LDL, which acquired autoantigenic properties, is the triggering factor, which stimulates the expression by endothelial cells of chemoadhesive molecules, which connect with the ligands of non-granular leucocytes. Activated monocytes/macrophages and T-cells, which penetrate

into intima, even during the most early stages of the AG, start a cascade of reactions, that makes sure the development of the early phase of the inflammation. Conclusion. The involvement of the intimal cells into the inflammatory reaction (macrophages, SMC and endothelial cells begin to produce class II antigens) transfers the acute phase of the inflammation into a chronic one, like the delayed hypersensitivity reaction.

PP3-295
TWO AUTOPSY CASES OF A NEW CORONARY DISEASE (ISOLATED EOSINOPHILIC CORONARY PERIARTERITIS)

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Ist case: A 40y male presented symptoms of angina pectoris for 9 years and expired with symptoms of unstable angina. At the time of admission, 26 days before death, blood examination showed WBC5,000/mm3(St 3%, Seg 38%, Ly 47%, Eo 4%, Ba 5% and Mo 3%). At the autopsy, the heart weighed 430g and both right and left coronary arteries of the subepicardial region were grayish white in color and elastic hard. Histologically, inflammatory infiltration was localized only in adventitia of coronary arteries located in the subepicardial region. Inflammatory cells infiltrated in the adventitia were mostly eosinophils. The medial smooth muscle cells were slightly hypertrophied and the intima showed irregular thickening with fibrosis. Inflammatory change could not be found, not only in the intramural coronary arteries, but also in the arteries of other tissue and organs. IInd case: A 28y female suffered from bronchial asthma for 4 years and showed symptoms of angina pectoris about 3 months before death. Blood examination showed WBC8,600/mm3 with slight eosinophilia. At the autopsy, the heart weighed 220g and epicardial coronary arteries were grayish white in color, the same as case I. Histologically, inflammatory cells, mainly of eosinophils, infiltrated also in the adventitia of both right and left epicardial coronary arteries. The medial smooth muscle cells were well preserved, but the intima of these arteries showed irregular thickening with fibrosis. No findings of angiitis could be detected in the blood vessels except subepicardial coronary arteries. As a result of these autopsy findings described above, this type of coronary arteritis could be termed "Isolated Eosinophilic Coronary Periarteritis", a new coronary disease.

PP3-296
RENOVASCULAR HYPERTENSION CAUSED BY GIANT-CELL ARTERITIS – CASE REPORT

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Background: Giant-cell arteritis (GCA) affects almost exclusively persons older than 50 years of age, and disease risk is highest among those who are 75 to 85 years of age. Two thirds of those affected are women. Giant cell arteritis typically causes vasculitis of the extracranial branches of the aorta and spares intracranial vessels. Transmural inflammation of the arteries induces luminal occlusion through intimal hyperplasia. Clinical symptoms reflect end-organ ischemia. We reported a case of a 15-year-old male teenager with severe heart and acute renal failure as the dominant clinical manifesations of renovascular hypertension (RVH) caused by atypical (GCA). Unrecognized RVH and treatment of the consequent heart failure by angiotensin-converting enzyme inhibitors (ACEI) probably contributed to progression of renovascular disease to bilateral renal artery occlusion. Contrast angiography of the abdominal aorta and renal arteries disclosed bilateral renal artery occlusion and the patient was reffered for surgical revascularization. The diagnosis of giant-cell arteritis is established by biopsy. Material and methods: Surgical specimen is a part of the wall of the right renal artery. The material was fixed in formalin, filtered and embedded in paraffin. Serial sections of the renal artery were stained:H&E, Masson trichrome and Elastic Van Gieson. Microscopic finding of the resected right renal artery consistent with giant cell arteritis: Numerous multinucleated giant cells mainly close to the internal elastic lamina surrounded by nonspecific lymphocyte infiltrates. The elastic lamina is partially fragmented or thickened. The lumen is partially occluded by intimal hyperplasia with superimposed organized thrombus. Disscusion: Fibromuscular dysplasia is the most common cause of RVH in children and GCA is an exceptionally rare cause of RVH. Primary or isolated involvement of renal arteries is more, often described in Takayashu arteritis. The onset of symptoms in GCA may be acute or insidious. Conclusion: Unrecognized RVH treated with ACEI probably provoked acute renal failure and contributed to recurrent heart failure and “flash” pulmonary edema in our teenage patient with atypical GCA. Prompt normalization of heart function and arterial hypertension occured after adequate therapy which is related with GCA.

PP3-297
BONE FORMATION IN CARDIAC VALVES

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Background: Dystrophic calcification is the most common pathological finding in operatively excised cardiac valves. Several reports describe bone in calcified cardiac valves (heterotopic ossification; bony metaplasia) and recent studies have identified bone proteins in such valves. Method: Histological and immunohistological examination of 1177 calcified cardiac valves operatively excised at the Department of Cardiac Surgery. Results: There were 128 patients (10.9 %) with bone and/or cartilage formation in the excised valve (119 aortic, 9 mitral). The incidence was higher in men (12.8 %) than in women (8.5 %). The average age of patients with metaplasia (64.1 years) did not differ from the average age of those without it (64.3 years). It was higher in females (66.9 years) than in males (62.6 years). Metaplastic changes were encountered in all types of calcific aortic valve disease (senile, bicuspid valve, postrheumatic). The marrow of the heterotopic mature lamellar bone was usually formed by adipose tissue. There was often chronic inflammation with an infiltrate composed predominantly of polyclonal plasma cells. Rarely, complete hematopoiesis was present. Conclusion: Heterotopic bone formation is closely related to the process of calcification, such that its presence has been described particularly in the aortic valve, in the calcified annulus of the mitral valve and, rarely, in arterial atherosclerotic plaques. In the past, calcific aortic disease was thought to be due to “degenerative”, time-dependent, wear-and-tear of the leaflets with passive dystrophic calcium deposition. Now, there is compelling data to suggest that calcific aortic disease is an active disease process similar to atherosclerosis. A crucial role in the process of calcific aortic stenosis is played by valvular interstitial (fibroblast-like) cells that, under various stimuli, become activated to myofibroblast-like cells. A subset of valvular myofibroblasts may differentiate into an osteoblastic phenotype capable of promoting formation of nodules of calcium and bone. Cartilage in calcified valves indicates that endochondral ossification, similar to that seen in bone fracture healing, is operative. Our results imply that bony metaplasia does not appear to be a late stage in the process of valvular calcification, but rather develops in parallel with it. Supported by the Research Project MZO (Grant 00179906)

PP3-298
HEPATOMA WITH CARDIAC METASTASIS: AN ADVANCED CANCER REQUIRING ADVANCED TREATMENT
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Background Hepatocellular carcinoma with cardiac metastasis is very rare and has a poor prognosis. Surgery is frequently reserved for symptomatic patients. This investigation first report the clinical and pathologic findings, and then demonstrates the pathophysiology. Methods This study included eight patients with hepatoma involving the heart, treated by surgical excision. Detailed clinical parameters were reported. Results The patient population comprised two (25%) women and six (75%) men. The mean age of subjects was 50 years old, with an age range of 40 to 70 years old. The presentations included: asymptomatic (75%), heart failure (25%), and pulmonary embolism (12.5%). All lesions involved the right atrium, and extended to the lung (12.5%), inferior vena cava (25%), and left atrium (12.5%). The level of tumor marker, alpha-fetal protein, was not correlated with the severity of metastasis or disease prognosis. Moreover, the availably estimated doubling time was less than 3 months. The pathological findings included variable hemorrhage and necrosis. The survival time following surgery also varied from one month to longer than 30 months. Discussion. Hepatoma metastasis to the heart was detected in all eight patients. This study demonstrates that surgery might help the outcome in such cases.

PP3-299
Z DISC PATHOLOGY IN CYPHER-NULL MICE: A 3D ELECTRON MICROSCOPIC STUDY
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Comparative studies of the relationship between structure and function offer a promising means of understanding the significance of differences in cytoarchitecture (Not sure what you mean, since you are not studying function?). The three-dimensional organization of the sarcomere in the striated muscle of Cypher-null mice, which exhibited dilated cardiomyopathy and skeletal muscle failure, was studied to assess the ultrastructure of the Z disc. Three-dimensional reconstruction of the Z disc was performed on 1 μm thick sections of the diaphragm and heart tissues using electron tomography in both embryonic and new-born Cypher-null mice. Three-dimensional information was extracted from thick sections using stereo pairs and tomographic reconstructions from single axis tilt series from ethanolic phosphotungstic acid (EPTA) staining sections. Oure results show that both the three-dimensional reconstructions and the selectively stained thick sections of the Z disc demonstrated a discontinuous appearance. This investigation used the Telescience Project merging technologies to clearly demonstrate severe fragmentation of the sarcomeric Z disc of embryonic heart and newborn diaphragm of Cypher-null mice, which had not been documented using previous 2D techniques. The analytical results of this investigation strongly confirm that Cypher plays an important role as a linker-strut in the Z disc of striated muscles.

PP3-300
MACROPHAGE-SUBENDOTHELIAL EXTRACELLULAR MATRIX CONTACT IN THE ATHEROGENESIS, ELASTIN CONTACT INDUCES TNF-α UPREGULATION
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[Background] One of the earliest events of atherogenesis is endothelial injury. When subendothelial tissues are exposed to the blood flow, first exposed is endothelial basement membrane or internal elastic lamina which are mainly composed of elastin, laminin, and collagen type-IV. These extracellular matrices (ECM) are reported to be recognized by elastin-laminin receptor (ELR). We hypothesize that macrophage (MΦ)-subendothelial ECM contact triggers atherosclerogenesis. We already reported that MΦs contacted to elastin release M-CSF. This time, we investigated TNF-α. [Methods] Primary cultured MΦs were prepared from mouse bone marrow cells. Insoluble elastin and collagen (type I) were suspended in αMEM, and applied to adherent MΦs. Total cellular RNA and protein were extracted, and northern blot analysis and western blot analysis for TNF-α were performed. To investigate whether this reaction is specific for elastin, we treated MΦs with galactose, which is said to uncouple the ELR molecular complex, and treated with both elastin and collagen. [Results and conclusion] MΦs contacted to elastin showed 11.8±1.7 S.D upregulation of TNF-α mRNA with its peak at 2 hours, and 1.34±0.05 S.D increase of TNF-α protein with its peak at 6 hours. Type-I collagen also induced TNF-α mRNA upregulation with its peak at 2 hours. The elastin induced upregulation was inhibited by galactose pretreatment while no inhibition was found in collagen-MΦ contact. MAP-K inhibition experiment revealed that MEK 1/2 and JNK/SAPK were involved in this reaction while p38 is not. These results strongly suggest that MΦ-subendothelial ECM contact is a key event of atherosclerogenesis.

PP3-301
MORFOLOGICAL STUDY OF CONGENITAL HEART MALFORMATIONS WITH ENDOCARDIAL FIBROELASTOSIS
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AIMS: Our study aimed to identify endocardial fibroelastosis (EFE), as a rare but important finding among hereditary heart endocardial malformations, which brings to persistence of fibrous thickening that is a cause of cardiac insufficiency and exitus letalis in early childhood. METHODS: We used autopsy material which has been collected during 19 autopsies, performed at the Institute of Pathology, Faculty of Medicine in Skopje. The patients were neonates and children, not older than 2 years. Histological sections of analyzed hearts were stained with HE, VanGieson-Elastica, Trichrome-Masson and Reticulin-Gomori. RESULTS: EFE was more frequent in males than in females (11/19 cases).Two of the patients showed presence of primary fibroelastosis as an isolated congenital heart malformation; in the rest 17 cases, secondary EFE combined with isolated or complex congenital malformations of the heart and large blood vessels, were found. The analysis of the anatomical location and place of the fibroelastosis in the ventricular system showed that it was more often situated in the left ventricle. More precise morphological analysis has revealed that the endocardium was thickened (to 2mm) along its wall diffusely and had porcelain white color. There was a hypertrophy of the heart, with bigger and wider LV; the apex belongs to the LV, lumen of the LV was wider, interventricular septum was pushed to the RV. The secondary EFE is usually associated with other heart malformations, the endocardium was confluent or focally thickened and accompanied with: SHLS (7 cases); DAP (3 cases); CoAo (2 cases) and pulmonary stenosis, SHDS, ASD, VSD and earlier closing of foramen ovale with one case of each. Histolopatological findings in endocardium showed proliferation of elastic tissue and collagen fibers which were parallel to the

surface. Inflammatory cells in both endocardium and myocardium were not found. The most frequent cause of death in the cases with EFE was insufficiency of LV (10/19) where severe pulmonary hypertension was found in one case. In the rest of the cases pulmonary hemorrhage 2/19 was present, bronchopneumonia in 2/19, aspiration in 1/19, enterocolitis in 1/19, brain hemorrhage in 1/19 and sepsis in 1/19 cases. CONCLUSIONS: Morphological analysis as well as histopathological findings has shown that most of the analyzed complications were due to mechanical obstruction of the blood flow. The most frequent was secondary EFE accompanied with other CHM, which was the cause of: "inlet" and outlet" obstructions; anoxia in utero; earlier closing of the FO.

PP3-302
INDUCTION OF MITOCHONDRIAL BIOGENESIS IS A MALADAPTIVE MECHANISM IN CARDIAC REMODELING

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Objectives. The purpose of this study was to clarify the molecular mechanisms linking mtDNA dysfunction to cardiac remodeling. Background. Defects of the mitochondrial genome cause a heterogeneous group of clinical disorders, including mitochondrial cardiomyopathies (MIC). The molecular events linking mtDNA defects to cardiac remodeling are unknown. Both energy derangements and increase of mitochondrial-derived reactive oxygen species (ROS) could play a role in the development of cardiac dysfunction in MIC. In addition, mitochondrial proliferation could interfere with sarcomere alignment and contraction. Methods. We performed a detailed morphologic and molecular analysis on failing hearts from three patients with MIC, failing human hearts due to ischemic heart disease (IHD) or dilated cardiomyopathies (DCM), and non-failing hearts. Results. MIC hearts showed marked mitochondrial proliferation, with myofibril displacement. Consistent with morphologic features, increase in mtDNA content per cell and induction of genes involved in mitochondrial biogenesis, fatty acid metabolism and glucose transport were observed. Down-regulation of these genes characterized DCM and IHD hearts. Furthermore, up-regulation of uncoupling proteins and a pronounced increase in mitochondrial-derived ROS was observed in MIC as compared to failing hearts due to other etiologies. The observed increase in free radical production was not paralleled by the induction of antioxidant enzyme activity, despite marked up-regulation of the corresponding genes. Conclusions. Our results suggest that mitochondrial biogenesis is a maladaptive response in MIC and, possibly, in other metabolic cardiomyopathies. In fact, increased oxidative stress, mechanical interference with sarcomere alignment and contraction, and, possibly, uncoupled respiration are detrimental factors to myocytes function, besides energy deficiency.

PP3-303
COULD SUDDEN CARDIAC DEATHS HAVE BEEN PREVENTED: AN AUTOPSY STUDY

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The objective of this study was to identify which, if any, sudden cardiac death (SCD)victims could have been identified before their death and considered for an implantable cardioverter defibrillator (ICD) on the basis of their previous cardiac history.Autopsy proven cases of SCD were identified over a one year period in a defined population. They were categorised by a modification of Davies method. Those with evidence of myocardial scarring, coronary narrowing or ventricular hypertrophy may have died as a result of a ventricular arrhythmia. However cases with clear evidence of acute coronary thrombosis or acute infarction were excluded. Hospital and GP records were examinaed for information on previous symptoms, investigations and past history of cardiac disease. Two cardiac electrophysiologists judged the appropriateness of each case against pre defined criteria. 366 of 975 post mortems were sudden cardiac deaths and in 232of these there was no evidence of acute thrombosis or infarction. 215 lived within the catchment area, 54% were male and the median age was 75 years. Agreement between experts was good (kappa score 0.64). One case was considered appropriate for ICD insertion. 41% of cases had no evidence of cardiac events or prior heart disease. 49% of the remainder had previous cardiac events or symptoms suggestive of cardiac arrhythmias but had not been referred for further investigations. In particular some patients with a previous myocardial infarction were not referred for 24 hour ECG tracings and some of those with suspected heart failure did not have echocardiography. Our study has shown that 40% of SCDs could not have been predicted A significant proportion of the remaining patients were not referred for appropriate investigations.

PP3-304
APOPTOSIS-RELATED FACTORS P53, BCL-2 IN ACUTE AND CHRONIC ISCHEMIC CARDIAC DISORDERS

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Background: Cardiac myocyte death during ischemic injury has been thought to occur exclusively by necrosis, but recently several studies have demonstrated that large numbers of myocytes undergo apoptosis in response to ischemic disorders. P53 limits cellular proliferation by inducing cell cycle arrest and apoptosis in response to cellular stresses such as DNA damage, hypoxia, and oncogene activation. P53 mediates apoptosis through a linear pathway involving bax activation, cytochrome c release from mitochondria, and caspase activation. The Bcl-2 family of proteins constitutes a critical checkpoint in cell death. These proteins contain agonists and antagonists of apoptosis, and alterations in their ratio determine the life or death of a cell. Proapoptotic proteins include Bax, Bak, Bad, and Bcl-xs whereas Bcl-2 and Bcl-xL are antiapoptotic. The aim of this study was to investigate the expression of apoptosis-related proteins p53, bcl-2 in acute and chronic ischemic cardiac disorders and their association with myocyte death in ischemic hearts. Method: We studied myocardial samples of hearts with histologic findings of acute myocardial infarction (group A, n=15), old myocardial

infraction (group B, n=15), and chronic ischemic heart disease (group C, n=15). Myocardial samples of normal heart were also included in this study (control group, n=15). An immunohistochemical method was performed with the use of p53 and bcl-2 antibodies, in order to investigate the expression of apoptosis-related proteins p53, bcl-2 in ischemic cardiac disorders. Results: The percentage of bcl-2 positive samples was 66% in acute myocardial infarction (Group A, 10/15 positive samples). In old myocardial infraction and chronic ischemic disease the bcl-2 positive samples demonstrated weak staining as in the control group. P53 positive samples was 20% in acute myocardial infraction (Group A, 3/15 positive samples), 80% in old myocardial infraction (Group B, 12/15 positive samples), 80% in chronic ischemic heart disease (group C, 12/15 positive samples) and 20% in control group (3/15 positive samples). Conclusions: The elevated expression of p53 is associated with the progressive loss of myocytes by apoptosis and may play a role in the evolution of the chronic heart failure in patients with old myocardial infraction and chronic ischemic disease. The increased expression of bcl-2 in acute myocardial infraction represents a possible compensatory mechanism of salvaged myocytes.

PP3-305
THE VASORELAXATION OF RAT RENAL ARTERY INDUCED BY WINE POLYPHENOL RESVERATROL

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BACKGROUND: Resveratrol, a stilbene polyphenol found in grapes and red wine, has recently been found to produce vasorelaxation in endothelium-dependent and endothelium-independent manner. The aim of this study is to determine the mechanism(s) of relaxation produced by resveratrol in the isolated rat renal artery (RA). METHOD: RA rings were precontracted with phenylephrine. In order to assess the endothelial integrity of the preparation we have used acetylcholine. Endothelium was removed mechanically by rubbing with a steel wire. Failure of arteries to relax to acetylcholine was considered as indicator of state of endothelial denudation. For detection of K+ channels in smooth muscle, peptide-specific antibodies in immunoperoxidase were used. RESULTS: Resveratrol (1–100 microM) produced concentration-dependent relaxation of RA rings with endothelium (EC50~10 microM, n=10) and without endothelium (EC50~15 microM, n=12). Methylene blue and L-NAME did not antagonize the resveratrol-induced relaxation of RA rings with endothelium. In order to analyze the contribution of different types of K+ channels in resveratrol-induced relaxation in the RA, various K+ channel blockers were used. The relaxation of RA was not blocked by glibenclamide, a selective ATP-sensitive K+ channel blocker, and tetraethylammonium, a non-selective blocker of calcium-dependent K+ channels. 4-aminopyridine, non-selective blockers of voltage-dependent K+ (Kv) channels, antagonized resveratrol-induced relaxation of RA in a noncompetitive manner. Margatoxin, highly selective blockers of Kv1.1-1.6 channels shifted the concentration response curves induced by resveratrol to the right without significant inhibition of maximal responses. Kv1.3 channels were detected in endothelium but not in the smooth muscle of RA using peptide-specific antibodies in immunoperoxidase. CONCLUSION: It is likely that endothelial Kv1.3 channels are involved in relaxation of RA produced by resveratrol. However, further experiments with smooth muscle peptide-specific antibodies need to be performed.

PP3-306
FATAL DRUG INDUCED HYPERSENSITIVITY MYOCARDITIS AFTER MINOCYCLINE-THERAPY

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Background: Acute Hypersensitivity Syndrome (HSS) is an allergic-type complication of drug therapy with associated symptoms such as rash and fever associated with blood-eosinophilia and multiorgan involvement. Allopurinol, anticonvulsants, sulphonamides and tetracyclines are amongst the most frequent causative agents. A genetic predisposition has been associated with HSS which shows a higher overall-prevalence in the African-American population, especially with minocycline. Visceral lesions such as interstitial pneumonitis, hepatitis, interstitial nephritis or myocarditis can be lethal. Case and clinical findings: A 34-year-old black African woman, without any prior medical history, was admitted to the emergency unit in October 2006 with fever, arthralgias and dyspnea. She had been treated by minocycline 2 weeks before admission for chlamydial genital infection. The physical examination showed tachycardia, erythematous papular exanthema on arms, erythematous angina, facial oedema and leucocytosis. No infectious source was identified. Drug induced HSS after minocycline was suspected and she received high dosed corticotherapy. After initial improvement of her dyspnea, she was discharged after 1 week, against medical advice, and stopped her medication. One month later, she was readmitted with severe hypotension, dyspnea and major left ventricular systolic dysfunction. Support by a central extracorporeal-membrane-oxygenation was provided during open-heart surgery and myocardial biopsy was performed. She died 6 days later after multiple organ failure. Microscopic findings: Myocardial biopsy showed mixed inflammatory infiltrates with interstitial and perivascular distribution, mainly consisting in lymphocytes, plasmocytes, histiocytes and an increased proportion of eosinophiles. This acute eosinophil-rich myocarditic pattern was associated with pronounced interstitial edema and recent necrosis of myocytic fibers explaining myocardial pump failure. Conclusion: Eosinophilic myocarditis (EM) may occur in the setting of a hypereosinophilic syndrome, parasitic infections, malignancy or vasculitis. EM has been sporadically described in HSS, but the majority of reports are post-mortem observations due to frequent fatal outcome. Minocycline is often prescribed as the first-line therapy in acne or genital infection. Although fatal side effects are rare, HSS should be taken into consideration by the pathologist when confronted to eosinophil-rich necrotizing myocarditis. In our case, endomyocardium biopsy has been helpful for the diagnosis which often remains unsuspected.

PP3-307
KAWASAKI DISEASE

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Introduction: Kawasaki disease (KD) is acute self-limited vasculitis effecting mainly children under 5 years. Etiology is still unknown. Currently debate is about infectious trigger leading to

self-directed immune response. Coronary arteries are predominantly involved; aneurysms may lead to sudden death, infarction and ischemic heart disease. Treatment in the acute phase is directed towards reducing inflammation of the artery wall and preventing thrombosis, at the late phase against myocardial ischemia and infarction. Case report: Three and a half year old boy was admitted to hospital, four days after he was taken ill, with fever and classical symptoms of KD. Viral tests were negative. Among usual therapeutics, he was treated with aspirin and repeated doses of iv-immunoglobulin, without success. Echocardiography showed diffuse dilatation of coronary arteries, which persisted till the death. After three and a half months of hospitalizations, after receiving infliximab, fever dropped and laboratory tests normalized for the first time, but he died 10 days later, four months after onset of the illness, with signs of myocardial infarction. At autopsy the heart was enlarged. Histology showed acute myocardial infarction of left ventricle with some older necrotic areas with granulation tissue. Main coronary arteries were diffusely dilated, diameter of RCA aneurysm was 8 mm, and of others between 3-4 mm. Due to progressive myointimal proliferation aneurysms had very reduced lumen diameter. In distal part of coronary arteries there were organized thrombi in recanalisation. Beside bronchopneumonia, hemorrhagic pulmonary infarction and cyanosis of internal organs, there were no other major abnormalities. Conclusion: Clinical picture of typical KD is characterized by fever and at least four of five principal features which are rash, conjuctival injection, cervical lymphadenitis, inflammation of the lips and oral cavity, swelling of the hands and feet. Especially in infants, some of the criteria are lacking. It is very important to diagnose these cases early, as they are at risk of developing coronary artery aneurisms and stenosis. Myocardial infarction is the principal cause of death and may occur in acute phase, but commonly year or even years later, especially in patients with giant aneurysms when measuring more than 8 mm in diameter. Surgical bypass procedures are effective when stenoses are proximal, what was not the case in our patient. There were reports in literature that a small group of KD patients can benefit from transplantation, with no report of recurrence.

PP3-308
CLINICAL AND PATHOLOGICAL FINDINGS IN FAST-PROGRESSING OBSTRUCTIVE HYPERTROPHIC CARDIOMYOPATHY (OHCM) IN CHILDREN AND ADULTS

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Aim: To compare clinical and pathological features in severe cases of obstructive hypertrophic cardiomyopathy in children and adults. Materials and methods. Nineteen children (of them, 17 survived and 2 died) and forty-seven adults (30 survived and 17 died) with OHCM were observed. OHCM diagnosis was verified by means of echocardiography, MRI, macro- and micromorphometry of affected tissues. Myocardial samples were taken during cardiac surgery or urgent autopsies. Microscopic preparations were stained with H&E, or van Giezon stain. The degree of disturbed tissue architecture, as chaotic rearrangement of cardiomyocytes, was estimated by a semiquantitative method using a 5-point morphometric score (M. Sutton et al., 1980). Results. Mid-ventricular OHCM location in pediatric group was as common as 63,2 % (12/19). In adult patients, this variant was diagnosed in 40.4% of cases (19/47). In general OHCM group, mid-ventricular OHCM was registered in 31 of 66 patients (46,7 %). Transmural myocardial scars have been revealed in 4 of 10 (40 %) autopsied HCM patients with mid-ventricular OHCM, and in 2 of 7 (28.5 %) deceased HCM patients with left ventricular outflow obstruction. In three adult patients with mid-ventricular OHCM, an apical aneurysm of top area was revealed, in absence of significant hemodynamic coronary stenosis, as evidenced by coronarograpy. In pediatric OHCM, a degree of cytoarchitectonic disorders in the right half of interventricular septum varied from 2.25 to 3.00 (2.87±0.05) units, thus being higher than at adults ($p < 0{,}001$). In adult patients, this parameter varied from 0.25 to 3.00 (1.92±0.10). Stromal portion of the right part of interventricular septum in the children varied from 1.08 to 6.67 % (3.62±0.30). In adults with OHCM, this parameter changed from 2.12 up to 10.60 % (5.36±0.34), thus being higher than in pediatric patients ($p < 0.005$). Conclusions. Increased grade of tissue «disarray» is a sufficient feature of OHCM in children. Meanwhile, enlarged area of myocardial stroma is a significant sign of OHCM progression in adults. In the patients with severe variants of the disease, occurrence of mid-ventricular OHCM is comparable to incidence of HCM with left ventricular outflow tract obstruction. Mid-ventricular OHCM progression is more often complicated by transmural myocardial scars. Higher frequency of apical aneurysm, developing at the top area, is detectable in adult patients with mid-ventricular OHCM.

PP3-309
RELATION OF MORPHOLOGICAL STRUCTURE OF HIBERNATING MYOCARDIUM TO CONTRACTILE RESERVE IN HUMANS

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Objective.The aim of this study was to investigate the morphologic characteristics of the hibernating human myocardium and to correlate its with dobutamine stress echocardiography (DSE). Methods and results.We evaluated 15 patients with coronary disease (58 +/- 12 years old, ejection fraction 38+/-14 %) with a corresponding wall motion abnormality on DSE (up to 10 micrograms kg (-1) min(-1) before coronary bypass surgery. During surgery, transmural myocardial biopsies from hypokinetic or akinetic area were perfomed (n=15). The samples of myocardium were analyzed by histopathology and immunohistochemistry to investigate the extent of interstitial fibrosis, intracellular and interstitial proteins. Among the 15 biopsied segments included in the study, 7 recovered function as assessed with DE (an echocardiography) one month after bypass surgery. Segments with DE viability showed less fibrosis and less vimentin expression, more glycogen, a higher ration of alpha-smooth muscle actin, actin and desmin then those without recovery. The degree of severity of the morphological changes (three stages) correlated well with the demonstration of inotropic reserve during DSE and with the extent of postoperative functional recovery (wall-motion score index, NYHA). Conclusion. Morphologic evidence of hibernating myocardium correlates with DSE findings wich has high diagnostic accuracy for the detection of myocardial viability.

PP3-310
THE ASSOCIATION FOR EUROPEAN CARDIOVASCULAR PATHOLOGY

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Although there are comparatively few pathologists who specialise in cardiovascular pathology all practicing pathologists are aware of the great importance of cardiovascular diseases. Ischaemic

heart disease, stroke and peripheral vascular disease are major causes of morbidity and mortality in all societies. Much of the improvement in life expectancy in the western world has been the direct result of improved prevention and treatment of these disorders. The Association for European Cardiovascular Pathology is an affiliated society of the European Society of Pathology. As a professional association it is committed to setting the standards of practice throughout Europe and providing post graduate education and training in cardiovascular pathology. We alternate our annual meetings between the European Society of Pathology and free standing meetings held in the late autumn. Our last meeting held in Aarhus, DK in November 2006 included three separate symposia, invited keynote presentations and clinicopathological presentations. In Istanbul we are organising a Surgical Pathology slide seminar on Cardiovascular Disease and a Symposium on Sudden Cardiac Death. We extend a warm invitation to all delegates to attend our evening meeting On Monday 10th September where a series of clinicopathological cases will be presented. The poster will describe the activities of our Association, the format and venue of future meetings and educational opportunities in Cardiovascular Pathology

PP3-311
REACTIONS OF MYOCARDIUM TO THE PRESENCE OF IMPLANTABLE ELECTRONIC MEDICAL DEVICES

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Background: In view of constantly increasing number of electronic medical devices (EMDs) implantations, the possible alterations of cardiac structures associated with the presence of EMD electrodes are studied. Although transvenous implantable leads are coated with inert materials to minimize the reaction of human tissues, the experiment on dog hearts showed inflammatory and degenerative changes developing in the atrial wall after implantation of pacemaker (Pacing Clin Electrophysiol. 2003 Mar;26(3):685-91). Methods: The hearts of 182 autopsy cases (130 males, 52 females) with EMDs (pacemakers and implantable cardioverter-defibrillators) were examined. The mean patients´ age at the time of EMD implatation was 76,8(±9,8). Different anatomic parameters of each heart were registered together with the number of fixations of leads to various cardiac structures. The tissue samples obtained from places of electrode insertions and each heart ventricles were processed using standard histological techniques. The tissue sections stained with hematoxylin-eosin were examined microscopically to identify inflammatory reaction, the presence of granulation tissue, fibrosis, hyaline scar, calcification and other changes in myocardium adjacent to the lead. Results: The mean weight of examined hearts was 544,48 g (±155,80), the mean left ventricle thickness was 16,22 mm (±4,19), the mean right ventricle thickness 4,86 mm (±1,94). 70% of all leads were attached to the cardiovascular structures by the band of fibrous tissue. 49,1% of all leads were fixed to the tricuspid valve or some of papillary muscles (potentially harmful fixation). The histological examination showed fibrosis and hyaline scar tissue in the electrode-myocardial interface. There was no correlation between the point of the fixation of lead and macroscopic parameters of right and left ventricles. The type of leads (pacing versus defibrillator), the type of stimulation (unipolar versus bipolar), and the percentage of stimulation did not influence the character of reaction in places of insertions. The mild reduction of the tricuspid valve circumference was observed in cases with long-time insertion of the lead. The occurrence of thrombi in heart cavities was minimal (2%)in association with electrodes. Conclusion: Implantation of EMD is followed by a reaction of the myocardium adjacent to the place of insertion. Potential harmful effect of EMDs was observed in cases with a long- time insertion of the lead. The research is supported by grant MH CZ 1A/8598-3.

PP3-312
CARDIAC MYXOMA WITH GLANDULAR COMPONENT: CASE REPORT

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Cardiac myxoma is the most common primary cardiac tumor. Glandular epithelial differentiation is a rare phenomenon. A striking observation, seen in approximately 1-5% of cardiac myxomas. Here, we present a case of a cardiac myxoma with glandular component removed from the left atrial wall of a 38-year-old woman. Cardiac myxomas are the most common primary cardiac tumor in adults. Myxoma constitute approximately 50% of primary tumors of the heart. The sporadic tumor occurs in middle-aged woman, usually in the left atrium. A glandular component in cardiac myxoma is uncommon. The histogenesis of this feature is enigmatic. The presence of the epithelial element as glandular structure is an example of one spectrum of the multidirectional differantiation. Alternative cardiac myxoma was described as entrapped foregut rest or intracardiac endodermal heterotopia in some literatures. The results suggest that cardiac myxomas is the occurence of a glandular component embedded within the myxomatous stroma. Malignancy of the glandular component in cardiac myxoma has been reported, but in many cases this needs to be set apart from tumor embolization and local recurrence.

PP3-313
SYPHILITIC AORTITIS WITH RUPTURED THORACIC AORTIC ANEURYSM, A CASE REPORT

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Background: Aortic aneurysm is an important cause of morbidity and mortality. The symptoms caused by it can mimic other diseases and hence it is usually under diagnosed. Therefore, ruptured thoracic aortic aneurysm is a not uncommon post-mortem finding. Syphilitic aortitis is a rare entity nowadays, but cases are still seen and its diagnosis needs a high index of suspicion. We report her post-mortem case of ruptured thoracic aortic aneurysm due to tertiary syphilis. Methods: We report post-mortem findings of a 71 years old man, who developed severe chest pain with vomiting and collapsed at home. man developed severe chest pain with vomiting and collapsed at home. Resuscitation was done with no benefit and the patient died after 30 minutes from reaching the hospital. No history of diabetes or systemic hypertension. Results: Post-mortem examination revealed a ruptured thoracic aortic aneurysm measuring 12x12cm involving the arch and descending parts of the aorta with hemothorax. The aortic valve showed mild dilatation and the same for the left ventricle. The heart weight was 475gm. No significant atherosclerosis was noted in any part of the aorta or in other major arteries. Microscopic examination of the aorta showed chronic inflammatory infiltrate, most sever in the adventitia and media with large number of plasma cells mostly around the vasa vasorum with endarteritis obliterans picture. Microscopy showed no significant atherosclerosis or cystic medial degeneration. The abdominal part of the aorta was

not dilated and showed no significant atherosclerotic changes. Post-mortem examination revealed no features of Marfans syndrome or connective tissue diseases. Conclusion: Syphilis continues to be a cause of significant morbidity and mortality. It is a great mimicker for many diseases in its different stages. Missing a diagnosis will deprive the patient from a chance of an effective and specific treatment with a high chance of cure. High index of suspicion is required for the diagnosis of aortic aneurysm as a cause of chest and abdominal pain; and tertiary syphilis should be considered as a possible cause for thoracic aortic aneurysms.

PP3-314
CAUSE, FREQUENCY AND TIME OF ONSET OF ASYMPTOMATIC ACUTE MYOCARDIAL INFARCTION IN ABRUPT UNEXPECTED DEATHS IN A FORENSIC AUTOPSY MATERIAL

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Background: Acute myocardial infarction is frequently found at autopsy in persons dying suddenly. The aims of this study was to determine the time between onset of an asymptomatic myocardial infarction to the time of abrupt deaths and to determine whether there are morphological signs of spasm of the epicardial coronary arteries at autopsy in persons dying abruptly and unexpectedly. Methods: In a forensic autopsy service cases of sudden deaths were selected consecutively, one group with a preliminary diagnosis of coronary heart disease, and a control group with cases of a non-coronary cause, mainly self-inflicted death. Complete autopsy was carried out with particular emphasis on the heart examination. Without knowledge of which group the case belonged, multiple histological sections from the coronary arteries and the myocardium were examined. The age of the lesions was estimated by observing several time-related phenomena: macroscopic evidence of myocardial infarct or, microscopic presence of C9-positivity, and increasing quantity of CD15 positive leucocytes in sections from the myocardium. The degree of spasm is quantified by picture analysis, measuring the degree of folding of the internal elastic lamina in cross sections of elastin stained proximal and distal part of the coronary arteries. Results: Acute coronary lesions (rupture of necrotic plaque, thrombi, etc.) were found in 13 coronary cases, and myocardial infarcts, with onset within 5-6 hours prior to death in 13 cases, no infarct in 2 cases. In the non-coronary group, acute coronary changes were found in 4 cases, small recent infarcts in 7. The degree of folding was significantly greater in the distal section of right coronary artery in cases of the coronary group compared to the folding in the same section of the non-coronary cases. There were no significant differences in the other arteries. Conclusion: Sudden, unexpected deaths in persons with coronary disease occur within the first several hours after the onset of an asymptomatic myocardial infarction. Small, acute infarcts were found incidentally also a minority of the non-coronary group. Also,our findings indicate that a spasmic contracture of an artery may be diagnosed post mortem. In several of the coronary cases spasm of the distal part of the right coronary artery may have caused focal ischemia in the central parts of the cardiac conducting system precipitating a lethal arrhythmia.

PP3-315
AN EXPERIMENTAL MODEL OF MYOCARDIAL INFARCTION IN PIGS: REPERFUSION DAMAGE, DYNAMICS OF HEALING PROCESSES

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Background: The goal of experiments was to create an animal model of myocardial infarction and to evaluate ischemic/reperfusion damage and dynamics of healing processes. The prospective use of the model is to evaluate fate, quantity and the best way of application of stem cells in the management of myocardial infarction. Material and Methods: Four experimental animals, female pigs, with weight from 30 to 40 kg were used. An experimental myocardial infarction was performed via occlusion of r. interventricularis anterior for 40 minutes. The hearts were examined 1 hour, 3 days, 5 days and 7 days after the procedure. Results: Macroscopically, red infarction, characteristic for ischemia with reperfusion was found. Microscopically, healing process with granulation tissue production/collagen deposition was remarkably accelerated in coparison with data in the literature. Also, besides of deposition of intracellular calcium, large deposits of extracellular calcium were found. Conclusion: dynamics of healing processes in myocardial infarctions in young female pigs is remarcably accelerated compared to data in the literature (probably because of examining tissues from significantly older human patients described in the literature). Also, not well described is a fenomena of large deposition of extracellular calcium, which may possibly interfere with healing processes.

PP3-316
PAPILLARY FIBROELASTOMA WHICH OCCURED IN THE POSTERIOR LEAFLET OF THE MITRAL VALVE: REPORT OF A CASE

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Papillary fibroelastoma (PFE) is a rare benign tumor arising from the cardiac endotelium.They are usually located on valves, mostly the aortic valve.These tumors can be responsible for strokes, transient ischemic attacs, anjina pectoris, infarction and sudden death.Echocardiographic diagnosis, followed by surgical excision, may prevent these complications.Here, we report a rare case of papillary fibroelastoma because of its uncommon apperance in the posterior leaflet of the mitral valve. CASE: A 33-year-old male was hospitalized with a diagnosis serebrovasculer accident.Echocardiography revelaled a mass attached to the posterior mitral leaflet.The mass was removed by surgery.It was 0.7x0,6x0,5cm. diameter graywhite color and contain small papillary projections. On the microscopic evaluation,there was a lining of endocardial cells covering a core of hyalinized hypocelluler stroma. We identified PFE and supported immunhistochemically. The endotelial cells on papillary projection are reactive for CD34 and the stromal cells are weakly reaktive for S100. DISCUSSION: Papillary fibroelastomas are primary cardiac tumors of valvuler tissue. They are usually seen in elderly patients. But PFE of the mitral valve in 3-year-old child has also been reported. Transthorasic echocardiography may lead to suspicion of a PFE, but transoesophageal echocardiograhy is required for confirmation. It is identified histologically. This tumor may occur as isolated lesion or concomitant valve stenosis or other cardiac abnormalities. It is an important source of emboli. The lesions consist of a slender or broad fibrocollagenous stalk from which numerous papillary villous projections. Promt surgical excision is indicated in most cases. Anticoagulation is only recommended in situations of high surgical risk and during the wait for surgery.

PP3-317
MORPHOLOGICAL CHANGES OF CEREBRAL VASCULAR STRUCTURES IN HYPERTENSIVE PATIENS DEAD WITH INTRACEREBRAL HAEMORRHAGE

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Background The purpose of this study was to assess the spectrum of cerebral vascular wall (VW) changes in patients with clinical suspicion of primary intraparenchymal hematoma, confirmed after autopsy. Method 82 cases, clinically diagnosed with hypertension (HT), died with stroke and confirmed with intracerebral haemorrhage at autopsy were selected. The studied material consisted of nervous tissue situated near and distant from the haemorrhagic focus (HF). The specimens were processed following the classical histological technique and stained with usual stainings (H-E, van Gieson and Goldner trichromes) and immunohistochemical stains for basement membranes (BM) (Collagen-IV) and endothelial cells (CD34). Results Extraparenchimal large arteries showed only classical atheroslerotic lesions (AthL) which were found in most of the patients. The severity and extention of AthL presented wide individual and interindividual variations and were not correlated with the patients' age. The atrophy of the middle layer resulted in significant loosening and subsequent aneurismal spindle-shaped dilatations in certain extracerebral arterial segments. In some cases, thrombi in the lumen of large arteries were found, which partially occluded them. Intraparenchymal arteries and arterioles showed the entire range of VW degeneration steps caused by HT: thickening and folding of the inner elastica, hypertrophy followed by degeneration of the middle smooth muscle layer (SML), substitutive progressive and extensive fibrosis, starting from the outer adventicial layer and hyaline degeneration (HyD) of the collagen fibres which replaced the middle and outer VW layers. All observed changes had a focal irregular distribution, not related with the proximity of HF. Capillary walls showed focal or circumferential thickening of BM caused by the encreasing of collagen-IV material, followed by HyD of collagen fibres. The CD34 immunostaining showed that endothelial cells kept their structural integrity. Spindle-shaped dilatations and rupture of impaired VW in areas with hyalinization were also observed. Conclusions The VW changes are different depending on vessel histological structure and function. The sequence of cerebral VW degenerative lesions results in HyD of excessive fibrillar material from arteriolar wall or from BM. Weakening of wall resistance by hyalin material and minimal resistance of the surrounding parenchyma, explains vascular rupture due to HT, with subsequent HF only in cerebral parenchyma. The more adequate term for describing the VW changes seems to be sclerosis with HyD.

PP3-318
ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY AS A CAUSE OF SUDDEN DEATH IN 12 YEARS OLD GIRL

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BACKGROUND: Arrythmogenic Right Ventricular Cardiomyopathy (ARVC) has recently been recognized as a cause of ventricular tachycardia and sudden death in young people. According to the WHO, ARVC is distinct from the more familiar forms of cardiomyopathy (dilated, hypertrophic and restrictive) and is characterized by three features: progressive fibrofatty replacement of right ventricular myocardium, strong familial transmission and presentation with symptomatic arrhythmias or sudden death. Although a gene defect was localized on chromosome 14, the pathogenesis remains obscure. The disease almost always occurs after puberty. METHODS: Autopsy and routine histology. CASE: Twelve years old girl was operated for ductus thyreoglossus persistens. Two hours after operation she started bleeding from operational wound. Despite of chemostasis and reanimation procedures , she died three hours later. Autopsy investigation revealed : Thymic follicular hyperplasia (95gr.) and adrenals atrophy ; Degenerative changes with apoptotic body between cardiomyocytes and its replacement by fibrous scars; Subendocardial and interstitial fibrosis and edema in right atrium wall and large amounts of fat in right subepicardium and around coronary vessels was seen as well. The right ventricular wall was severely thinned with extensive fatty infiltration and loss of myocytes. Subepicardial fatty tissue and myocardial fatty tissue replacement was most severe in apical parts of right ventricle. Thymic follicular hyperplasia was accompanied with necrotic Hassal's body and many eosinophyles and neutrophyles in medulary sone. Edema and inflammatory cells infiltrates composed from eosinophils, plasma cells, lymphocytes, neutrophils and mast cells was in subepithelial connective tissues of pharynx and epiglottis CONCLUSION: Cause of death was shock which first clinical symptom was bleeding in site of operation. Bleeding is consequence of acute congestive heart failure due to arrithmogenic right ventricular cardiomyopathy. In this case ARVC is accompanied with thymic hyperplasia, adrenal glands atrophy and ductus thyreoglossus persistens, which are probably involved in pathogenesis of ARVC.

PP3-319
EXPRESSION OF TELOMERASE AND Ki-67 IN MYOCYTES AFTER MYOCARDIAL INFARCTION

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Background: The presence of myocyte proliferation in the adult heart remains controversial. Telomerase replaces telomeric repeat DNA lost during the cell cycle, restoring telomere length. This enzyme is present only during cell replication and its activity reflects the extent of proliferation. Ki-67 is a nuclear antigen, strictly correlates with cell proliferation and is expressed in all phases of the cell cycle except G0. The aim of this study was to investigate the presence of myocyte replication by analyzing the expression of telomerase and Ki-67 in myocytes after myocardial infarction. Method: We studied myocardial samples of twenty hearts with histologic findings of myocardial infarction. Myocardial samples of ten normal hearts were used as controls. An immunohistochemical method was performed with the use of telomerase and Ki-67 antibodies, in order to investigate the expression of these markers in infarcted hearts. Results: The percentage of Ki-67 and telomerase positive expression samples was 85% (17/20 positive samples) and 70% (14/20 positive samples) in myocardium infarction, respectively. Moreover, we report that 70.5% of Ki-67 positive samples expressed telomerase. Positive expression of telomerase and Ki-67 appeared rarely in control group. Conclusions: The increased expression of Ki-67 and telomerase in infarcted myocardium, suggest the presence of myocyte proliferation and this may be a compensatory mechanism that could be replace damaged myocardium.

PP3-320
IMUNOHISTOCHEMICAL STUDY OF THE ADIPOSE TISSUE IN A FATAL CASE OF ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA
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Arrhythmogenic right ventricular dysplasia (ARVD) is a heritable disorder characterized by progressive degeneration and fibro-fatty replacement of right ventricular myocardium, causing ventricular tachyarrhythmias and resulting in sudden death at a young age. We report a young man without prior complaints dying suddenly during minor physical effort. The gross autopsy findings in the heart were minimal. The histologic features in both right and left ventricles were typical of ARVD, and consisted of fatty infiltrates with typical cardiomyocyte degeneration of the right ventricle and subepicardial regions of the left ventricle. Several lines of evidence suggest that impairment of cell-to-cell adhesion and involvement of desmosomal proteins may be the underlying pathogenic mechanism in ARVD, via accelerating apoptosis of myocardial cells. As fat replacement is most essential for the diagnosis of ARVD, the aim of the present study is to analyze immunohistochemically ARVD-related adipocytes compared to these from the subcutaneous fat. It is becoming increasingly evident that adipose tissue is a multifunctional organ that produces and secretes multiple factors that can act in both paracrine and endocrine fashion. So it is our opinion that adipose tissue in ARVD is not an innocent bystander and adipocyte-secreted cytokines probably play a substantial part in ARVD pathogenesis.

PP3-321
ONE STEP CLOSER TO DISCLOSING THE MORPHOLOGICAL SUBSTRAT OF SYNDROM X
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Background: The name and existence of several definitions of the syndrome X point to vagueness of this condition. In present research we adhere to opinion, that the X-syndrome is the phenomena of angina pectoris and myocardial ischemia documented by electrocardiography without evidence of stenosis or a spasm of heart coronary arteries. Some researchers explain such pains through spasm of fine arteries of heart. There are no methods to study lifetime morphology of syndrome X at the same patient. We started with the assumption, that among died from first heart attack the number of persons suffered from X-syndrome at lifetime should be much more, than among healthy people. The aim was to clarify whether quantitative spatial peculiarities of intramyocardial arterial bed exist at died from the first heart attack? Method: Present research is carried out on 80 hearts received from practically healthy persons died of the violent reasons and on 60 hearts of patients died from acute myocardial infarction. Methods of X-ray micro-angiography of hearts; planimetry of X-ray films of myocardial strips; specimens cutting in three planes; scanning of histological slides under microscope; stereological calculation of volume density of arterial bed and statistics were used. Results: Both X-ray films data and data from histological slides show statistically reliable differences of the arterializations in different parts of a left ventricle wall. It has been revealed almost ninefold difference in mean value of parameters of volume density of arteries calculated for injected hearts in comparison with the data of non-injected hearts. The wide variation of value of a parameter both in healthy and in hearts with a myocardial infarction is found out. Thus the degree of arterializations in heart muscle with infarction was significantly much lower than arterializations of healthy hearts. The difference was obvious both between the data of X-ray films and data of stereological analysis of injected and non-injected hearts. Conclusion: The types of myocardial angioarchitectonics possessing low volume density are less favorable for heart in respect to blood supply of a myocardium and these types most frequently meet at hearts attacked by acute myocardial infarction. It is possible to assume, that the syndrome X meets more often at persons, who have less favorable angioarchitectonics, which brings to faster ischemia even by week spasms of coronary or small intramuscular arteries.

PP3-322
PRIMARY MALIGNANT PERICARDIAL MESOTHELIOMA: A CASE REPORT
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BACKGROUND: Primary malignant pericardial mesothelioma (PMPM) is extremely rare and overall poor prognosis. On reviewing the literature it appears that approximately 72% of all PMPM were actually diagnosed on autopsy. This obviously indicates a silent nature of this highly malignant and lethal tumor. The case of primary pericardial mesothelioma presenting as constrictive pericarditis is reported here, because of rarity. CASE: A 42-year-old male presented with two months history of progressively worsening breathlessness and central chest discomfort on effort. The diagnosis of constrictive pericarditis was made based on CT evidence of pericardial thickening and hemodynamic evidence of constrictive physiology. The history did not reveal any pertinent information such as exposure asbetos fibers. At surgery, the pericardium was thick, white, homogeneous, and very strongly adherent to the myocardium. As a result, resection of the pericardium was incomplete. Postoperatively the patient's condition deteriorated rather rapidly with low cardiac output state and he died soon after (one month) surgery. No post-mortem was possible. Several hard pericardial samples with irregular shapes were analyzed, the largest measuring 5x5x2cm. Microscopic examination revealed cancer infiltration of the pericardium with a multiple connected nodule morphology. These nodules consisted of large eosinophil cytoplasm, a vesicular nucleus and prominent nucleolus. The cells formed cord-like or tubular structures, confirming the mesothelial origin of the malignancy. Immunohistochemistry was positivity for calretinin, EMA and P53, while staining for desmin was negative. The final diagnosis was "malignant pericardial mesothelioma, epithelial type". CONCLUSION: Pericardial mesothelioma is extremely rare, although it is the most common primary pericardial tumor. Its incidence was <0.0022% among 500,000 cases in a large necropsy study. Approximately 200 cases have been reported so far and only 25% of these were antemortem diagnoses. PMPM is an aggressive disease and in our patient this was responsible for his death within one month of diagnosis. Herein, we report this rare case of PMPM and present a brief review of the relevant literature.

PP3-323
PEDIATRIC CARDIAC SARCOMAS, A SERIES OF 16 NEW CASES WITH LITERATURE REVIEW
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Background: Malignant primary cardiac tumors are extremely rare in infants and children, and there are no series published to date. We present a pathological series of pediatric cardiac sarcomas with clinical follow-up. Methods: Pathologic and clinical information from 23 primary heart tumors in patients ≤ 16 years at presentation were retrospectively reviewed. Chart record and histologic material were available in all cases. Follow-up information was obtained from contacting health care provider or patient family in 10 patients. A literature review for primary heart sarcomas in children published since 1980 was performed. Results: Of the initial 23 tumors in the series, 7 were reclassified as inflammatory myofibroblastic tumor (IMT) or benign fibrous histiocytoma, based on pathologic features and benign clinical follow-up. Sixteen cases remained as primary heart sarcomas. There were 14 males and 2 females, mean age of 10.4 years (range 0-16). Clinical presentation was variable and included recurrent pericardial effusion (1), respiratory distress (2), pulmonary emboli (1), shortness of breath (5), arrhythmia (2), chest pain (4), congestive heart failure (1) and constrictive pericarditis. Six patients presented with tumor in the left atrium, 2 in the left ventricle, 4 in the right atrium, 2 in the right ventricle, 2 in the pericardium and 1 in the mitral valve. Histologically, there were 5 leiomyosarcomas, 3 undifferentiated round cell sarcomas, 2 pleomorphic undifferentiated sarcomas, 3 angiosarcomas, 2 rhabdomyosarcomas, 1 osteosarcoma, and 1 fibrosarcoma. The youngest patient with angiosarcoma was 14 years, the youngest patient with pleomorphic sarcoma 12 years, and the ages at presentation of leio-, rhabdo- and undifferentiated round cell sarcomas ranged from 1 – 16 years. Three patients presented with metastatic disease, all with lung metastases. In cases with follow-up, mean survival was 8.7 months; all patients were dead of disease, with one alive with recurrence. Of the review of 12 published reports, 4 were considered likely IMT, based on histologic description and benign follow-up. The remaining 8 were angiosarcomas (2), rhabdomyosarcomas (3) liposarcoma (1), fibrosarcoma (1) and sarcoma, not otherwise specified (1). Prognosis was uniformly fatal, with survival up to 13 months. Conclusions: Primary pediatric sarcomas of the heart have a dismal prognosis, are of varied histologic types, have a male predominance, occur most frequently in the atria, and should be clearly separated from cardiac IMT.

PP3-324
INFLAMMATORY PSEUDOTUMOR OF THE HEART: A CASE REPORT

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Introduction: Inflammatory pseudotumor is a rare entity which most commonly involves the lung and the orbit. It rarely occurs in the heart: only 14 cases are reported in the literature mainly in children and adolescents. In this report, we review the relevant literature and discuss the pathogenesis, clinical manifestation, the diagnosis and the treatment of cardiac inflammatory pseudotumor. Case report: A 31 year old woman presented since seven days a fever which was followed by chest pain. The clinical examination was normal. Viral serologies and the assessment immunological were normal. Radiological explorations (ultrasonography, thoracoabdominal tomography and magnetic resonance imaging) revealed the presence of a cardiac mass which measured 24x13mm suggesting a thrombus. Anti coagulant treatement was administrated, but no improvement was observed. Consequently, the patient was operated. Histological examination of the mass showed spindle shaped cells with eosinophilic cytoplasm and elongated bland nuclei; these cells were accompanied by a variable number of inflammatory cells including lymphocytes, macrophages, plasma cells and eosinophils. En immunohistochemical stain, the spindle cells expressed actin and vimentin, but were negative for keratin, S-100 protein, CD34 and CD1a. Basing of this finding, the diagnosis of inflammatory pseudotumour of the heart was made. Conclusion: Inflammatory pseudotumour of the heart is rare, occurs mainly in children. Clinical presentation is variable. Tumor location and its characteristics can be observed by radiological findings but histological examination is necessary to make diagnosis. Complete surgical resection is the treatment of choice.

PP3-325
UNUSUAL CARDIAC TUMORS OTHER THAN MYXOMA. PRESENTATION OF 2 CASES

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Background: The presentation of heart tumors is very rare. About 75% of primary tumors are benign, and 75% of these are atrial myxomas. Malignancy is found in fewer than half of primary tumors of the heart. The majority (95%) are sarcomas, primarily angiosarcomas and undifferentiated sarcomas. Primary cardiac tumors are high-grade sarcomas with a high metastatic potency that often becomes evident early after surgery.The presence of metastatic tumor to the heart usually indicates widespread metastases. Lung carcinomas are the most commonly encountered tumor followed by breast and pancreas cancer and melanoma. We present 2 cases with rare heart tumors Case 1: 24 year old woman presented with cardiac arrhythmia and was found to have a mass on mitral valve and left atrium. Histopathology of the resected specimen was a high grade osteosarcoma composed of fibroblastic, chondroblastic and osteoblastic components. An immunohistochemical study was performed by using S-100 antigen to differentiate the osteosarcoma from malignant peripheral nerve sheath tumor. There was positive staining in histiocytes but negative staining for tumor cells. So the histological and immunohistochemical findings of the permanent sections of the mass were consistent with primary cardiac osteosarcoma. Case2: 44 year old man presented with chest pain. His radiological findings revealed a mass in right atrium. Histopathology of the incisional biopsy was an undifferentiated malignant tumor with partial epitheloid pattern, showed extensive necrosis and invasion through pericardium and myocardium. Metastatic undifferentiated carcinoma was taken into differential diagnosis, but keratin was negative and no suspicious primary focus was found. Immunohistochemical results didn't support the relatively common primary malignant cardiac tumors such as rhabdomyosarcoma and angiosarcoma. Malignant paragangiioma was excluded due to chromogranin negativity and malignant melanoma was excluded due to HMB 45 and Mart-1 negativity. Conclusion: Sarcomas as unusual primary cardiac tumors should be taken in differential diagnosis when cardiac myxoma and metastatic carcinomas are excluded.

PP3-326
THE RELEVANCE OF CO-MORBIDITIES FOR IN HOSPITAL LETHALITY OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION: CLINICO-MORPHOLOGICAL STUDY

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Aim: To assess the impact of co-morbidities on myocardial infarction outcomes during acute phase of the disease. Methods: The survey vas based on retrospective analyze of necropsy

protocols for a 10-years period. 1269 cases of in-hospital death of patients (mean age 67,9±0,55 years) with myocardial infarction were studied. Statistical analysis included clinical features of myocardial infarction and its complications; co-morbidity; results of morphologic study. The aim of statistical analysis was to determine the factors which might have crucial role for the evolution of myocardial infarction and which could be used for predicting some fatal complications: acute ventricular aneurysm, cardiac rupture, pulmonary oedema, acute cerebrovascular accident, etc. Results: The group included 698 (55%) men and 571 (45%) women. Mean hospitalization period was 10,9±0,88 days. The frequency of co-morbidities were: arterial hypertension (27,1%); diabetes mellitus (21,2%), pulmonary diseases (11,5%), kidney pathology (11,5%), hepatitis/cirrhosis (3,7%), gastro-intestinal pathology (4,2%), obesity (7,6%). Causes of death were: pulmonary oedema – 40,3%, acute heart failure – 29,7, cardiogenic shock – 19%, sudden death- 14%, left ventricular parietal wall rupture – 12,2%. Other important complications: rhythm disturbances (chronic atrial fibrillation – 12,7%, high gradation extrasystoles- 4,6 %), stable conduction abnormalities (atrio-ventricular block – 2,36%, complete left bundle block – 2,36%), development of left ventricular endocavitary thrombosis (6,7%), periinfarctic pericarditis (1,73%). Among clinical parameters, responsible for the development of myocardial infarction complications and death, discriminant analysis revealed myocardial infarction characteristics: depth (transmural – 33,9%), location (46,3% - anterior, 30% - posterior), width (anterior extended – 27,5%, circular – 23,5%, posterior extended – 19%), evolution (repeated – 39,5%, recidivant – 13,5%), presence of left ventricular aneurysm (total – 12%, acute – 4,4%, chronic – 8,2%). Co-morbidities were not found as specific determinants of lethal outcomes in acute phase of myocardial infarction.

PP3-327
DYNAMICS OF ADAPTABLE RESERVES AT THE PATIENTS SUFFERING FROM ISCHEMIC HEART DISEASE AGAINST THE BACKGROUND OF TREATMENT WITH CITOPROTECTOR BIOR

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Both pharmacological means and the program for improvement of quality of a life and increase of tolerance to physical load of the patient with IHD should be used reasonably, render positive influence on the basic links of pathogenesis of the illnesses and increase nonspecific resistance of an organism. The purpose of research - to study the dynamics of adaptable reserves of the cardiovascular system at the patients with the ischemic heart disease during treatment. Material and methods of research. 135 patients with ischemic heart disease from which 21 (15,5%) had stenocardia I CF, 98 (72,6%) – a stenocardia II CF and 16 (11,9%) – a stenocardia III CF were under supervision. The most frequent risk factors were: an arterial hypertension -78 the patients (57,8%), hypercholesterolemia - 82 (60,7%). All patients received traditional medicamentous treatment, 82 patients (the basic group) in addition received cytoprotector BioR by 1, 0 ml intramuscularly daily during 20 days. Results of research. The analysis of dynamics of the clinical symptoms and syndromes against the background of the carried out therapy has shown, that in patients of the basic group pains in heart, a short breath, instability of arterial pressure decreased for 6-7 days earlier, than in group of comparison. It is established, that in patients with initial depression of processes of tissue respiration the increase of the constant of the speed oxygen absorption (from 0,032±0,003 up to 0,040±0,002 sec, $p < 0,001$), alongside with the reduction of the exhaustion time of oxygen stocks (from 155,2±10,8 up to 131,7±11,2 sec, $p < 0,001$), and after treatment time of exhaustion of half of oxygen stocks (from 39,6±1,2 up to 32,7±1,6 sec, $p < 0,001$) has been revealed. That demonstrates the improvement of processes of recycling of oxygen by the tissue. In patients of the control group positive changes have also been revealed, but they are less expressed and are statistically doubtful. The improvement and increase of compensative-adaptive possibilities of the organism during application BioR are also testified by the positive changes of the hemodynamics parameters in the patients. So, the heart index has increased with 1,41 ± 0,03 up to 1.56±0,04 l/mines/m², ($p < 0,001$), while in group of the control - with 1,43 ± 0,05 up to 1.48±0,06 l/mines/m², ($p > 0,01$); the Volume of performed work in the patients of the basic group has increased by 32,7%, in group of the control – by 6,7%; tolerance to loading has increased by 19,8% ($p < 0,001$) in the patients of the basic group, and only by 5,85% ($p > 0,01$) in control group. The involvement of cytoprotector BioR in the complex therapy of patients with IHD promotes the increase of the compensative-adaptive reactions of an organism and clinical efficiency of the treatment.

PP3-328
SUDDEN DEATH FROM TUBERCULOUS MYOCARDITIS

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Tuberculosis is an important public health problem considering to spare four organs: heart, skeletal muscle, thyroid and pancreas. We present a case of tuberculous myocarditis diagnosed on a post-mortem cardiac biopsy to a girl patient of fifteen years old, presented with unknown history of tuberculosis, but having a juvenile rheumatoid arthritis for who received corticosteroids for a long period of time. Clinical evaluation revealed rhythm disturbances on electrocardiogram, right cardiac failure and right upper lobe consolidation on chest X-ray. Microscopical examination of necroptic biopsies evidentiated specific lung giant-epithelioid granulomas with caseous necrosis and extensive infiltrative tuberculous lesions on the right lung associated with myocardial epithelioid granulomas without evident caseous necrosis. Tuberculous etiology was confirmed by applying a specific stain for Mycobacterium tuberculosis, obtaining a positive Ziehl-Neelsen result. We hypothesize that the mechanism of death was severe ventricular arrhythmia due to granulomatous proliferation in the structures of the interventricular septum. Because the involvement of myocardium in tuberculosis is rare, the increasing recognition of the entity and the use of endomyocardial biopsy may help us for detecting of more cases of this "curable" form of cardiopathy.

PP3-329
CARDIOVASCULAR CAUSES OF SUDDEN UNEXPECTED DEATH; SIX YEARS PROCESS IN COUNCIL OF FORENSIC MEDICINE

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Background: Cardiovascular deaths constitute the major cause of sudden and unexpected deaths. Aim: To evaluate the pathological changes of 1424 cases reported as cardiovascular deaths,

autopsied in Council of Forensic Medicine in Istanbul, Turkey Methods: Autopsy reports of sudden unexpected deaths of cardiovascular origin between the years of 1999-2001 were retrospectively analyzed. In the sudden unexpected cardiovascular originated deaths between 2002 and 2004 whole hearts were sampled and examined systematically after being fixed. Findings of these two periods were compared in order to the role of cardiac sampling and systematic evaluation in the diagnosis of sudden unexpected deaths. Results: 1424 autopsy cases were analyzed. 1240 (87,1%) of these cases were male and their age range was 56± 15, 184 (12,9%) were female and the age range was 49± 20. 657 (46%) of these cases were died suddenly, 619 (43,5%) were found death and 98(6,9%) were died at the hospital. We found chronic ischemic changes in 724 (50,8%), acute infarction in 177 (12,4%), valvular disease in 44 (3,1%), myocarditis in 21 (1,5%), cardiomyopathies in 7 (0,5%) and left ventricular hypertrophy in 326 (22,9%) cases and the rupture of an aortic aneurysm was the cause of death in 32 (2,2%) cases and 79 (5,5%) cases were morphologically negative. When compared to the group of only myocardial sections are carried out at autopsy, the incidence of cardiomyopathies (7,8%), congenital heart diseases (3,1%) and acute infarction (14,1%) was detected to be high in the group where heart were whole sampled. Discussion: Pathological changes detected during the autopsy in a systematic manner will not only support the clarification of the cause of death, but also provide in depth knowledge about sudden and unexpected deaths.

PP3-330
DETECTION OF APOPTOSIS IN ENDOMYOCARDIAL BIOPSY SPECIMENS FROM PATIENTS WITH DILATED CARDIOMYOPATHY

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Background: Apotposis is a regulated energy-consuming process and physiologically found in embryogenesis and the maturation of multiple cell systems, and pathologically in various diseases. Apoptotic cardiomyocyte death is encountered in various heart diseases such as DCM, myocarditis, coronary artery disease, reperfusion injury, as well as right ventricular dysplasia, and hypertensive heart disease. The most widely used method to identify apoptosis in human histological material is the TUNEL assay. Design: The aim of the present multicentric study was to asses the apoptotic index by TUNEL method in endomyocardial biopsies from patients with DCM. Apoptosis was detected by using the TUNEL method in 33 patients. The values of apoptotic index were expressed as means with standard deviations. Biopsies were performed at the Instutute of Cardiovascular Diseases Dedinje, Belgrade. Results: The mean age of patients with dilated cardiomyopathy (DCM) was 29 males and 4 females. The mean age of patients was 44 (31-59 years). All cases of DCM were diagnosed both clinically and pathologically DCM was detected in 26 cases (79%). In this group of patients, the most common diagnosis was advanced DCM (9 cases or 35 %), followed by mild DCM (7 cases or 27 %), early stage of DCM (3 cases or 11%); recovery phase (6 cases or 23%) and hereditary CM (1 case; 4%). In 3 cases (9 %) Idiopatic dilated cardiomyopathy was found and the most common diagnosis was early DCM (1 cases;33 %), mild DCM (1 case; 33%) and focal myocarditis (1 case; 33%).DCM and insuffitientio valvulae mitralis were found in 4 cases (12 %) and in this cases pathohistological diagnosis were: early DCM (2 cases; 50 %); advanced DCM (2 cases; 50 %). TUNEL-positive myocytes were found in 26 of 33 cases with DCM (78,8%). The apoptotic index ranged 1,4 - 10,4%. The mean apoptotic index for the whole analyzed group of DCM was 3,3 ± 2,485%. Conclusion: The present study focused on the morphological analysis of apoptosis in DCM in endomyocardial biopsies specimens. We have found relatively high percentage of apoptosis in our series, in 78.8% of cases, as well as relatively high apoptotic index. These results could be explained by manifested cardiac failure of our patients, and represent midle values in other reported series.

PP4 - Displayed on Wednesday, September 12

Gynecopathology

PP4-1
DIFFERENTIAL DIAGNOSIS PROBLEMS IN CHORIOCARCINOMA

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Background: Choriocarcinoma is a relative rare malignant tumor of gestational trophoblastic disease. Most cases present myometrial invasion, but rarely locoregional metastases (vulva, vagina, broad ligament) or in other organs (lung, brain, spleen, gastrointestinal tract, liver) can be found. Material and method: We present a case of a 41-years-old woman who was admitted in emergency in Universitary Colentina Hospital for massive vaginal bleeding; a tumoral abdominal mass was present at clinical examination. For these reasons the patient underwent a surgical intervention (total hysterectomy and bilateral oophorosalpingectomy). Results: Gross appearance: the uterus is much larger (16/12/7,5cm), fluctuent at palpation; the uterine cavity was occupied by numerous cysts measuring 0,3 to 1 cm across with serous/serohematic content forming an adherent mass to the endometrial mucosa; there are necrotic areas especially in fundic zone. Microscopical examination revealed rare hydropic villi and a tumoral proliferation composed of trophoblastic cells with insular/ cordonal pattern invading the myometrium. Tumoral cells have a marked cellular and nuclear pleomorphism, with frequent atypical mitoses. Occasionally, in periphery of the tumor, giant multinucleated tumoral cells with marked cytonuclear atypia could be identified. This atypical aspect of trophoblastic cells invited us to a large debate of the differential diagnosis with invasive mole caused by the presence in a limited aria of the hidropic villi. Hormonal tests have been performed (beta HCG >40 000 mU/ml) and immunohistochemical tests: positive reaction for betaHCG in hydropic villi and in trophoblastic elements; p53 was positive 10-15% and Ki67 was positive 3-4% in trophoblastic elements, all of them confirmed the histopathological result. Conclusion: We presented this case both for the rarity of gestational trophoblastic disease and for the difficulty to sustain the histopathological diagnosis. We want to evidentiate the utmost importance of the periodical examinations in pregnancy.

PP4-2
EXPRESSION OF AURORA KINASES A AND B IN OVARIAN CARCINOMA: CORRELATION WITH CLINICOPATHOLOGICAL FEATURES

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BACKGROUND: Ovarian carcinoma is the most important cause of gynaecological cancer-related mortality in the western world with a poor 5-year survival. Combination platinum-paclitaxel chemotherapy has become a standard first line treatment. Aurora kinases are involved in mitosis and cell division process. Overexpression of Aurora kinases, particularly Aurora-A, has been reported in many cancers. The aim of this study was to evaluate the expression of Aurora kinases A and B in ovarian carcinomas and to correlate their expression with clinicopathological features. MATERIALS AND METHODS: The study was conducted on 68 ovarian carcinomas treated by surgery followed by paclitaxel plus carboplatin-based chemotherapy. A complete response to chemotherapy was observed in 66.2% of cases. Median follow-up of survivors (38.2%) was 33 months. Antibodies against Aurora-A (Novocastra, 1:100), Aurora-B (Bethyl, 1:50), estrogen receptor (ER) (Novocastra, 1:100), progesterone receptor (PR) (Dako, 1:100), p53 (Novocastra, 1:100) and Ki67 (Dako, 1:100) were applied using the Envision (Dako) method. The immunohistochemical expression of Aurora-A and –B was evaluated as present or absent, regardless of intensity and percentage of cells that exhibit immunostaining. Additionally, we analyzed the amplification of Aurora-A gene by FISH. RESULTS: Overall, 58.8% and 92.1% of ovarian carcinomas showed overexpression of Aurora-A and –B, respectively. Expression of Aurora-A and –B did not correlate with FIGO stage. However, disease free survival (DFS) was significantly higher among patients with Aurora-A protein expression (P=0.02). A clear trend for longer DFS was also observed in Aurora-B expressing carcinomas (p=0.06). Additionally, a trend for positive correlation between Aurora-A expression and complete response to chemotherapy was also observed (p=0.09). Amplification of Aurora-A was found in 26.7% of cases examined, and was not associated with overexpression of Aurora-A by immunohistochemistry. There was no significant association of Aurora-A amplification and the recorded clinicopathological variables. Expression of ER, PR, p53 and Ki67 did not show association with clinico-pathological variables. CONCLUSION: Aurora-A expression seems to have a prognostic value in patients with ovarian cancer treated with a platinum-taxol based regimen. The present results are consistent with a role of Aurora kinases as a determinant of chemosensitivity of ovarian carcinomas. However, these results should be confirmed in a larger series of ovarian carcinomas. Grant (MEC) SAF2004-0825-C02-02

PP4-3
A CASE REPORT: EXTENSIVE IN SITU SQUAMOUS CELL CARCINOMA OF THE ENDOMETRIUM AS SUPERFICIAL EXTENSION OF INVASIVE CERVICAL CARCINOMA

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Background: We report a case of 67-year-old woman with cervical squamous cell carcinoma (SCC) with extensive in situ squamous cell carcinoma of endometrium.SCC is the most common tumor of the female genital tract.However primary SCC of endometrium is extremely rare and its diagnosis requires absence of cervical malignancy. It is commonly encountered in postmenopausal women, where chronic pyometra or endometritis and long-lasting use of an intrauterine device are proposed etiologic factors.Ichthyosis uteri is a rare condition describing epidermalisation of the uterine cavity.It was described as an endometrial response to iatrogenically-introduced caustic substances and with a variety of inflammatory conditions of the endometrium. Case:A multiparous woman with new onset

vaginal bleeding administered to our Gynecology and Obstetrics Department's outpatient clinic.Physical examination revealed a massively enlarged, barrel-shaped cervix.Following a biopsy, she underwent a type III radical hysterectomy, bilateral salpingo-oophorectomy and pelvic-paraaortic lymph node sampling.Macroscopic examination revealed a 4 cm × 4 cm exophytic mass obliterating the endocervix and extending into the lower uterine segment.Sectioning revealed stromal invasion to almost 100% of the cervical wall.The uterus has become "pyometria" and uterine wall was extremely thinned.The endometrium was flat and glistening but was otherwise unremarkable.Microscopically,cervical neoplasm was a moderately differentiated,large cell keratinizing SCC.Extensive sampling of the endometrial wall revealed complete epidermalisation and areas where cells displayed high grade squamous intraepithelial lesion or carcinoma in situ.There was no evidence of extrauterine disease, parametrium was free of disease, 2 lymph nodes were metastatic.The patient received chemotherapy, now alive without diseases. Conclusion: Our case is a cervical SCC associated with extensive ichthyosis uteri-like changes of the endometrium that, additionally,had superimposed carcinoma in situ. These coincident findings may be explained that a SCC in the cervix and the associated HPV extended proximally,colonizing a pre-existing ichthyosis uteri.The second potential explanation is that within a background of extensive ichthyosis uteri, a SCC developed in the lower uterine segment.Neoplastic potential of ichthyosis uteri is largely unknown and theoretically longstanding,mature and even keratinizing squamous epithelium in the endometrium would be subject to the same potential changes in other visceral sites.

PP4-4

OVARIAN MUCINOUS CYSTADENOCARCINOMA WITH MURAL NODULE

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Solid mural nodule within a mucinous cystic ovarian tumor is extremely rare. Sarcoma-like mural nodules are not just limited to mucinous tumor. They can also seen in squamous cell carcinoma, cystic teratoma and granulosa cell tumor. A 78 year-old woman presented with abdominal pain caused by a mass. USG detected a 199x150 mm cystic mass.The mass contained a solid area of 73x47 mm and had a rich neovascularisation. The serum levels of CA125, CA15.3, AFP, CEA were normal and CA 19.9 was slighly eleveted at 50,9 U/ml. Grossly the ovarian tumor measured 17x16x11 cm. The outer surface was smooth and gray-white in colour. The cut surface showed a hemorrhagic mural nodule of 8x8x5 cm. Microscopically, tumor showed features of a malignant mucinous epitelial tumor. Mural nodule composed of round or ovoid cells with pleomorfic nuclei and prominent nucleoli. There was clear nuclear atypia and mitotic figures were inconspicous. There were scattered multinucleated giants cells with plemorfic hyperchromatic nuclei. Lymphovasculer and stromal invasion were present around the tumoral focus. Immunohistochemically the atypical cells in the mural nodule were negative with SMA and desmin. They expressed vimentin and multinuclear giant cell were CD68 positive. There were scattered CK positive atypical glands and isolated cells. In the literature, rare cases of ovarian mucinous tumors have been described that contain foci of sarcoma-like nodules. This case report describes the pathologic features of this rare entity with review of the literature.

PP4-5

DISTRIBUTION OF OVARIAN TUMORS IN ZONGULDAK KARAELMAS UNIVERSITY MEDICAL FACULTY HOSPITAL BETWEEN 2001-2007 YEARS

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Introductıon: Ovarian tumors are one of the common form of neoplasia in women. It is the fifth leading cause of cancer mortality in women. There are numerous types of ovarian tumors, both benign and malignant. Majority are benign. The purpose of this study is to evaluate the epidemiology of ovarian cancer in Zonguldak and surrounding cities, retrospectively. Methods: Between May 2001 and March 2007, 769 ovarian biopsies from 494 cases which undergo operation for variable cause are reviewed in Karaelmas University Medical Faculty Pathology Department. Tumors and other pathologic changes were classified. Results: Patients ages are between 10 days to 85 years and average is 45.46 (SD±13,45) years. Ovarian tumor's ratio is (n: 123) 24,9% in all cases. Distribution of this 123 ovarian tumor: 90 (%73,2) are benign, 9 (%7,3) are borderline and 24 (%19,5) are malignant. 68 of the cases (55,3%) are tumors of surface epithelial origin of the ovary {26 (38,2%) are benign serous, 18 (26,5%) benign mucinous, 2 (2,9%) benign brenner, 7 (10,3%) borderline serous, 2 (2,9%) borderline mucinous, 9 (13,2%) malignant serous, 1 (1,5%) transitional cell carcinoma, 1 (1,5%) endometrioid carcinoma, 1 (1,5%) clear cell carcinoma, 1 (1,5%) malignant mixt mullerian tumor}; 34 of the cases (27,7%) are germ cell tumors {33 (97,1%) mature cystic teratoma, 1 (2,9%) teratoma with malignant transformation}; 10 of the cases (8,1%) are sex-cord stromal tumors {1 (10%) adult granulosa cell tumor, 1 (10%) juvenil granulosa cell tumor, 4 (40%) fibroma, 2 (20%) fibrothecoma, 1 (10%) sclerosing stromal tumor, 1 (10%) sex-cord stromal tumor of not other specified}; one of the cases (0,8%) is vascular tumor (cavernous hemangioma); one of the cases (0,8%) is undifferantiated carcinoma and 9 are (7,3%) metastatic tumors. Conclusion: Among cancers of the female genital tract, the incidence of ovarian cancer ranks below only carcinoma of the cervix and the endometrium. Ovarian cancer mortality takes the first place when compared with all other gynecological malignancies. In our study cystadenoma and cystadenocarcinoma are the most frequent benign and malignant tumors respectively and most of them are serous. When compared with the literature, tumors of surface epithelial origin of the ovary are less frequent, but metastatic tumors to ovary are more frequent in both whole and malignant groups. Germ cell tumor's ratio is higher according to literature. We also have cavernous hemangioma which is an extremely rare tumor of ovary.

PP4-6

CLINICO-MORPHOLOGICAL PECULIARITIES OF SEPTICEMIA IN LABOR AS A SEPARATE NOSOLOGICAL FORM AMONG THE CAUSES OF MATERNAL DEATH

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Background -Pyoseptic diseases account for 15% of the main causes of maternal death and are usually classified as postnatal sepsis Methods - We studied 15 autopsies of puerperas who died over a 10 year period (1996 - 2006) from pyoseptic diseases in the Rostov Province, and we analyzed their afterbirths and partum histories. Microscopic examination of uteri, spleens and lymph nodes, as well as fetal membranes and placentas, was

performed by standard methods of light microscopy, with hemotoxillin and eosin staining; PAS reaction was also used, and immunohistological verification of cells from the inflammatory infiltration in the above organs. Results - Microscopic and immunohistochemical examination of the uterus showed, in 50% of cases, shallow invasion of the cytotrophoblast cells in the placental bed, incomplete gestation restructuring of the endometrial segments of the uteroplacental artery; in 60% of cases we found focal, low-grade infiltration with granulocytes, T-lymphocytes, and macrophages; myometry revealed, in 60% of cases, focal low-grade, mostly perivascular, infiltration with granulocytes, T-lymphocytes, and macrophages. The inflammatory infiltrate in the placental fetal membranes was represented by a great number of granulocytes, plasmacytes, macrophages, and rare T-lymphocytes. The inflammatory infiltrate contained no B-lymphocytes. In 73% of cases we observed effacement of the spleen pattern, dysfunction of the humoral and cell protection - depletion of the lymphoid tissue and its replacement with granulocytes and macrophages; the lymph nodes in 86% cases also showed effacement of the pattern, with only fragments of the lymphoid follicles preserved, while the parafollicular zone was characterized by depletion of T-lymphocytes and diffuse infiltration with granulocytes and macrophages. Conclusion - Early antenatal infection of the afterbirth, which is these cases is the entrance gate for penetration of bacterial, fungous, and viral agents into the mother's blood flow. We found a relative intactness of the uterus, because the revealed inflammatory changes in the basal endometry and myometry should be regarded as normal inflow of leucocytes and lymphocytes to the area affected by necrosis and rejection of the decidual tissue and remains of the fetal membranes. The rapidity of development of the clinical manifestations of septicemia in labor, and of the infection and toxic shock, leading to death of the puerperas within the first days of the postpartum period, is accounted for by the apparent deterioration of the immune system function.

PP4-7

HIGH-GRADE ESS ARISING FROM OVARIAN ENDOMETRIOSIS, WITH ASSOCIATED FOCI OF ENDOMETRIOID ADENOCARCINOMA: REPORT OF A CASE.

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Background: Endometriosis malignant transformation is a well documented phenomenon that occurs most commonly in the ovaries, frequently associated with endometrioid or with clear cell adenocarcinomas. Endometrioid stromal sarcoma (ESS) is a sarcomatous tumor characterized by a diffuse proliferation of neoplastic cells similar to the normal stromal cells present in the proliferative endometrium. Among these, ESS arising in endometriosis of the ovary is an extremely rare tumor. Here we present, for the first time, a case of an high-grade ESS arising from ovarian endometriosis associated with endometrioid adenocarcinoma foci. A 51-years-old white woman, with no significant gynecological history, presented with increasing abdominal distension, and weight gain. After an exploratory laparotomy a 10 cm right ovarian mass with neoplastic ascitis and multiple tumoral nodules in the omentum and in the peritoneum was found. Given the advanced stage, debulking surgery was performed. No alteration of the uterine corpus were revealed under clinical, ultrasonographic and laparoscopic examinations. The patient died after 1 month for the diffuse metastatic disease. Method Immunohistochemical studies were performed by indirect staining methods using antibody against cytocheratin 7, 20, 8-18, vimentin, epithelial membrane antigen (EMA), inhibin, calretinin, S100 protein, HMB45, smooth muscle actin, desmin, estrogen receptor (ER), progesterone receptor (PR), NSE, cromogranin, sinaptofisin, CD99, CD45, CD30, Ki-67 (Mib-1), CD10. Results The ESS tumor nodules were composed mostly by sheets or cords of closely packed round or oval uniform cells, with pale and scanty cytoplasm and intersected by dense fibrous band. Some of these neoplastic cells occasionally were found around numerous small thick-walled blood vessels. More than 10 MF per 10 HPF were counted. Extensive necrosis and hemorrhage and vascular and perineural invasion were present in the tumor. Endometriosis, identified in the cystic areas, in some areas was associated with endometrioid adenocarcinoma foci (G2, nuclear grade 3). ESS tumor cells immunohistochemical stain was positive for vimentin, CD10 and focally for EMA and around 80% of nuclei were Ki-67 (Mib-1) positives. All the other immunohistochemical markers performed were negative. In the multiple foci of of endometrioid adenocarcinoma the neoplastic glands resulted diffusely positive for EMA and CK7, and negative for CD10. Conclusion: According to our knowledge this is the first report of a high-grade ESS associated with concomitant endometrioid adenocarcinoma foci.

PP4-8

MULTIPLE OXYPHILIC TYPE ADENOMATOID TUMORS OF THE UTERUS COEXISTING WITH ENDOMETRIAL ADENOCARCINOMA IN A RENAL TRANSPLANT RECIPIENT.

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Backround: Uterus is the most common site of occurrence of adenomatoid tumors in the female genital tract. Multifocal cases, as well as oxyphilic variants, have rarely been described. To our knowledge, this is the second case, coexisting with endometrial adenocarcinoma of the uterus and the fourth case, in a renal transplant recipient. Case: This is a report of the coexistence of a multifocal uterine adenomatoid tumor with a poorly differentiated endometrioid adenocarcinoma, FIGO stage IB. Both were incidental findings in a 56 years old woman, undergoing hysterectomy because of large leiomyomas. Two of the adenomatoid tumors, found macroscopically, had gelatinous appearance while the third was found microscopically. All consisted of large oxyphilic epithelioid type cells, with a solid or trabecular pattern resembling leiomyoblastoma. Slit–like and adenoid pattern was rarely found. Immunohistochemically, the tumor cells stained for Vimentin, Pankeratin, Calretinin, Keratin 5/6 and HBME-1, while no immunoreactivity for Actin, Desmin. H-Caldesmon, CD34 and CD31 was found. The Ki67 index was low. Conclusion: Uterine adenomatoid tumors are rare tumors, which may cause differential diagnostic problems, but the knowledge of their morphological spectrum and the characteristic immunoprofile allows their diagnosis. Very interesting features of our case are the multicentricity and the oncocytic appearance of the tumor, as well as the coexistence with a poorly differentiated carcinoma, in an immunocompromissed status of a renal transplant recipient.

PP4-9

PRIMARY VAGINAL MUCINOUS ADENOCARCINOMA ARISING ON A PREEXISTING ADENOMA. A RARE CASE REPORT.

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Background: Primary vaginal mucinous adenocarcinomas (PVMA) are rare neoplasms that develop in patients with in utero exposure to diethylstilbestrol (DES). We report a case of PVMA of intestinal type arising on a preexisting adenoma, in a patient without intra-uterus DES exposure. Awareness of this lesion is important as it must be distinguished from metastatic

adenocarcinomas especially from colon or endocervix. Method: A 13mm polypoid mass was found on the posterior vaginal wall of a 52-year-old menopausal woman during routine gynecological check up. The patient did not have any symptoms of vaginal bleeding, mucus secretion nor a history of diethylstilbestrol exposure. Complete excision of the polypoid mass was performed and the histological examination showed adenocarcinoma of intestinal type arising on a tubular adenoma. To preclude possible metastasis from the gastrointestinal tract, sigmoidoscopy, colonoscopy and gastroscopy was ordered showing no primary gastrointestinal lesions. Results: Grossly, the specimen was a lobulated, circumscribed, brown-colored solid lesion measuring 13mm. Histologically, it was a tubular adenoma of medium degree of dysplasia with glands made of columnar and goblet cells without any Paneth and neuroendocrine differentiation. The adenoma focally transformed to areas with higher density of glands, placed back-to-back, with multistratification of nuclei, loss of cell polarization and cribriform architectural arrangement, similar to in situ intestinal adenocarcinoma arising on an adenoma. The adenocarcinomatous area was showing early focal invasion of the underlying stroma with accompanying chronic inflammatory infiltration and slight desmoplasia. Dysplastic glandular epithelium was replacing the normal vaginal non-keratinized stratified squamous epithelium in adjacent sites. The immunohistochemical profile of the lesion revealed expression of CK 20 and CEA and negativity in CK 7. Alcian blue positivity was indicative of intestinal type mucin. Conclusion: PVMA is a rare tumor of the lower female genital tract and its occurrence is higher among women who have been exposed to DES during intra-uterus life. However, cases of no such association to DES have also been reported. The differential diagnosis involves metastatic mucinous adenocarcinoma of the endocervix and of the intestines. In the first case, the adenocarcinoma cells contain sulfomucin, a mucin specific for adenocarcinoma of the endocervical type. In the second case, the mucin is the intestinal type o-acetylated sialomucin.

PP4-10

HIGH CELLULAR LEIOMYOMA WITH EXTENSIVE CYSTIC DEGENERATION MIMICKING LOW GRADE ENDOMETRIAL STROMAL SARCOMA. A CASE REPORT WITH PECULIAR MACROSCOPIC APPEARANCE

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Background: High Cellular Leiomyoma (HCL) is a subtype of Cellular Leiomyoma (CL) with accentuated cellularity composed of small, round or spindle cells lacking moderate to severe cytological atypia and necrosis. HCL is often misdiagnosed as Endometrial Stromal Tumor (EST). We present a case of HCL in the form of an intramural, solitary, well-defined myometrial cyst. Method: A 38-year-old woman presented with a pelvic mass, causing urinary urgency and stagonoid hemorrhage. On palpation, and ultrasonographically, an enlarged cystic uterine mass was observed, displacing the urinary bladder and slightly obstructing the ureters causing dilation of the pyelocalyceal system. On CT and MRI scan no other notable lesions were discovered. Total hysterectomy was performed with bilateral salpingectomy. Results: Grossly, the uterus measured 13.5x11x10.5cm and the left and right salpinx measured 3.5 and 3.7cm respectively. On dissection, a voluminous cystic mass was observed in the ventral myometrial wall measuring 9cm in greatest diameter and filled with a yellowish serous liquid content. On cross section, the mass was whitish-yellowish-to-tan, nodular-appearing, with extensive cystic degeneration and soft in consistency. A small leiomyoma measuring 1.5cm was also found. The endometrial surface was not affected. Histologically, the tumor was characterized by a monotonous appearing hypercellular mass with slightly irregular peripheral borders. It was composed of relatively uniform cells showing no or minimal cytological atypia, having round or ovoid nuclei with finely granular and evenly dispersed chromatin, small inconspicuous nucleoli and scanty cytoplasm. At foci, typical smooth muscle bundles and few thick walled vessels were encountered. Furthermore, cystic changes were also spotted among the stromal neoplastic cells. Low mitotic activity (infrequent mitotic figures, <5/10HPF) was evident. The margins of the tumor were relatively well-delineated with focal finger-like irregularities of 3mm and isolated neoplastic cells infiltrating the adjacent myometrium. Necrotic foci and invasion of the lymphatic and vascular channels were not observed. Conclusion: Differential diagnosis includes Endometrial Stromal Nodule (ESN) and low grade Endometrial Stromal Sarcoma (ESS). Although morphologically the neoplasm is consisted with low grade ESS and despite the slightly irregular peripheral borders of the neoplasm, HCL is strongly considered. Our case was positive in vimentin/ER/PR/desmin/SMA and negative in CD10, the latter typically staining endometrial stromal tumors.

PP4-11

DIVERSITY OF MORPHOLOGY, PROGNOSIS AND CLINICS OF ENDOMETRIAL CARCINOMA

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Endometrial carcinoma (EC) is the 3rd common cancer of female in Latvia with 30,8%o and there is tendency for increase. The aim of our research is to evaluate stage, variants, prognostic factors and immunohistochemical spectrum of EC. Materials and Methods. We have analyzed retrospective material of operated uterus from 60 cases with the characteristics of lymph nodes, tubes, ovaries and expression of estrogen, progesterone, CD 44 and synaptophysin markers. Coherence between clinical symptoms we analyzed by Speerman rank system, but correlations between morphological changes by diagrams. Results. 60% of EC were endometroid adenocarcinomas, 23%-villoglandular variant, 15%-with squamous differentiation, 2%-clear cell adenocarcinoma. Age of patients: 44-82 years. EC were of I to IV stage and myometrial invasion from 0, 1 to 2 cm. Clinical information showed uterine bleeding in 43%, obesity - 5% of patients. Number of deliveries and abortion were variable. In 31, 6% of cases EC were proved in such precursor lesions as endometrial polypus and atypical hyperplasia. Mean age of menopause – 52 year. Late menopause was in 36% of patients. The size of the uterus in majority of cases was normal. Clinical diagnosis of benign process was changed to malignancies by pathologists in 14, 6 % of cases. Metastasis were proved only in 3, 3% of female. In 92% of patients lymph nodes were not exceeded for histological examination. In ovaries were different cysts. Expression of estrogen, progesterone markers were: negative in 23, 8 %, positive-57, 2 %, but in 19 % of cases estrogen was negative, but progesterone –positive. Conclusions.1.Diagram analyses showed the most aggressive invasion in myometrium by villoglandular variation of EC. 2. There were no statistically significant differences between different clinical data but we proved direct correlations between nononcological changes in ovaries with increased risk ofendometrial carcinoma at earlier age of patient. 3. Immunoreactivity of estrogen and progesterone receptors was more expressed in G1, G 2 of EC but lack of lymph nodes in our research group decreased the possibility of real prognosis.

PP4-12
HOW OFTEN HSIL ACCOMPANIES LSIL: IS AGE IMPORTANT?
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BACKGROUND: High-grade squamous intraepithelial lesion (HSIL) has much greater potential for progressing than does low-grade intraepithelial lesion (LSIL). So it may be the important to identify the subset of women at higher probability of having "LSIL harbouring the HSIL" lesions in follow-up biopsy specimen whose cytologic specimens show atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells; cannot exclude HSIL(ASC-H) and LSIL. METHOD: Sixty-five consecutive patients whose follow-up cervical biopsies were available and have ASC-US, ASC-H and LSIL lesions in their conventional smears were enrolled. We divided them in two groups: age 40 and under and over age 40. All follow-up biopsy results were evaluated and cases were excluded which the histologic examination didn't show any squamous lesion. RESULTS: Six patients were excluded because of not showing any squamous lesion in their follow-up biopsy specimen.There were 39(66%) women age 40 and under and 20(34%) women over age 40 who have cytologic abnormalitiy as ASC-US, ASC-H and LSIL. In younger age group; 30(77%) women showed LSIL and 9(23%) women showed HSIL in their biopsy specimen. In over 40 group; there were 8(40%) LSIL and 12(60%) HSIL showing biopsy specimens. CONCLUSION: Compared to younger women with abnormal low-grade cervical cytology, women over 40 years of age, had higher rate of having 'LSIL harbouring HSIL' in their follow-up biopsy. Since current study has small sample group further studies involving large number of cases and detailed statistical analysis must be made. If these findings confirmed in future studies then it may be possible to say that HSIL lesion is more expectable in follow-up biopsy of women over 40 years of age with a diagnosis of ASC-US, ASC-H or LSIL in cervical cytology.

PP4-13
ENDOCERVICAL-LIKE MUCINOUS BORDERLINE TUMORS OF THE OVARY : A CLINICOPATHOLOGIC ANALYSIS OF 9 CASES
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Endocervical-like mucinous borderline tumors (EMBTs) are rare distinct entity of the ovary that seems to be under recognized. These tumors are characterized by a papillary architecture reminiscent of serous tumors but composed of endocervical-like mucinous epithelium. The latter may be admixed with other mullerian-type epithelium, including those of serous, endometrioid, squamous and eosinophilic epithelial cells. We have studied nine EMBTs including 2 cases with intraepithelial carcinoma and one case with stromal miroinvasion. The patients averaged 35.5 years of age. None tumor was bilateral. Endometriosis was identified in three cases and one patient was known to be pregnant at the time of diagnosis. All patients were stage I. Of the 5 patients which have a follow-up data, only one patient was complicated by recurrence in the conserved contra lateral ovary with a peritoneal implant, three years later. The present study describes our experience with endocervical type mucinous tumors. The frequency of occurrence, criteria for the diagnosis of intraepithelial carcinoma and microinvasion are discussed.

PP4-14
GENOMIC INTEGRATION OF ONCOGENIC HPV AND GAIN OF THE HUMAN TELOMERASE GENE TERC ARE STRONGLY ASSOCIATED EVENTS IN PROGRESSION OF UTERINE CERVICAL DYSPLASIA TO INVASIVE CANCER
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Background: Recently proposed markers for progression of CIN II/III to cervical carcinoma include integration of HPV into the host genome, genomic instability and an increase in chromosome 3q copy number. In particular, the gene coding for the RNA component of telomerase (TERC) at 3q26 has been implicated as possible candidate gene. Since it is not known to date how these events are temporarily related during cervical carcinogenesis, the aim of the present study was to assess the correlation between TERC copy number and physical status of HPV in high grade solitary precursor lesions of the uterine cervix (CIN II/III, n=17), lesions associated with a microinvasive cervical carcinoma (CIN III&mCA, n=13), and advanced invasive cervical carcinoma (invCA, n=7). Method: Fluorescence in situ hybridization (FISH) was applied to assess the copy number of the TERC gene and HPV integration and to correlate TERC gain with HPV integration during progression in cervical neoplasia. The TERC gene probe was applied in a mixture together with probes for the centromeres of chromosomes 3 and 7, used to determine the ploidy of these lesions. Results: Our study revealed that CIN II/III lesions with episomal HPV frequently show tetrasomy for TERC. In these cases the TERC gene copy number parallels the DNA ploidy of the lesions. Furthermore, TERC was increasingly gained with progression of CIN II/III (3 of 17), via CIN III&mCA (7 of 13) to invCA (5 of 7). In the cases exhibiting gain of TERC the virus was predominantly integrated. The finding that the combination of gain of TERC (three copies) and genomic integration of HPV was seen in 8 out of 10 diploid lesions, indicates that these events occur prior to overall aneuploidization. Our data show that gain and aneusomy for the TERC gene is strongly correlated with viral integration, which in turn is associated with the progression of CIN III to mCA and invCA ($p<0.001$). Conclusion: Genomic integration of oncogenic HPV and gain of the human telomerase gene TERC are strongly associated events in progression of uterine cervical dysplasia to invasive cancer.

PP4-15
HPV DNA DETECTION AND TYPING AS AN AID IN THE CLASSIFICATION OF SECOND NEOPLASMS IN WOMEN WITH UTERINE CERVICAL CANCER
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INTRODUCTION: The vast majority of uterine cervical carcinomas are human papillomavirus (HPV) related neoplasms. In women with a cervical cancer and a distant lesion the histologic distinction of metastatic cervical cancer versus another primary tumor can be difficult and has important clinical implications. OBJECTIVE: To study the presence of HPV DNA in primary cervical cancer and in second neoplasms in the same patient. MATERIAL AND METHODS: Four patients with squamous cell carcinoma (in bone, lung, lateral neck lymph node and pleural fluid) and two with adenocarcinoma (in ascitic fluid) and a past history of uterine cervical cancer (diagnosed 1 to 11 years before) were studied. HPV DNA detection was performed both in the cervical tumors and in the second neoplasms (formalin fixed, paraffin embedded tissues) using a PCR based technique with GP5+/6+ primers for detection of the HPV L1 gene followed by reverse line blotting hybridization for genotyping. RESULTS: In five patients DNA of the same HPV type was found in the cervical tumor and in the second neoplasm (HPV16 in 4 squamous cell carcinomas and HPV18 in one adenocarcinoma), thus allowing to classify the second tumors as metastatic. In the remaining patient HPV DNA was not detected in any of the tumors tested. CONCLUSIONS: In women with a past history of an HPV-related cancer and a second neoplasm detection and typing of HPV DNA is helpful in the differential diagnosis between metastases and a second unrelated malignancy.

PP4-16
EFFECTIVENESS FOR EVALUATION OF LYMPHATIC INVASION IN ENDOMETRIOID ADENOCARCINOMA OF THE UTERINE CORPUS

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Backgrounds: Lymph-vascular involvement is one of the important prognostic factors in the patients with carcinoma of the uterine cropus. Conventionally, it has been determined morphologically using hematoxylin and eosin sections. In addition to that, various immunohistochemical stains have been attempted in order to confirm the accuracy of diagnosis of lymph-vascular involvement with section stained H&E. Recently, D2-40 immunostaining was demonstrated as the best immunohistochemical marker for the endothelium of the lymph vessel. The aim of this study is to clarify the effectiveness of immunohistochemical staining with D2-40 in determination of lymphatic invasion. Methods: We investigated that 40 patients with adenocarcinoma confirmed to the uterine corpus, who were underwent radical hysterectomy. The stages of tumors were 26 cases in pT1b, 14 cases in pT1c, and 7 cases were positive node among 40 cases. From each material, one section containing the deepest invasive part was selected for examination and stained with H&E and D2-40 immunohistochemically. Lymphatic invasion was evaluated by either H&E staining or D2-40. The concordance of these results was assessed using the kappa statistic (κ). Additionally, the relationship between lymph vascular involvement and lymph node metastasis was examined by using Fisher's exact test. Results: The evaluation of lymphatic invasion with H&E staining resulted in 24 positive cases (positivity of 60%) and 16 negative cases (negativity of 17.5%). The result with D2-40 immunostaining showed 7 positive cases (positivity of 17.5%) and 33 negative cases (negativity of 82.5%). The concordance between H&E and D2-40 was moderate (57.5%, κ=0.53). The negative cases in the evaluation with H&E staining were always negative in the evaluation with D2-40. As a result, lymph vascular involvement might have been over-estimated by H&E staining. The correlation between lymphatic invasion and lymph node metastasis showed statistical significance, in both results with H&E and D2-40 (p=0.011 and p=0.0001, respectively). Conclusion: Lymphatic invasion determined by D2-40 staining could be much more accurate predictive factor for lymph node metastasis.

PP4-17
ENDOMETRIAL CYTOLOGY IN DIAGNOSIS OF ENDOMETRIAL CARCINOMA

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Cytodiagnosis of the precancerous lesions of the endometrium has remained unclear compared to that for cervical lesions. Endometrial cytology and biopsy have been performed for patients with atypical genital bleeding, who were over fifty, postmenopausal or nulliparous. The cytologic diagnosis of endometrial cancer using material obtained with the Inocurette endometrial sampler was assessed for 64 patients.The cytologic findings for benign and malignant samples are described and illustrated in detail. Relative to other endometrial sampling devices, the Inocurette is inexpensive and was easily used by the gynecologist and well tolerated by the patients, with no complications and minimal discomfort.

PP4-18
EVALUATION OF COMBINED BCL-2/MDM-2 IMMUNOHISTOCHEMICAL EXPRESSION AS A PROGNOSTIC FACTOR IN EARLY STAGES OF INVASIVE CERVICAL CARCINOMAS

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BACKGROUND: The present study was designed to evaluate the immunohistochemical expression of apoptosis regulating proteins (bcl-2, mdm-2 and p53) in correlation with proliferation (Ki-67), human papillomavirus (HPV) infection and other histopathological and clinical parameters in early stage cervical carcinomas and the estimation of their prognostic significance. Special attention was given to combined bcl-2/mdm-2 immunophenotypes in predicting the recurrence of the disease. METHOD: The subject of this study was a series of 83 surgically treated patients with cervical carcinoma confined to the uterine cervix (pT1b1/1b2), who subsequently received complete radiotherapy. The presence of HPV DNA was determined by the conventional method of in situ hybridization (ISH) and catalyzed reporter deposition signal amplification ISH. The immunostaining was performed using avidin-biotin-peroxidase complex method and the expression of the biological markers was semiquantitatively evaluated as the percentage of immunostained cells. RESULTS: During the clinical follow-up (mean 120.7, range 4.4-181 months) a relapse was diagnosed in 9 (10.8%) patients and the expected 5-, 10- and 15- year disease-free survival was 92.7%, 90.8% and 86.6%, respectively. The results of the univariate analysis indicate that significant predictive indicators for recurrence are: lymphonodal status, maximal tumor diameter, depth of stromal invasion, histological type and HPV DNA presence and type. Immunohistochemical markers showed the following correlations: increased expression of Ki-67 (P=0.031) and bcl-2 negativity (P=0.047) correlated with poor disease-free survival, while mdm-2 positivity showed borderline significance (P=0.051) and p53 expression had no influence on disease-free survival. Additional evaluation of combined bcl-2/mdm-2 expression showed that cases with bcl-2+/mdm-2- and bcl-2-/mdm-2+ immunophenotype had better survival (P=0.048) compared to bcl-2+/mdm-2+ and bcl-2-/mdm-2- phenotype. In the multivariate analysis, histological type, HPV DNA presence and the expression of Ki-67 have been selected as

the most significant independent prognostic parameters (P=0.0024). CONCLUSION: The evaluation of combined bcl-2/mdm-2 immunohistochemical expression provides more relevant information for the prediction of the recurrence of the disease than their individual expression. However, neither individual expression of bcl-2 and mdm-2 nor their combined immunohistochemical expressions are independent predictors of prognosis in early stages of invasive cervical carcinomas.

PP4-19
SCLEROSING STROMAL TUMOR OF THE OVARY WITH SEX CORD ELEMENTS

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Screrosing stromal tumor of the ovary is a distinctive type of benign stromal tumor characterized by a cellular pseudolobular pattern. The tumor cell population is composed of fibroblasts and round cells separated by hypocellular, oedematous or collagenous tissue. In the recent literature there have been very rare reports of the coexistence of sex cord elements in, otherwise typical, sclerosing stromal tumors of the ovary. We present a case of a seventeen-year-old girl with a sclerosing stromal tumor in which sex cord elements were observed. Multiple histological sections were studied and immunohistochemistry was performed with a wide spectrum of antibodies. Morphological and immunohistochemical findings led to the diagnosis of sclerosing stromal tumor with sex cord elements. The tumor cells stained positive for desmin, vimentin and SMA. Sclerosing stromal tumors comprise a very distinct type of ovarian stromal tumors with very characteristic histology, which is presented in detail.

PP4-20
THE IMPORTANCE OF THE TUMOR SUPPRESSOR GENE DESIGNATED PTEN AND OF THE PROLIFERATION MARKERS Ki-67 AND PCNA IN THE EVALUATION OF THE MALIGNANCY POTENTIAL IN ENDOMETRIAL HYPERPLASIA

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Introduction: Endometrial Hyperplasia (increase in gland/stroma ratio, disorder number and shape of the glands like modifications of the glandular epithelium: exhibits loss of nuclear polarity, stratification, etc) is a lesion frequently associated with the uterine leiomyomas Purpose: The Study was realized at the Universitary Emergency Hospital Bucharest, Romania on 210 endometrium biopsies taken in the period 2004-2005 from women with ages between 45 and 55. Material and methods: Hematoxylin –eosin stained slides of endometrial formalin fixed, paraffin embedded tissue has emphasized in 105 cases the simple hyperplasia (SH), in 75 cases the complex hyperplasia (CH), in 11 cases the simple atypical hyperplasia (SAH) and in 23 cases the complex atypical hyperplasia (CAH). We performed the indirect tristadial ABC method of IHC for 3 antibodies: PTEN, Ki-67 and PCNA on formalin fixed embedded tissue taken by biopsies from 50 cases (8 SH, 8 CH, 34 SAH and CAH). Results: PTEN was focal positive for SAH, diffuse for CAH and for 1-2 cases of SH and CH. Ki-67 and PCNA were also very frequent in group SAH and CAH. Conclusion: PTEN, Ki-67 and PCNA take part in the process of endometrial carcinogenesis following probably molecular pathways and determine the malignity potential of atypical hyperplasia of endometrium.

PP4-21
EGFR AND HER2/NEU EXPRESSION IN ENDOMETRIAL CARCINOMA. CORRELATION WITH TYPE AND STAGE

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BACKGROUND: Epidermal Growth Factor Receptor (EGFR - Her1) and Human epidermal growth factor type II (Her2/neu) are members of the subfamily of tyrosine kinase transmembrane receptors. The expression of EGFR and Her2/neu has been documented in a variety of malignant neoplasms of epithelial origin as breast, ovary and lung. The expression of these markers correlates with advanced stage, poor prognosis and metastasis. The status of these oncoproteins in endometrial carcinoma, however, is not entirely clear. Aim of this study was the evaluation of EGFR and Her2/neu in endometrial carcinoma (endometrioid and serous –type I and II), the relation to other prognostic factors and the possible therapeutic implications. MATERIAL and METHODS: Forty one cases of endometrial carcinoma were selected, 22 endometrioid (type I) and 19 serous (type II). Formalin fixed-paraffin embedded tissues were immunohistostained with EGFR (clone 31G7, Zymed, CA, USA) and Her2/neu (clone CB11, Bio Genex, CA,USA). Over-expression of Her2/neu was defined as moderate or strong membranous staining (≥2+ staining) in more than 10% of the cells. EGFR's expression was interpreted as positive (any membranous staining) or negative. Tumors were subdivided into two stage groups, 1 and ≥2. RESULTS: Patients ranged in age from 27-84 years, mean age 59,81 in endometrioid and 68,26 in serous carcinomas. Her2/neu was over-expressed in 9,9% of type I and 41,1% of type II (P< 0,001) and in stage ≥2 in comparison to stage 1 (54,5% vs 25%). EGFR's expression was demonstrated in 40,9% and 31,6% of type I and II carcinomas, respectively. Stage 1 type I carcinomas expressed EGFR in statistically significant percentage, compared to stage ≥2 (77,8% vs 15,4% , P<0,05). CONCLUSIONS: Her2/neu usually is over-expressed in serous type of endometrial carcinoma. Moreover, the expression increases with stage. EGFR especially is expressed in endometrioid type carcinoma and mainly in low stage. Newly developed drugs that target these receptors may have a role in the treatment of endometrial cancer. These results, obtained by immunohistochemistry, have to be confirmed using molecular methods (CISH or FISH).

PP4-22
EXPRESSION OF MASPIN IN TYPE I AND II ENDOMETRIAL CARCINOMA, ITS CORRELATION WITH VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND CLINICAL PARAMETERS

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BACKGROUND: Maspin (mammary–specific serpin) is a tumor suppressor gene that inhibits the invasion and angiogenesis. Maspin is expressed in normal human mammary and prostate epithelial cells but is down –regulated during cancer progression. Interestingly, the protein's prognostic significance in various cancers is highly impact by its cellular localization (nuclear vs cytoplasmic).Vascular endothelial growth factor (VEGF) plays important role in angiogenesis. The aim of this study is to determine the maspin's pattern expression in endometrioid (EC) and serous uterine carcinomas (SC), its correlation with VEGF and the clinicopathologic variables. MATERIAL AND METHODS: We examined maspin and VEGF expression in 22 endometrioid and 19 serous uterine carcinomas. Paraffin blocks

from each case were immunostained, using antibodies against maspin (clone EAW24, NeoMarkers, USA) and VEGF (clone CI 153-694, DD Pharmigen ,USA) by established methodology. The immunoreactivity of maspin and VEGF was semi-quantitatively scored, based on the intensity and percentage of positive cells (0- +3). Moreover, was estimated the maspin's cellular distribution as cytoplasmic and/or nuclear.. RESULTS: The mean age was 59,8 years for patients with endometrioid carcinoma and 68,3 years for patients with serous carcinoma. Patients' distribution by FIGO grading of EC was as follows: 15 G2 and 7 G3. FIGO surgical stage was as follows: 17 stage I, 6 stage II and 18 stage III. Maspin's expression was detected in 5/22 (22,7 %)of ECs-5/7 (71,4 %) of G3 tumors- and 11/19 (58 %, P<0,001)of SCs. In squamous metaplasia of ECs, the stain was absolutely nuclear. There was not correlation between maspin's expression and vascular invasion on one side and stage on the other. All tumors expressed VEGF (ECs showed lower intensity and had heterogeneity- 36,36 % 1+, 50% 2+, 13,64 % 3+ - despite of SCs which all had strong intensity- 3+). CONCLUSIONS: Maspin and VEGF expression was found in uterine carcinomas related to type (I or II) and grade. Maspin cytoplasmic expression in contrast to other carcinomas is directly associated with the biological aggressiveness (serous phenotype, and grade) and may result in deregulation of tumor inhibitor properties.

PP4-23

PRIMARY VULVAR EWING'S SARCOMA/PERIPHERAL NEUROECTODERMAL TUMOR: DIAGNOSTIC CONFIRMATION WITH CD 99 IMMUNSTAINING AND REVERSE TRANSCRIPTASE –POLYMERASE CHAIN REACTION

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BACKGROUND: Ewing's sarcoma (ES) is a rather common, highly malignant primary malignant tumor of bone and soft tissues, and a single pathologic entity with primitive neuroectodermal tumor (PNET). They most commonly involve the deep soft tissues. Situation of this tumor in superficial locations is rare, female genital tract is also an unusual location. We describe a case of ES/PNET occuring as a primary neoplasm in the vulva. To our knowledge, this is the seventh such case reported in the literature METHODS Tumor tissue sections were stained with hematoxylin and eosin, periodic acid schiff (PAS) and PAS-diastase. Immunohistochemical staining for the MIC2 gene product CD99, pankeratin, chromogranin A, synaptophysin, desmin, leucocyte common antigen (LCA), HMB-45 were performed. The t(11;22)(q24;q12) translocation in tumoral tissue was revealed by reverse transcriptase polymerase chain reaction (RT-PCR). CASE REPORT. A 23-year-old woman with 4x4cm left labia majora mass and bilateral pelvic lymphadenopathies is presented. An incisional biopsy revealed a small round cell tumor. The patient underwent left radical hemivulvectomy, inguinal, femoral and paraaortic lymphadenectomy. Tumor was solid, hemorrhagic mass, 6 cm in maximal dimension with 6x4 cm skin ellipse on it. Cut surface was grayish-yellow and friable with infiltrative margins. Diffusely growing cell masses, lobules were seperated by fibrovascular connective tissue.The tumor cells were characterized by dark or vesicular, round nuclei with one to two indistinct nucleoli and scant pale cytoplasm. Immunohistochemical stains revealed that the tumor cells were diffusely immunreactive for vimentin and CD99 (the product of MIC2 gene). Cytokeratin, desmin, HMB-45, LCA, chromogranin, synaptophysin were all negative DISCUSSION: Most frequently extraskeletal ES/PNETs are localized in soft tissues of lower extremities, paravertebral region, chest wall and retroperitoneal region. Gynaecological system is an unusual cite for ES/PNET as is rare involvement of vulva. ES/PNETs have to be kept in mind in spite of its rarity when small round cell tumor is seen at superficial locations as vulva especially in children and young adults.

PP4-24

IMMUNOHISTOCHEMICAL STUDY OF PAX-2 IN ENDOMETRIAL HYPERPLASIA AND CARCINOMA

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BACKGROUND: Pax-2 is a paired box gene which has been reported to be activated by estrogen in endometrial carcinoma but not in normal endometrium. However, there are limited studies about Pax-2 expression in endometrial hyperplasia and carcinoma. This study examines the Pax-2 expression in endometrium, aiming to discover any help in the differential diagnosis between atypical hyperplasia and well differentiated endometrioid adenocarcinoma, knowing the major problem in inter observer variability. Moreover, the distinct pattern of Pax-2 expression would be help in the comprehension of endometrial carcinogenesis, related to unopposed estrogen stimulation. METHODS: Immunohistochemical analysis of Pax-2 expression was performed on 57 endometrial specimens: 18 non atypical hyperplasia, simple or complex (SH/CH), 13 atypical hyperplasia (CAH) and 26 endometrioid carcinomas (EC). We used a rabbit polyclonal antibody, Pax-2 (1:50 dilution, Zymed, CA, USA) in established methodology. We estimated only nuclear staining, using a semi-quantitative method as follows: 0=< 5%, 1+=5-25%, 2+=26-50%, 3+=51-100%.Normal endometrial glands, as were uniformly strong positive, were served as positive control. RESULTS: Pax-2 was uniformly expressed in normal endometrium (atrophic or functional). Non-atypical hyperplasia's epithelial nuclei were positive in 16 cases (88,9%, 2+or 3+) while only in 4 cases of CAH (30,76%).Mainly, mild to moderate Pax-2 expression demonstrated in 8 endometrioid carcinomas (30,8%, 4:1+ , 3:2+, 1:3+). CONCLUSIONS: Normal endometriun expresses Pax-2 uniformly and strongly .There is a loss of expression from non- atypical to atypical endometrial hyperplasia and endometrioid carcinoma. The same expression between atypical hyperplasia and endometrioid carcinoma doesn't permit the use of antibody as a tool in the differential diagnosis .There is a need of further studies in order to be comprehended the relation between Pax-2 expression and ER, the methylation status of the gene and the possibility to convert this polyclonal antibody to a monoclonal one.

PP4-25

PRIMARY INVASIVE SQUAMOUS CELL CARCINOMA OF THE VAGINA: HPV DNA DETECTION, AND P16INK4A, p53, AND PRB IMMUNOEXPRESSION IN 22 CASES

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Background: Primary invasive squamous cell carcinoma (SCC) of the vagina is rare, and the role of human papilloma virus (HPV) in its pathogenesis remains unclear. Alterations of the cell cycle regulatory proteins p16INK4a, p53 and pRb are poorly known in primary invasive SCC of the vagina. The aim of our study was to investigate HPV DNA, and p16INK4a, p53 and pRb immunoexpression in 22 cases of primary invasive SCC of the vagina. Material and Methods: Tumors (n=22) were classified according to the WHO classification. HPV genotyping (INNO-LiPA HPV Genotyping v.2) was performed in 21 cases. Immunohistochemical staining for p16INK4a, p53 and pRb was assessed in the whole series. Statistical analysis was performed with Fisher's Exact Test and with Student's t-test. Results: The

patients' age ranged from 36 to 88 (mean 65.17) years. Six cases were keratinizing SCC, and the remaining 16 cases were non-keratinizing SCC (8 "common" non-keratinizing, 3 basaloid, and 5 warty types). The median age of patients with keratinizing SCC was 73.83 yrs. and that of non-keratinizing SCC patients was 62.93 yrs. (p=0.057). HPV DNA was detected in 17 cases (80.9%): 13 (86.7%) non-keratinizing SCC (in one case HPV genotyping was not performed) and 4 (66.7%) keratinizing SCC (p=0.31). The HPV genotypes identified were: 6, 11, 16, 18, 31, 33, 35, 40, and 58. HPV 16 DNA was the most prevalent, being identified in 7 specimens (33.3%); 16 cases (76.2%) had high-risk HPV DNA. In 4 cases (19.0%) multiple HPV genotypes were isolated. p16INK4a was express in 15 cases (68.2%), p53 in 18 cases (81.8%), and pRb in 12 cases (54.5%). Most HPV DNA positive cases were p16INK4a positive (HPV positive: 70.6% vs. HPV negative: 50%), without statistical significance. Likewise, there was no statistical association between HPV DNA detection, and p53 or pRb expression or patients' age. Conclusions: 1) Similarly to uterine cervix SCC, primary SCC of the vagina is frequently associated with HPV DNA, most cases having high risk HPV DNA, with HPV 16 predominance. 2) There was no statistical difference between HPV DNA detection in keratinizing and non-keratinizing SCC, in contrast to vulvar SCC. 3) p16INK4a expression was not associated with HPV DNA detection. 4) p53 is frequently overexpressed in primary SCC of the vagina.

PP4-26
DIFFUSE OVARIAN STROMAL TYPE METAPLASIA IN TUBAL SUBEPITHELIAL TISSUE

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BACKGROUND: Even there were few numbers of reports about 'ovarian thecal metaplasia in adrenal glands' in the pathology literature, no case reports describing diffuse ovarian stromal metaplasia of tuba uterina exist. CASE REPORT: A 48-years-old woman, gravida 2, para 2, presented with a 3 weeks history of vaginal bleeding. Pelvic ultrasound (USG) examination revealed a simple adnexal cyst. Also USG revealed 6 cm diameter leiomyomatous mass in uterus. The patient underwent total abdominal hysterectomy bilateral salpingooophorectomy. Grossly: the right fallopian tube was cystic measuring 6cm in diameter and was containing serous fluid.The outer surface of the cystic tube was smooth while inner surface was thick and folded diffusely and had a tan-white color. Other tube and bilateral ovaries had not any specific feature. Microscopically; the stroma of the cystically dilated tuba showed diffusely transformation to dense ovarian stroma under the typical tubal epithelium..There were luteinized stromal cells singly or in the form of small clusters.. These cells were immunreactive for alpha-inhibin. DISCUSSION Other than endometriosis the tubal epithelium may undergo metaplastic changes. Despite of these few cases of epithelial type metaplasia, we couldn't find any ovarian stromal type metaplasia mentioning report in the literature other than focal fimbrial stromal transformation to ovarian stromal type especially in postmenopausal women.In current case; the change is diffuse and related with hydrosalpinx. To our knowledge, we report the first case of diffuse ovarian stromal type of metaplasia in fallopian tube and suggest that it can be the origin of any type of sex-cord stromal tumors of ovarian type.

PP4-27
MICROVESSEL DENSITY AND IMMUNOHISTOCHEMICAL EXPRESSION OF THE ANGIOGENIC PROTEINS VEGF, VEGFR1 AND VEGFR2 IN NORMAL, HYPERPLASTIC AND NEOPLASTIC ENDOMETRIUM

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Background: Vascular endothelial growth factor (VEGF) may play an important role in the multistep process of endometrial carcinogenesis by its effect on angiogenesis and tumor cell proliferation. Therefore, the immunohistochemical expression of VEGF, its specific receptors VEGFR1 and VEGFR2, and the microvessel density (MVD) were analysed in normal, hyperplastic and neoplastic endometrium in relation to MIB1/Ki67 and p53 protein expression as well as to clinicopathological parameters. Materials and Methods: The expression of VEGF, VEGFR1 and VEGFR2 proteins was studied by immunohistochemistry in 31 cases of normal endometrium (15 proliferative and 16 secretory), 36 cases of endometrial hyperplasia (19 simple and 16 complex) and 80 cases of endometrial carcinomas (56 endometrioid and 24 non-endometrioid). MVD was also estimated in all specimens after immunostaining for CD34 antigen and evaluated as the mean (+SD) vessel count/high power microscopic field (HPF). Spearman's correlation coefficient test was used for the statistical analysis. Results. The expression of VEGF protein was estimated in epithelial cells. In normal endometrium, a significant higher VEGF protein expression was observed in proliferative than in secretory endometrium ($p<0.0001$). Moreover, VEGF protein expression was significantly higher in hyperplastic than in normal endometrium and in carcinomatous than in hyperplastic endometrium ($p=0.002$ and $p<0.0001$, respectively). In endometrial carcinomas, significant positive correlations were found between VEGF/VEGFR1 ($p<0.0001$), VEGF/VEGFR2 ($p=0.049$), VEGF/MVD ($p<0.001$), VEGF/histological grade ($p=0.007$), VEGF/MIB1 ($p=0.005$) and VEGF/p53 ($p=0.01$). Conclusions: The results of this study suggest that VEGF may play an important role in endometrial carcinogenesis by acting a) as a paracrine growth factor promoting angiogenesis and b) as an autocrine growth factor promoting tumor cell proliferation. Moreover, VEGF protein expression may be regulated by p53 in endometrial cancer.

PP4-28
HEPATOID CARCINOMA OF THE OVARY

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Hepatoid carcinoma (HC) is a rare malignant tumour defined as a primary extra-hepatic tumour morphologically mimicking hepatocellular carcinoma (HCC). It has been described in several organs including the ovary.Differentiating this tumor is not only a challenge but also critical, since treatment modalities and operative strategies are dependent upon the exact nature of the hepatoid cancer. We present a rare case of HC of the ovary .A 51-year-old woman was hospitalized with abdominal pain and distention. Physical examination revealed pelvic mass, and abdominopelvic computed tomography showed bilateral ovarian and multiple colonic masses. There was no tumoral mass in the

hepatic parenchyma, other than capsular penetrations of the colonic tumor. Serum CA 125 level was high (638 U/ml), but serum Alpha Fetoprotein (AFP) was not elevated (1.5 ng/ml). The patient was treated with hysterectomy and bilateral salphingooopherectomy, followed by chemotherapy. Colonic tumours were accepted as inoperable. Histopathologic examination revealed a tumour consisting of large neoplastic cells having vesicular nuclei and abundant eosinophilic cytoplasm resembling hepatocellular carcinoma. Neoplastic cells expressed both CA 125 and AFP .The patient had a poor survival and she died after 20 months following the diagnosis.Based on a review of the literature, we discuss the guidelines for differentiating these tumours and utilize these criteria to differentiate these tumours irrespective of their primary tissue of origin. In conclusion, HC of the ovary should be included in the differential diagnosis of ovarian tumors composed of large eosinophilic cells.

PP4-29
MASPIN EXPRESSION IN ENDOMETRIAL HYPERPLASIA AND CARCINOMA, AND ITS RELATION WITH ANGIOGENESIS

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Background: The purpose of this study was to evaluate the prognostic significance of maspin expression in endometrial hiperplasia and cancer, and also to investigate its relation with angiogenesis. Methods: A total of 19 women with complex atypical hyperplasia, 44 patients with simple hyperplasia without atypia, and 67 patients with endometrial carcinoma were included. Maspin expression was assessed by immunohistochemistry and tested for possible significant relation with age, FIGO stage, histologic type, grade, depth of myometrial invasion, lymphovascular space involvement, lymph node metastasis, and overall survival . Angiogenesis was determined by vascular endothelial growth factor staining. Results: Maspin expression was detected in only three patients with endometrial hyperplasia. In patients with endometrial cancer, cytoplasmic and nuclear maspin expressions were detected in 36 (53.7%) and 18 (26.9%) patients, respectively. No significant relation was noted between prognostic variables and staining localizations. The 5-year OS rate for patients with cytoplasmic staining was 91%, compared to 87% for patients without staining (p=0.31). The values for nuclear expression were 100% and 87%, respectively (p=0.16). The cytoplasmic and nuclear maspin expressions were found to be significantly correlated with VEGF (r=0.278, p=0.02; and r=0.295, p=0.02, respectively). Conclusion: This is the first study investigating the relation between maspin expression and angiogenesis in endometrial cancer. Although no significant correlation was noted between the expression of maspin and clinicopathologic prognosticators and no survival difference was noted for cytoplasmic and nuclear expression of maspin, a tendency was detected for nuclear staining similar to the literature. Further series will clarify the exact prognostic role of maspin in gynecologic malignancies including endometrial cancer.

PP4-30
RETROSPECTIVE ANALYSIS OF GYNECOLOGICAL SARCOMAS:

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Background: Gynecological sarcomas, comprised mostly of uterine origin, are rare and generally care a poor prognosis. Histological prognostic features are confusing due to absence of large patient data. The aim of this study was retrospective re-analysis of all the gynecological sarcomas in our institution to evaluate the morphological prognostic factors. Method: Out of 182 cases treated in our institution with gynecological sarcoma diagnosis between 1968-2007, 149 cases were available for pathological examination. Clinical data were obtained from patients' medical records. All cases were examined by the same pathologist. The histologic type, presence and percentage of necrosis, mitotic count per 10 HPF, the grade of atypia, cellularity, lymphovascular space involvement (LVI) and lymph node metastasis (LNM) were evaluated. Survival analysis (Kaplan-Meier) and long rank test were used for statistical analysis. A new scoring system was developed by combining mitotic count (1-5=1, 6-10=2, >10=3), percentage of necrosis (<25%=1, 25-50%=2, >50%=3) and nuclear atypia (mild=1, moderate=2, severe=3). Results: Of the 149 gynecological sarcoma cases, 49.7%(74) were leiomyosarcoma (LMS), 32.2%(48) were carcinosarcoma and 10.1%(15)were endometrial stromal sarcoma (ESS). The uterus was the site of origin in 86.6%(129) of cases. The age at diagnosis varied between 16 - 84 years (mean: 53). Patients were followed-up for a period of 1 to 197 months (mean: 26.5). Considering all the sarcoma cases clinical stage, histologic type, grade of atypia, percentage of necrosis, LNM and mitotic rate were found to be statistically significant for survival (p<0.005). Necrosis and LNM were found to be the prognostic parameters among LMS cases; whereas in carcinosarcomas necrosis, LVI and LNM emerged as statistically significant factors. In addition, the analysis of LMS cases using the newly developed scoring system was found to be a valuable prognostic factor (p=0.0004). Conclusion: Whatever the histologic type and site of origin, this study shows that mitotic rate, percentage of necrosis and grade of atypia are the histological prognostic factors-with varying influence-in gynecological sarcomas. Besides, LVI and necrosis for carcinosarcoma, and necrosis for LMS come out to be the the prominent and statistically significant factors. There appears that no single histological factor is enough to determine the prognosis in gynecological sarcomas. However, this scoring system, combining some of the morphological features, was found to be a valuable prognostic factor for LMS.

PP4-31
HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CORRELATIONS BETWEEN ADENOMYOMATOUS POLYPS, HYPERPLASTIC POLYPS AND ENDOMETRIAL MALIGNANCIES

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Background: Endometrial polyps are considered markers of increased cancer risk, revealing endometrium tendency to develop proliferative lesions. Beside well recognized precursors of endometrioid endometrial carcinoma represented by diffuse endometrial hyperplasia and, in a lesser degree, by hyperplastic endometrial polyps, when associated with cytological atypia, a large interest in recent years has centered on endometrial polyps with myomatous stroma, including those with atypical features. Polypoid adenomyomas cover a large spectrum of proliferative benign and premalignant changes, being designated as low malignant potential lesions. The aim of our study was to investigate the correlations between histopathological and immunohistochemical features of these neoplasias. Method: As a material, we used 46 polypoid adenomyomas and hyperplastic polyps, selected from our files after excluding frank cancers, conventional polyps, functional polyps, atrofic polyps, mixed

endometrial-endocervical polyps, and decidual pseudopolyps. Results: Histopathological examination identified cytological atypia and associated areas of malignancy. Immunohistochemical staining for ER, PR, MMP-2, MMP-9, Ki-67, and cyclin D1 revealed a significant correlation with hormonal status, expression of stromal components and proliferative activity in cases that associated great cytologic atypia and malignant transformation respectively. Differential diagnosis was difficult due to the typical fragmentation of curretage specimens and included florid hyperplasia, adenomyoma, adenocarcinoma with polypid growth, and mesenchymal tumors. Conclusion: Correct identification of stromal and cellular alterations often provides a crucial clue to the therapeutic orientation and assures prognostic improvement. Identification of histopathological and immunohistochemical markers of intense proliferation associated with invasion activity is important in positive and differential diagnosis with carcinomas, carcinosarcomas and adenosarcomas.

PP4-32
EXPRESSION OF EGFR AND C-KIT (CD117) IN UTERINE LEIOMYOSARCOMAS

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Objectives: Determine the expression of epidermal growth factor receptor (EGFR) and a growth factor receptor with tyrosine kinase activity, c-kit (CD117), in uterine leiomyosarcoma and correlate with clinical and pathologic characteristics. Materials and Methods: Twenty-seven cases of uterine leiomyosarcomas were immunohistochemically evaluated for the expression of EGFR and c-kit (CD117) and correlated with clinical and pathological features. Results: EGFR was positive in 13 cases (48.1%), 71% of patients were younger than 50 years (p=0.002). Statistical analysis showed that expression of EGFR was not an independent predictive factor for recurrence (p=0.22). EGFR expression was also not related to clinical stage or other clinical and pathological features (p=0.7). Mean survival in cases positive for EGFR was 17 months in comparison with EGFR negative cases, for which mean survival was 60 months (p=0.068). C-kit (CD117) was positive only in one case of uterine leiomyosarcoma (3%), therefore, no valid statistical conclusions could be established. Conclusions: Treatment of uterine leiomyosarcomas is generally poor; the use of targeted therapies may help to improve long term control and survival. Expression of two specific targets for cancer therapy, EGFR and C-kit, did not appear to demonstrate any specific correlation with survival or clinical and pathological features in uterine leiomyosarcomas.

PP4-33
HISTOGENESIS OF LIPOMATOUS COMPONENT IN UTERINE LIPOLEIOMYOMAS

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Background: Uterine neoplasm composed of an admixture of smooth muscle and adipose tissue are relatively common and have been designated as lipoleiomyomas. The origin of this tumor is still controversial and it has not been sufficiently studied. Aim of our research was to investigate the immunohistochemical phenotype of the fat cells in uterine lipoleiomyomas for clarifying their origin. Methods: Archived tissue samples of 10 uterine lipoleiomyomas were selected and analyzed immunohistochemically for vimentin, desmin, and HMB45 expression. Results: The patients ranged from 31 to 63 years of age (mean age 53.5±9.9). Seven tumors affected the uterine corpus, and were located intramural; two cases were subserosal, and the one was in the cervix. All tumors were constituted by irregular bundles of smooth cells and mature large adipose cells. The amount of adipose component varied from 5 to 95 % of the tumor mass. Cytological atypia and necrosis were not seen. The immunohistochemical investigations revealed obvius reactivity to vimentin and desmin in perivascular and tumor smooth muscle cells. Adipose cells in the tumors demonstrated uniformly vimentin expression and inconsistent desmin immunreactivity. All adipose cells were negative for HMB45 antigen. However, HMB45 antigen was weakly positive in spindle shaped tumor cells of two cases. Conclusions: The immunohistochemical findings suggest a complex histogenesis for these tumors, which may arise from perivascular immature mesencyhmal cells or direct transformation of smooth muscle cells into adipocyte by means of progressive intracellular storage of lipids.

PP4-34
VULVAR CARCINOSARCOMA: A CASE PRESENTATION

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Vulvar carcinosarcoma is extremely rare and the least frequent of all genital localizations with just a few reported cases. In the limited data in the literature, the additional problem of estimating the rarety of this tumour represents inconsistent terminology of these neoplasms. Carcinosarcoma in the genital tract, often reported as malignant Mullerian mixed tumour, in most cases was accepted as being metaplastic or sarcomatoid carcinoma. A proportion of these cases in the vulvar region were confirmed as keratin-positive sarcomatoid carcinoma or squamous cell carcinoma with spindle cell sarcoma-like transformation of the stroma. However, carcinosarcoma is defined as mixed epithelial and mesenchymal tumour with malignant both epithelial and mesenchymal component, either homologous or heterologous by cellular origin. Recently, the clonal origin of the first case of squamous carcinomatous and leiomyosarcomatous diffefrentiation in the same tumour has been genetically proved. We report a case of vulvar carcinosarcoma diagnosed in a 66 year-old woman who underwent radical vulvectomy with bilateral inguinal lymphadenectomy because of rapidly grown tumour in the right labial region. Histopathological analysis presented ill-defined mass measuring 70 mm in maximal diameter and revealed the admixture of well differentiated squamous cell carcinoma and distinctive spindle cell sarcomatous areas with fibrosarcoma-like aspect. Final report showed FIGO II stage without deposits in any of 14 lymph nodes. Immunohistochemical analysis were performed on 4 microns sections from formalin fixed and paraffin-embedded samples using LSAB+ immunostaining method with AEC visualization for: vimentin, smooth muscle actin - alpha, desmin, S-100 protein, CD34, HHF35, GFAP, CD117, NSE, EMA, AE1/AE3, High molecular weight cytokeratins, p53 protein, and proliferative activity measured by Ki-67 protein labelin index. Morphologically readily seen sarcomatous component immuno-histochemically showed the presence of strong cytoplasmic positivity only for vimentin, contrasting to strong positivity for all epithelial markers in the carcinoma; Ki-67 protein labelling index was 42% and immunopositivity for p53 (about 55%). In conclusion, we believe that morphological and immunohistochemical characterizations of two neoplastic populations represent true carcinosarcoma. Besides differential diagnostic significance, it remains unclear whether biclonality or sarcomatoid metaplastic carcinomatous or stromal transformation bear more unfavourable prognosis in these aggressive tumours.

PP4-35

P53, COX-2 EXPRESSIONS AND MICROVESSEL DENSITY IN OVARIAN CARCINOMAS

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Ovarian carcinomas are the sixth of the most common carcinomas. Surface epithelial and stromal tumors are the most common neoplasms in ovarian carcinomas. In molecular studies, the mutation of p53, which is a tumor supressor gene, is positive in %50-60 of invasive serous carcinomas. Neoangiogenesis is an important factor in carcinogenesis. The growth of tumor is thought to be prevented by antagonists of angiogenesis and it can be an effective treatment method. Cyclooxygenase is an important catalyser enzyme in the synthesis of prostoglandins from arachidonic acids. It is suggested that by the epidemiological studies, inhibition of cox-2 with the help of nonsteroidal antiinflamatuar drugs can prevent ovarian carcinomas. We studied on 17 primary serous adenocarcinomas, 3 primary musinous cystadenocarcinomas, 6 borderline serous tumors, 4 borderline musinous tumors,10 serous cystadenomas,10 musinous cystadenocarcinomas retrospectively and researched clinical and histopathological features. For cox-2 expression, the difference between musinous tumors and serous tumors was meaningful.For p53 positivity, the difference between malignant, borderline and benign ovarian tumors and serous versus musinous tumors was meaningful. The difference between malignant, borderline and benign ovarian tumors for MVD>60 was meaningful. There was no correlation between cox-2, p53 and MVD and survival. For reducing the diameter of tumors by neoadjuvant and adjuvant therapies, the methods for reducing the vascularity can be useful. For the treatment of ovarian carcinomas, the experimental studies of p53 gene therapy should be developed. In current pathology practice; in ovarian tumors suspicious for malignancy by the histomophological findings; the positivity of p53, high microvessel density, old age, bilaterality are the helpful factors for differentiating malignant and borderline tumors. For evaluating the role of cox-2 in ovarian tumors there is need for larger series of cases which are chemotherapy resistant and /or chemotherapy responsive in oncology services.

PP4-36

ATYPICAL IMMATURE METAPLASIA OF CERVIX-THE SIGNIFICANCE OF KI-67 IN DIFFERENTIAL DIAGNOSIS WITH SQUAMOUS INTRAEPITHELIAL LESIONS

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Background: Atypical immature metaplasia (AIM) of cervix is an epithelial alteration with uncertain biological and clinical significance. The main issue is to differentiate AIM from squamous intraepithelial lesions, especially high grade squamous intraepithelial lesion (HGSIL) with which it has many morphological similarities. The histological criteria of AIM are a uniform basal cell population, minimal nuclear crowding, variable hyperchromatism, preserved cellular polarity, enlarged or multiple nuclei confined to suprabasal areas and absence of abnormal mitoses. Method and Result: In our study histological feaures and Ki-67 proliferation index were evaluated in the cervical biopsy specimens from 23 cases; including 5 AIM , 6 low grade squamous intraepithelial lesions (LGSIL), 12 HGSIL. The mitotic index and Ki-67 counting in the basal one-third, middle one-third and superficial one-third of epithelium were assessed seperately.For Ki-67 immunoreactivity percentage, at least 200 cells from informative areas were evaluated. The mean Ki-67 indices of AIM, LGSIL and HGSIL were 10,4; 7,9; 37,3 respectively. Overall Ki-67 index was higher than that of LGSIL and lower than HGSIL though the range of Ki-67 indices of AIM overlapped that of both LGSIL and HGSIL. When the Ki-67 indices were evaluated for each 1/3 layer seperately; the discriminative values were found to be for upper and mid third of epithelium rather than the basal third. When the histological features and the Ki-67 indices were evaluated together; 1 biopsy which was thought to be AIM turned out to be HGSIL. Conclusion: AIM is a poorly defined entity and the pathologist can have some hard time when dealing with this lesion especially in the differential diagnosis with HGSIL. Histological criteria are inadequate in such situations and Ki-67 index should be used in assesment of such atypical proliferations of cervix .

PP4-37

COMPARISON OF ESTROGEN, PROGESTERONE EXPRESSION AND ANGIOGENESIS WITH OTHER PROGNOSTIC PARAMETERS IN ENDOMETRIAL CARCINOMAS

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BACKGROUND: Besides the established prognostic parameters in endometrial carcinomas, recent researches revealed that estrogen, progesterone status, angiogenesis, HER-2/neu expression and tumour DNA ploidy have significant prognostic value. This study investigates the relationship between estrogen, progesterone receptor expressions, microvessel proliferation (angiogenesis) and established prognostic parameters. METHOD: Sixty two endometrial carcinoma diagnosed at Dr. Lutfi Kırdar Kartal Educational and Research Hospital are included in this study. Estrogen, progesterone and CD34 receptors are assessed immunohistochemically at sections from the blocks which are the best representative of histological grade, which have the deepest myometrial invasion and in which stroma can be viewed easily. Results are compared with other prognostic parameters including patient age, tumour type, histological grade, myometrial invasion, vascular invasion, lymph node metastasis, endocervical involvement and stage. RESULTS: A statistically significant difference is found between estrogen receptor positivity and tumour type or histological grade ($p<0.01$). A statistically significant difference is found also between progesterone receptor positivity and tumour type or grade ($p<0.01$). Any significant relation is not established between either receptors and other parameters. A significant relation is not found between microvessel proliferation and other prognostic parameters. Although a consistency is seen between estrogen and progesterone expressions, they do not have a significant relation with angiogenesis. CONCLUSION: For the endometrial tumours whose clinical behaviour can not be precisely assessed pre-operatively, the contributions of prognostic parameters investigated post-operatively are important. However, all of the cases do not act in accordance with available prognostic parametres. Such cases act more agressively. The aim is to detect those cases during planning treatment and step up to a more agressive treatment plan. There are several parameters in literature and we chose to assess steroid receptor status and angiogenesis. Although we found significant relation between hormon receptor status and tumour type and histological grade, unlike some reports, we could not find any relation with angiogenesis

PP4-38

A MOLECULAR STUDY ON SELECTED BRCA-1 AND BRCA-2 GERMLINE MUTATIONS IN BREAST, OVARY AND FALLOPIAN TUBE CANCER CASES IN TURKEY

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Background: Germline mutations in BRCA1 and BRCA2 genes increase the susceptibility to ovarian, peritoneal, fallopian tube, and breast cancers. 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 are well known, common mutations among Ashkenazi Jewish people and as frequent as 2.6% of the population. In a background of the mosaic Turkish population and its close relationship with Middle Eastern countries, we aimed to detect these three mutations in a series of young cancer patients. Materials and Methods:A total of 112 histologically confirmed breast (invasive ductal carcinomas), ovary, and fallopian tube (serous carcinomas) cancer patients under the age of 45 regardsless their family history are included. All cases were genetically tested for the three BRCA1-2 founder germline mutations (185delAG and 5382insC in BRCA1 and 6174delT in BRCA2). The analysis was performed on genomic DNA extracted from paraffin embedded tumor tissues. We used a simple and rapid method for the simultaneous detection of these three mutations. Allele-specific oligonucleotide primers designed on the basis of BRCA1 and BRCA2 sequences were used. Mutation in one of the alleles was detected by the presence of two bands. For the 185delAG mutation, the mutant and wild-type amplicons were 354 and 335 bp, whereas those of 5382insC were 295 and 271 bp, and those of 6174delT were 171 and 151 bp. Results:All breast carcinoma cases were negative for the three mutations. Among others, a case of ovarian serous carcinoma from a 42-year old woman, showed mutation in BRCA1 5382insC. The patient had no history of familial ovarian and/or breast cancer. The tumor was unilateral and revealed high grade morphologıc features. Conclusion: Detection of a BRCA1 mutation in a series of younger ovarian cancer patients is an important finding and may point more mutations among the population.

PP4-39

ADULT GRANULOSA CELL TUMOR OF THE OVARY: STUDY OF 20 CASES

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Background: Among various ovarian neoplasms, granulosa cell tumors of the adult type (GCT) correspond to less than 5%. They have low malignancy and are capable of recurring even after many years. They present many diverse histological aspects, whose differential diagnoses must be made with other primary or metastatic tumors in the ovary. Objective: to describe and to analyze clinical and pathological aspects of GCT and relate them to the evolution of the disease. Method: in a 10-year (1995-2004) review of the UNICAMP Clinical Hospital files, twenty CGT diagnoses were found, and these records were reviewed, correlating clinical aspects with morphological patterns, mitotic index, stage of the disease and frozen section diagnosis accuracy. Results: the ages varied from 27 to 79 years (mean: 53 years) and the follow-up from 12 to 96 months (mean: 42). The main symptoms were: postmenopause bleeding (45%), abdominal pain (35%) and palpable mass (25%). In gross analyses, most of the tumors were yellowish (60%) and the solid aspect was more common (40%) than the cystic or solid-cystic. Only three of the nine cases submitted to intra-operative frozen section biopsies were diagnosed as GCT (33%). The histological patterns were: 40% solid, 15% macrocystic and 45% of combined patterns, with low mitotic index. The clinical stage was: 13 cases in Ia (65%), 2 cases in Ic and 5 in IIIc. Only the stage of the disease was related to the recurrences. In 3 of 14 hysterectomies there was simple endometrial hyperplasia without atypia. Conclusion: The diagnosis of CGT generally occurs after the menopause, and intra-operative biopsies are seldom conclusive. Only the advanced stage was related to the worst prognosis.

PP4-40

DIFFUSE LARGE B-CELL NON-HODGKIN LYMPHOMA (DLNHL) - MIMICKING A PRIMARY GYNECOLOGICAL TUMOR

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BACKGROUND: Bilateral ovarian involvement mimicking a gynecologic malignancy in adults is extremely rare. Here, we report a patient with DLNHL, mimicking a gynecologic tumor. Aim: To determine the origin of the tumor, using immunophenotyping, with the introductory help of cytological imprints that had been taken simultaneously with the intraoperative frozen-section tissue samples. The very cytological imprints gave the clue way and reveal the possible true origin of the tumor-cells, differentiating lymphoblasts from others like “small round blue cell”- tumors. METHOD: A 57 year old Serbian rural woman, presented with the complaint of persistent lower abdominal distension with dull pain, and night sweats. Imaging studies revealed a huge ovarian mass, suggesting a large ovarian tumors. Surgery-laparatomia with total hysterectomia and bilateral adnexetomia and total omentectomia with intraoperative frozen-section analyses was performed. DLNHL diagnosed by a combination of: cytology, morphology and immunophenotype together with clinical features (“B-symptoms”-associated with lymphoma), managed with surgery and chemotherapy (MABTHERA). Subsequently patient staged, according Ann Arbor classification-IV B. RESULTS: The average diameter of the ovaries was 16 cm (range 12-20 cm), grossly- lobular surface, pink-grey color, soft consistency. Microscopic findings: ovaries, myometrium as well as peritoneal implants were completely replaced by the monotonous, uniform population of tumor cells-centroblasts. By the use of IHH: tumor cells have shown excessive immunopositivity towards CD20, CD79a, Ki67 (80%) and bcl2 (40%) while negative towards CD3, CD5, CD138, MPO, ALK and CK. CONCLUSION: Although rare secondary ovarian tumors i.e.lymphomas should be considered in the differential diagnosis of female adults with ovarian masses. Bilateral ovarian masses or large masses indistinguishable from the ovaries, particularly in the presence of other metastatic foci (secondary deposits in lungs), may help distinguish primary from secondary ovarian malignancies.

PP4-42

PREDICTORS OF RESIDUAL DISEASE ON HYSTERECTOMY AFTER LOOP ELECTROSURGICAL EXCISIONAL PROCEDURE WITH POSITIVE MARGINS

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Background: Loop electrosurgical excisional procedure (LEEP) is the treatment of choice in patients with cervical intraepithelial neoplasia (CIN). Positive surgical margins may be the most important prognostic factor for residual disease and patients have to undergo hysterectomy for definitive treatment with a rate as high as 50% of specimens negative. This study was performed in intent to identify predictors for residual disease in hysterectomy specimens of patients with positive margins in LEEP specimens. Material and methods: Pathological files were reviewed from 1998 to 2003. Cases with LEEP and positive margins that were undertaken to hysterectomy were identified. Histopathologic slides from the LEEP and hysterectomy were reviewed to confirm margin status, grade of lesion, and residual neoplasia. Variables such as age, parity, number of sexual partners, time from LEEP to hysterectomy, size of lesion, percent of affected fragments, and number of positive margins (endocervical, exocervical, or both) were analyzed. Univariate analysis was performed using Chi-square test, t test, and Wilkoxon Rank Sum. Multivariate logistic regression analysis was used to test relationships between preoperative factors and presence of residual disease on the final pathologic report. Results were considered statistically significant if $p < 0.05$. Results: 98 specimens were evaluated, median age was 42 years (range 25-78), age at first intercourse 17y (range 12-34), number of sexual partners 2 (range 1-10), parity 5 (range 1-16), and days prior to hysterectomy 48 days. Of LEEP specimens median size of lesion was 0.5 cms (range 0.1 to 2cm.). Median of percent of fragments affected was 40% (5-100%). Exocervical involvement was present in 6%, endocervical 64%, both 30% respectively. Median number of fragments with endocervical margins affected 3 (range 0-11), exocervical 0 (range 0-10). Of hysterectomy specimens 56 (58%) were negative residual disease, 2 (2.1%) CIN II, 2 (2.1%) CIN III, 31 (32.3%) In situ carcinoma, 2 (2.1%) microinvasive, 3 (3.2%) invasive carcinoma. On multivariate analysis variables that independently predicted residual disease were age (OR= 1.049, p= 0.05) and percent of affected fragments (OR= 9.74, p=0.038). Conclusion In our institution we have a high rate of negative hysterectomy specimens which is similar to other reports. Age and extent of disease (percent of affected fragments) are statistically significant predictors of residual disease rather than positiveness of margins as well as number of positive margins.

PP4-43

RARE TYPES OF CARCINOMAS LOCALIZED IN THE ENDOMETRIUM

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Background: There have been reported rare examples of unusual carcinomas arising in the endometrium, but the data consist largely of case reports precluding a detailed clinicopathologic analysis. Material and method: We present 3 cases of endometrial carcinomas, each of them with different clinicopathologic features. The specimens were surgically treated, routinely processed and stained with H&E, PAS, van Gieson, and a panel of immunohistochemical antibodies. Results: One case was histopathologically diagnosed as synchronous carcinoma of the endometrium and uterine cervix, one was a metastatic mucous adenocarcinoma in the endometrium, primary located in the ovary, and the last one was diagnosed as poorly differentiated endometrioid carcinoma of the endometrium, developed in a background of inflammation. Conclusions: Cervicitis, salpingitis, endometritis, and myometritis may represent an important background for the development of an endometrial carcinoma. Immunohistochemical studies may be helpful for distinguish a metastatic carcinoma from an independent tumor, but the differential diagnosis usually can be determined by conventional clinicopathologic criteria. The distinction is very important because the prognosis and treatment are different.

PP4-44

REAPPRAISAL OF OVARIAN METASTASIS FROM GALLBLADDER AND EXTRA-HEPATIC BILE DUCT CARCINOMA

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Background: Metastatic ovarian tumor derived from gallbadder or extra-hepatic bile duct (GB/BD) is estimated about 7% in overall ovarian metastasis. Especially, tumor-forming metastasis (classical or tubular Krukenberg tumor; KT) is extremely rare and most of all are limited in case reports. The aim is to reappraise ovarian metastasis originating in GB/BD. Methods: From autopsy files, 40 female GB/BD carcinomas were reviewed. We evaluated presence of the ovarian metastasis, number of other metastatic organs, presence of peritoneal dissemination, histology. Furthermore, 11 KTs from eight (7; surgical) cases were clinicopathologicaly evaluated. Results: Patients were 56 to 97 years of age. In 12 (30%) cases, ovarian metastasis was identified (bilateral: 4, unilateral: 8). The mean number of involved organs other than ovary was 4.42 (0-7) in contrast to 2.96 (0-6) in cases without ovarian metastasis. 11 cases were involved by peritoneal dissemination. All but one ovary was macroscopically normal or atrophic. Only one (1/12: 8.3%) KT, which measured 5x4 cm, showed multilocular appearance. Histologically, the metastatic cases consisted of 8 tubular adenocarcinomas, 2 signet-ring cell carcinomas. The rest was one case of clear cell, papillary adenocarcinoma, respectively. KT patients were 49 to 71 years of age. Five primary sources were gallbladder and three were bile ducts. With surgical cases, two were at stage II, III, respectively other than three at stage IV. The duration between biliary surgery and oophorectomy were 5 to 68 months. The laterality was shown as unilateral; 3 as bilateral; 5. In 11 ovarian tumors, the specimens ranged from 3.1 to 17 cm in greatest diameter and four were just described such as newborn's or adult head's size. They showed predominantly solid (4), solid and cystic (2) and multilocular (5) appearance. Eight tumors composed of tubular adenocarcinoma (one with foci of signet-ring cells) and each one was papillary adenocarcinoma and signet ring cell carcinoma. All cases were associated with cancerous ascites or peritoneal dissemination. From 7 to 12 months (mean; 9.8) after oophorectomy, 5 patients were died of disease and two was alive with disease for 12, 13 months, respectively. Conclusion: Ovarian metastasis from GB/BD carcinomas is not unusual at the advanced stage, but rare with KT. Classical KT is rare while most cases are tubular ones. KTs may be early or primary manifestation of metastasis in some cases, but frequently associated with peritoneal dissemination, the prognosis is poor.

PP4-46

PERITONEAL INCLUSION CYSTS: A CLINICOPATHOLOGIC STUDY OF 20 CASES

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Background: Peritoneal inclusion cysts are uncommon mesothelial proliferations that typically occur in the peritoneal cavity of women in the reproductive age. It was reported that they have tendency for recurrence, however; malign transformation has never been reported in the literature. This study was planned to determine the clinicopathologic features of peritoneal inclusion cysts. Methods: All patients with a diagnosis of peritoneal inclusion cyst were studied retrospectively from a retrospective

database at Ege and Pamukkale University Hospital between January 1995 and March 2007. Results: Twenty patients with a confirmed diagnosis of peritoneal inclusion cyst were identified. All the patients were women ranging in age from 20 to 56 years old (mean 37 years). The associated clinical conditions were endometrioid adenocarcinoma for 1 patient, endometriosis for 3 patients, mature cystic teratoma for 2 patients, pregnancy for 4 patients, leiomyoma of the uteri for 7 patients. The commonest location of the tumor mass was below the pelvic diaphragm. The size of the tumor was larger than 5 cm in 5 patients. The tumors presenting in the peritoneum were multilocular and large. The rest of the tumors were small. The septa consisted of loose fibrovascular tissue. No smooth muscle and no significant amount of inflammatory cells were present. Microscopically, the majority of cysts were lined with a single layer of flattened or cuboid mesothelial cells. These cells stained positively for calretinin and were negative for endothelial marker CD31. Conclusion: Peritoneal inclusion cyst is a rare and distinctive lesion of mesothelial origin. Most of the patients have a history of a previous pelvic operation, and endometriosis without recurrence, suggesting a role for inflammation in the pathogenesis of the cysts as a peritoneal reactive proliferation rather than the neoplastic process.

PP4-47

"FETAL-PLACENTAL UNIT": PROTOCOLIZED STUDY OF 2847 PLACENTAL SPECIMENS AND 432 FETUS IN THE LAST 7 YEARS.

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Introduction: Both placenta and fetus have a common origin (Fetal- Placental Unit) for that reason it should be studied together in cases of fetal death. In other cases, obstetric and clinical information should be carefully recorded. In this condition, placental examination and their study will be useful for patients, obstetricians and pediatricians. The indications for placental examination should be recomended in diverse causes of fetal, placental, maternal u obstetric disorders possibles of maternal or fetal problems. This could be use to prevent and control pathologies in following pregnancies. Material and Methods: In our Pathology Department have been examined 2847 Placentas since year 2000. They were received fresh and the handling of the placentas was according to a stablished protocol that includes: reception, description, cultures, frozen sections of umbilical cord, postdelivery drainage, membranes roll, disc weight after removing cord, membranes and clots, formalin fixation, disc sectioning and standarized sampling for microscopic examination. Fetus were all autopsied, Rx taken, photographs if needed and any anomaly described. Results: Every pathology report was sent to the Obstetrician and Perinathology Departmens, and those with funiculitis in frozen section by urgent shipment. Every autopsy report included the placental report, and a summary about the correlation of findings. The fetal reports were also sent to the Obstetrician and Perinathology Departments, and rewied in the Clinical- Pathological Conference. Conclusions: 1- The application of this Protocol developed in consensus with the Obstetrics Department, has been very useful in the clinical-pathological correlation of the feto placental pathology, and in the handling of the obstetrical pathology in following pregnancies. 2- Frozen sections of umbilical cord have been useful in early detection of fetal inflammatory response and adverse neonatal outcome.

PP4-48

PREVALENCE AND CHARACTERISTICS OF ENDOMETRIAL POLYPS IN PATIENTS WITH ABNORMAL UTERINE BLEEDING

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Background/Aim. The prevalence of endometrial polyps in the general female population is about 24%. Abnormal uterine bleeding is frequently the presenting symptom of endometrial polyps. The aim of this study was to determine prevalence and characteristics of endometrial polyps in patients with abnormal uterine bleeding. Methods:. The prevalence and characteristics of endometrial polyps were investigated in 961 patients with abnormal uterine bleeding who underwent dilatation and curettage (D&C) between January and December 2006. Statistical analysis was performed. Results: Endometrial polyps were found in 211 (21.94%) patients with abnormal uterine bleeding. Histopatologically, 175 (82.93%) polyps were benign, 29 (13.74%) polyps had simple and complex hyperplasia, 5 (2.37%) polyps had atipical hyperplasia, and 2 (0.95%) polyps had endometrial carcinoma. According to the malignant potential, endometrial polyps were divided into a group of benign (benign polyps and polyps with simple and complex hyperplasia) and a group of premalignant and malignant (polyps with atypical hyperplasia or endometrial carcinoma). Group of premalignant and malignant polyps were associated significantly with older age, postmenopause and hypertension. Conclusions. The prevalence of endometrial polyps in patients with abnormal uterine bleeding according our data was 21.94%. Atypical hyperplasia and endometrial carcinoma were rarely confined to a polyp. Older age, postmenopause and hypertension may incrise the risk of premalignant and malignant changes in endometrial polyps.

PP4-49

THE PROGNOSTIC IMPORTANCE OF GLUT 1 AND KI-67 EXPRESSION IN OVARIAN EPITHELIAL TUMOURS

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BACKGROUND: In epithelial malignancies, aberran expression of glucose transporter 1 (Glut 1), a glucose transport protein located at cell surface and its relation with neoplastic progression is well known. Beside this, protein derived Ki-67 antigen which is observed during proliferative cell cycle indicates proliferation of tumour cells. We studied Glut 1 and Ki-67 expression in benign, borderline, malignant serous and mucinous ovarian tumours, their significance as a prognostic factor in borderline and malignant tumours, their relation with serum Ca 125 levels and other prognostic parameters. METHOD: Ninety two cases (34 serous carcinoma, 4 mucinous carcinoma, 9 borderline serous tumour, 6 borderline mucinous tumour, 19 benign serous cyctadenocarcinoma, 15 benign mucinous cystadenocarcinoma, 5 ovarian endosalphingiosis) in which Glut1 Ab-1 and Ki-67 investigatement of immunohistochemical studies on sections obtained from paraffin blocks were diagnosed at Dr. Lutfi Kırdar Kartal Education and Research Hospital. Patients were analysed according to age, tumour grade, stage and serum Ca 125 levels. RESULTS: In benign, borderline, malignant serous and mucinous tumours and ovarian endosalphingiosis, regarding Glut 1, Ki-67 expression and serum Ca-125 levels were found to be significantly different ($p<0.01$). Glut 1 did not show any staining in benign cases and endosalphingiosis while 86.7% of borderline cases and 97.4% of malignant cases revealed positive staining. More than 1% positive nuclear staining was observed with Ki-67 in 35.3% of benign cases, 80% of borderline cases and 89.5% of malignant cases. Moreover, Glut 1 staining degree with serous

tumours was found significantly higher than mucinous tumours ($p<0.05$). Malignant and borderline cases were significantly correlated with each other due to their Ki-67 and Glut 1 staining degrees ($p<0.05$). Also, statistically significant correlation was found between Glut 1 staining degree with tumour stage and tumour grade ($p<0.05$). Ca 125 levels of serous tumours were found higher than musinous tumours ($p<0.05$). Invasive tumours had higher Glut 1, Ki-67 staining degrees and mean serum Ca 125 levels than borderline tumours ($p<0.05$). CONCLUSION: This study revealed that Glut 1 expression in serous and mucinous ovarian tumours is related with malignancy progression and other prognostic parameters. Although Ki-67 expression is related with malignancy progression, any other significant relation is not found with other prognostic parameters of malignant tumours.

PP4-50

A TEN YEAR REVIEW OF THE PATHOLOGY OF IMAGE-GUIDED PERITONEAL CORE BIOPSIES IN WOMEN PRESENTING WITH PERITONEAL CARCINOMATOSIS

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BACKGROUND: The commonest cause of peritoneal carcinomatosis in women is ovarian or primary peritoneal carcinoma. Presentation in the vast majority of women is with disseminated abdominal disease. Surgery in advanced cases is unlikely to achieve optimal debulking in some patients and therefore, primary neoadjuvant chemotherapy with the aim of achieving substantial cytoreduction to be followed by interval debulking surgery is a management option. Other patients with advanced disease are too ill for major cytoreductive surgery and chemotherapy is the treatment of choice. Therefore, an accurate histological diagnosis to establish the primary site of origin is paramount for the management of these patients. Image-guided peritoneal biopsy is a simple and safe technique to provide sufficient tissue in the majority of cases to reach an accurate diagnosis. METHOD: A series of 201 women who presented to a teaching hospital with peritoneal carcinomatosis over a ten year period underwent image-guided (abdominal ultrasound or computer tomography) peritoneal core biopsy, following multidisciplinary team review. Multiple cores were obtained and examined with haematoxylin and eosin. Ancillary techniques were used where indicated by the clinical situation; this was particularly helpful in the distinction of endometrioid adenocarcinoma from metastatic colorectal carcinoma. The slides of any known previous malignancies were reviewed and compared with the current biopsy to exclude recurrence. RESULTS: A primary site of origin was identified in 188 women; of these 164 were mullerian in origin, the majority of which were serous papillary carcinoma (130). The remainder were endometrioid 7, mucinous 4, clear cell 3, squamous cell carcinoma 1, mixed endometrioid and serous 2, carcinosarcoma 7, poorly differentiated mullerian carcinoma 10. 24 originated from other sites (gastrointestinal tract 9, breast 6, lymphoma 3, pseudomyxoma 2, hepatobiliary 2, renal 2). In 7 women the biopsy showed poorly differentiated carcinoma with no primary site identified and in 5 women the biopsies showed benign changes. In 1 patient the initial biopsy led to a misdiagnosis and a laparoscopic biopsy was required to make the definitive diagnosis. In 10 women there was insufficient material on the initial biopsy to make a diagnosis, a final diagnosis was made on repeat biopsy. CONCLUSION: In conclusion, peritoneal core biopsy in a multidisciplinary setting, together with appropriate biochemical markers, is a quick, efficient and safe method of making a reliable, definitive diagnosis in the vast majority of women with peritoneal carcinomatosis.

PP4-52

EXPRESSION OF MATRIX METALLOPROTEINASE-2 AND ITS TISSUE INHIBITOR IN ASSOCIATION WITH CD44V6 IN THE NEOPLASTIC SEQUENCE OF SEROUS OVARIAN TUMOURS

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Background: Variants of CD44, a cell surface adhesion molecule, have been suggested to be important in cancer invasion and metastasis and proposed as a potential prognostic marker in many epithelial and non-epithelial malignancies. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are also known to play a role in invasion and metastasis of many tumours. There have been controversial results regarding the significance of MMPs, TIMPs and CD44v6 for the development and progression of ovarian tumors. Therefore, the aim of the present study was to investigate the expression of CD44v6, MMP-2, and TIMP-2 in ovarian serous neoplasms and to evaluate their clinical significance. Methods: A total of 56 cases, which include 9 serous cysts, 10 serous cystadenofibromas, 3 borderline serous tumours and 34 serous carcinomas was included in the study. Clinical parameters such as age of the patient, tumour size, grade, stage were retrieved from the patient files. Tissue microarray blocks were constructed by taking 3 cores 2 mm in diameter for each case and immunohistochemistry was performed for the evaluation of the expression of CD44v6, MMP-2, TIMP-2 in sections of 3 μm thick. Expression of CD44v6 was evaluated by scoring intensity and percentage of staining, while expression of TIMP-2 and MMP-2 were evaluated only by scoring staining intensity on an arbitrary grading scheme of (+)-(+++). Kruskal- Wallis and Pothoc test were used for statistical analysis. Results: Both MMP-2 and TIMP-2 were expressed by the fibroblastic cells in ovarian stroma as well as vascular endothelium as well as the epithelium while CD44v6 was only expressed in the epithelial tissue. No difference between stromal and epithelial expressions of MMP-2 and TIMP-2 in any of the study groups was observed.The expression of TIMP-2 and MMP-2 were significantly higher in borderline and malignant group than benign group ($p<0,05$), while no such difference was observed between borderline and malignant cases. TIMP-2 expression was higher in serous cystadenofibromas compared with serous cyst ($p<0,05$). There was no significant difference in the CD44v6 expression between the study groups. No correlation was found between the expressions of all three markers and the clinical features of the patients. Conclusion: MMP-2 and TIMP-2 expressions seem to correlate with the neoplastic sequence of serous ovarian neoplasms whereas no such correlation was observed in CD44v6 expression. These findings suggest that there is increased degradation of extracellular matrix by MMPs in parallel with neoplastic progression.

Kidney Pathology

PP4-53
NEPHROBLASTOMA WITH BONE METASTASIS IN A 30-YEAR WOMAN, A CASE REPORT
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Background: Nephroblastoma is rare in adults and thought to have a worse prognosis than its counterpart seen in children. Bone metastasis of nephroblastoma are very rare in the 2 age groups. Method: We report a case of 30 year-old woman, that consulted for abdominal distension associated to pain in the left hip. Results: Imaging findings showed a right renal tumor measuring 18 cm with a condensing lesion of the superior extremity of the left iliac bone. The patient underwent a right nephrectomy and biopsy of the left iliac bone. Histologically, the renal tumor was a biphasic nephroblastoma without cellular anaplasia. The bone biopsy showed a round cell proliferation similar to the blastema cells seen in the renal tumor. The patient had 3 courses of chemotherapy without clinical improvement then has been out of sight. Conclusion: Nephroblastoma in adult with bone metastasis is very rare with a poor prognosis.

PP4-54
PAPILLARY CARCINOMA OF THE KIDNEY: A RETROSPECTIVE STUDY OF 6 CASES
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Background: Papillary carcinomas of the kidney are relatively rare tumors, counting for 4% of renal cancers. Two types are actually recognized, that differ by their morphological and prognostic features. Method: In a retrospective study, we have studied the clinicopathological and imaging features of 6 cases of papillary carcinoma retrieved in an 11- year period (1995-2005) at the pathology department of F. Hached hospital. These tumors were graded according to the new 2004's OMS histopathological classification of renal tumors. Results: In our study, there were 5 men and one woman. The age median was of 68 years with extremes ranging between 57 and 83 years. The tumors were asymptomatic in 3 cases, they were revealed by lumbar pain in 3 cases. A lombar contact and a lombar sensibility were found in 2 cases. Imaging findings were various, but ultrasonography and CT-scan found a neoplastic process in 5 cases. Histopathological examination found that type 2 papillary carcinoma was correlated to a higher nuclear grade, to an advanced infiltration stage and to a poorly prognosis. Treatment consisted in a radical nephrectomy in 5 cases and in a tumorectomy in one case. Conclusion: Actually, it is important to classify papillary carcinoma into type 1 or 2 because of the prognostic implications.

PP4-55
THE MORTALITY AND CLINICALLY MISSED DIAGNOSIS OF AA AMYLOIDOSIS IN RHEUMATOID ARTHRITIS
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Introduction AA amyloidosis (AAa) is one of the most important complications of rheumatoid arthritis (RA). Aim of this study was to determine: 1. the mortality due to AAa in RA, 2. the clinically missed diagnosis of AAa in RA, 3. the correlations between severity, mortality, and clinical recognition of AAa. Patients and Methods A randomized (non-selected) autopsy population of 161 in-patients with RA was studied (diagnosed clinically according to the criteria of the American College of Rheumatology-ACR). The basic disease, the complication(s), and the cause of death were determined and analyzed retrospectively, reviewing the clinical and pathological reports, tissue samples, and the histological slides. Amyloidosis and severity of amyloidosis was determined histologically. The correlations were determined by χ^2-test, comparing the mortality and clinically missed diagnosis of AAa with mild (<) amyloidosis. Results≤1), or severe (1 1. Systemic secondary (AA) amyloidosis was observed in 34 (21.1%) of 161 patients. Thirteen (38.2 rel%) of 34 patients revealed a "severe" degree of amyloid A deposition, massively involving many tissue structures of examined); and 21 (61.8≤organs (with average amount of amyloid A deposits / patient 1 rel%) cases were regarded as "mild", involving only a few tissue structures in some organs (with average amount of amyloid A deposits / patient <1). 2. Seventeen (50 rel%) of 34 patients died of uremia, and 9 (26 rel%) of 17 were diagnosed clinically. 3. There was a significant and positive correlation between severity and mortality of AAa (χ^2=7.97, p<0.004), or clinical recognition of AAa (χ^2=5.98, p<0.01), furthermore between mortality and clinical recognition of AAa (χ^2=9.67, p<0.001). Conclusions AA amyloidosis should be regarded as one of the most insidious complications of rheumatoid arthritis (RA), which may furtively lead to death (Bély, 1993). The clinical recognition of AAa is quite effective. In half of the lethal cases amyloidosis has been recognized clinically, but all of these were in a late, advanced stage of amyloidosis, with massive renal amyloid deposits and uraemia. The early diagnosis of AAa is important, because early recognition of amyloidosis allows an early start of therapy and therefore entails a better prognosis. Biopsy is suggested in all hospitalised RA patients, with or without clinical signs of amyloidosis.

PP4-56
STAGE DEPENDENT QUANTITATIVE DIFFERENCES OF (AA) AMYLOIDOSIS IN RHEUMATOID ARTHRITIS
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Introduction Development of systemic AA amyloidosis (AAa) is a progressive, cumulative process. Aim of this study was to determine 1. the quantitative differences of amyloid A deposits in RA patients complicated with AAa at death, 2. the quantitative differences of amyloid A deposits in different tissue structures of various organs. Patients and Methods A randomized (non-selected) autopsy population of 161 in-patients with RA was studied. RA was diagnosed clinically according to the criteria of the American College of Rheumatology. AAa was detected histologically. The quantitative differences of amyloid A deposits on different structures of various organs at death were evaluated by semi-quantitative, visual estimation of amyloid deposits on a 0 to 3 plus scale. Results AAa was observed in 34 (21.1%) of 161 patients. Thirteen (38.2 rel%) of 34 patients revealed a "severe" degree of amyloid A deposition, massively involving many tissue structures of examined organs (with average amount of); and 21 (61.8 rel%) cases were regarded as≤amyloid A deposits / patient 1 "mild", involving only a few tissue structures in some organs (with average amount of amyloid A deposits / patient <1). Discussion The process of amyloid A deposition is a progressive and time dependent phenomenon. Minimal or moderate amyloid A deposits (with average amounts of amyloid A deposits / patient <1), involving only a few tissue structures in some organs representing the early stage of amyloidosis. Massive amyloid A deposits (with average amounts of), massively involving many tissue structures of≤amyloid A deposits / patient 1 examined organs represents the late (advanced) stage of amyloidosis.

During the progression of amyloidosis, amyloid A protein deposition begins in the organs and tissue structures that are frequently involved and later show marked deposition of amyloid. Where deposits are infrequent or less marked, deposition starts later. Amyloid deposition in the wall of blood vessels (capillaries, arterioles, small arteries) will be followed by deposition of amyloid A along collagen fibers, basal membranes, and reticulin fibers (fat tissues). The amyloidosis of peripheral nerves is a late, end stage phenomenon. The chronologic sequence of amyloid A deposition regarding the organs is: GI tract, heart, kidney, spleen, liver and the adrenal glands. Other organs like lungs, pancreas, thyroid gland, aorta, skeletal muscle, synovial membrane, lymph nodes, peripheral nerves, bones and skin are involved less frequently and amyloid A deposition starts later in them.

PP4-57

NEPHROTIC SYNDROME AND ACUTE RENAL FAILURE IN NON-HODGKIN LYMPHOPLASMOCYTIC LYMPHOMA WITH MONOCLONAL GAMMOPATHY

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Background. Lymphoplasmacytic lymphoma is a rare disease that occurs in older adults, characterized by proliferation of small B lymphocytes, plasmacytoid lymphocytes or plasma cells with or without production of serum monoclonal protein. Renal lesions may occur quite rarely and may be the result of amyloidosis or depositing of monoclonal immunoglobulins or their subunits in one or more renal compartments including glomeruli, tubules, interstitium and blood vessels. Case report. We report two patients with lymphoplasmacytic lymphoma and monoclonal gammopathy of IgM and IgG type, nephrotic syndrome and acute renal failure. A 58-year-old man previously treated for pre-B acute lymphoblastic leukemia, developed 3 years later nephrotic syndrome as a complication of lymphoplasmacytic lymphoma and high paraprotein IgM kappa type. Biopsy of the kidney with light microscopic examination revealed 12 glomeruli with increase of mesangial matrix and mesangial cells. This proliferation was of segmental character with nodular appearance. Glomerular basal membrane was thickened and duplicated. Five glomeruli showed capsular adhesions. There were moderate tubular atrophy and interstitial fibrosis. The small arteries showed hyalinization of the walls. Imunofluorescence revealed mesangial and capillary wall positivity for IgM and kappa light chains and negativity for lambda light chains. Diagnosis of membranoproliferative glomerulonephitis was made. Treatment with cyclophosphamide was ineffective and patient died 2 months later. The second patient is 42-year-old female diagnosed with lymphoplasmacytic lymphoma and paraprotein IgG lambda type. Kidney biopsy disclosed 9 glomeruli, among them one was completely sclerotic. Glomeruli showed hypercellularity due to mesangial cell proliferation and infiltration with mononuclear leukocytes. There were moderate to severe tubular atrophy and interstitial fibrosis. Few tubules were extremely dilated filled with amorphous eosinophilic material with impression of microcystic formations. There were patchy interstitial infiltrates with mononuclear leukocytes. Immunofluorescence staining of glomeruli revealed mesangial and tubular wall positivity for immunoglobulin G, component of complement C3 and lambda light chains. Haemodyalisis and cytostatic therapy were without response and she died after 45 days. Conclusion. In our both cases light chain deposits were found. These light chains have probably caused glomerular as well as tubular changes which were observed.

PP4-58

RENAL MEDULLARY CARCINOMA IN A CHILD WITHOUT SICKLE CELL DISEASE: CASE REPORT

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Background: Renal Medullary Carcinoma is a rare primary neoplasm of the kidney more commom in young adults and frequently associated to sickle cell disorders. It was described by Davis et al in 1995 as epithelial tumor arising from the renal calix of patients with sickle cell disorders. Case Report: A 15-year-old black child admitted with flank pain lasting one year, hematuria and weight loss. Examination showed a palpable mass in the left flank. Laboratory tests were performed and showed: hematocrit 25%, leukocytes 11,400mm3, normal values of electrolytes and renal/hepatic function parameters. Hemoglobin electrophoresis and falciform anemy test were negative. Computed tomography of abdome showed a centrally located mass in left kidney. Then, radical nephrectomy was performed and the pathological result was a well-circumscribed tumor with 10,0 x 8,0cm located in kidney medulla. Microscopically, the neoplasm consisted of solid sheets of cells, eccentric nuclei, prominent nucleoli and eosinophilic and granulated abundant cytoplasm with dense infiltrate of lymphocytes and polymorphonuclear leukocytes. Immunohistochemical stains performed were positive for vimentine, CK7 and CK8 and negative for CK20 and CD10. Despite of treatment stablished the patient died within twelve months. Conclusion: Renal Medullary Carcinoma, known as seventh sickle cell nephropathy, a rare and extremmelly aggressive renal tumor, leading to the death of the majority of patients, as happened in the present case.

PP4-59

RENAL INFLAMMATORY PSEUDOTUMOR: A CLINICOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY OF A NEW CASE

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Renal inflammatory pseudotumor is an uncommon benign tumor that has been classified into separate group, but there is a risk that this lesion could be misdiagnosed. The aim of this work is to report a new case of 57 year old man presented in our hospital with hematuria, minimal grade fever and right flank pain. Magnetic resonance imaging (MRI) and sonography revealed a tumor of the right mediorenal parenchyma, 2,5 cm in diameter. The patient underwent right nephroureterectomy under the diagnosis of renal cell carcinoma. Macroscopically examination carried out the removed kidney showed a 2/2/1,5 cm yellowish, gelatinous, well circumscribed, mediorenal and pericaliceal mass. Material and methods: Fragments of the tumor were fixed in formaldehyde 10%, included in paraffin and the sections were stained with HE,VG and immunohistochemically: vimentine (VIM), MNF116, SyN, smooth muscle actin (ACT), DESMINE, CD68, S100. Results: The histological examination revealed a compact spindle cell proliferation, a hypocellular fibrous area in a

oedematous myxoid background infiltrated by small lymphocytes, histiocytes ,some plasma cells and small bone area. The spindle cells were diffuse positive for VIM, ACT, CD 68 and negative for desmin, MNF 116, SyN and S 100. Conclusions: The pathologic diagnosis was renal inflammatory pseudotumor and raises the problem of differential diagnosis as the clinical and imagistic aspects are similar to those of a renal carcinoma and the problem in establishing a preoperative correct diagnosis.

PP4-60
PAPILLARY RENAL CARCINOMA – A MORPHOLOGIC STUDY OF 36 CASES

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ABSTRACT: The study consisted of 36 patients diagnosed and surgically treated in the Center of Urological Surgery, Dialysis and Renal Transplant from Fundeni Clinical Institute between January 2002 and December 2006. We have evaluated the histological spectrum of PRCCs and assessed significance of conventional pathologic prognostic parameters (Fuhrman's nuclear grade, pathologic stage, tumour size, multifocality, necrosis, and foam cells) and correlated these with outcome. The conclusion that we reached from our study are: 1. PRCC is a malignant tumour, diagnosed at a lower stage, with a distinct potential for progression and aggressive behaviour. 2. Evaluation of these tumours according to cell type, amount of foam cells, presence or absence of vascular invasion, nuclear grade, and pathologic stage provides useful prognostic information. 3. The better 5-year survival rate of papillary RCC (overall and for stage I tumours) compared with that of conventional RCC suggests that it is a tumour with lower malignant potential. 4. Differential diagnosis must be made with urothelial carcinomas, collecting duct carcinomas, metastatic papillary carcinomas (lung, thyroid, etc.)and clear cell carcinoma.

PP4-61
NESTIN EXPRESSION IN MEMBRANOUS GLOMERULONEPHRITIS

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Background. Nestin, an intermediate filament protein which has role in regulating cellular cytoskeletal structure, is restrictedly expressed in the podocytes of human adult kidneys. In the present study nestin expression was investigated in biopsy specimens of membranous glomerulonephritis (MGN). Method. During the last two years, 52 MGN were collected out of 534 kidney biopsy received to be diagnosed at our institution. The diagnoses were performed by light and immunofluorescent microscopy and in some cases by electron microscopy. Clinical data (including immunological analysis wiht ANA and antiDNA antibodies) were also taken into account. For immunomorphology, monoclonal anti-nestin antibody from mouse (SC-23927, clone 2C1.3A11, Santa Cruz Biotechnology) diluted 1:100 was applied on cryostat or paraffin sections using Labeled StreptAvidineBiotin (LSAB+ Dako®) method. Visualization was performed by Dako® AEC substrate. Ten kidney biopsies of patients without nephrotic syndrome, mainly with mesangioproliferative GN, were used for control staining. Results. 52 patients were middle-aged adults (mean age 46) and 4 patients were under age of 15, ratio female : male was 26 : 30. In idiopathic MGN routine immunofluorescence analysis revealed granular deposits of IgG and C3 along glomerular basement membrane (GBM), and by light mtcroscopy the glomeruli showed thickened basement membrane, but appeared normocellular. Mesangial proliferation in addition to uniform thickening of GBM was crucial finding for secondary MGN and immunofluorescence microscopy usually showed a "full house" pattern (fine granular deposits of IgA, IgG, IgM, C1 and C3 along the GBM). In 12 cases (21.4%) idiopathic MGN was diagnosed, and in 35 cases (62.5%) secondary MGN. In 9 cases (16.1%) etiology was undetermined. Nestin expression was variably present in glomeruli in different cases of MGN. Decreased expression was predominantly found in MGN of undetermined or secondary etiology. Conclusion. MGN revealed heterogenity concerning nestin expression. Nestin expression was especially diminished in MGN associated with so-called "full house" immunofluorescence findings.

PP4-62
SEVERAL PATHOGENIC MECHANISMS OF CONGENITAL GLOMERULOPATHIES

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At present time, problems of kidney pathology of fetus and newborn at complicated pregnancy are not completely investigated. Different factors cause delay of fetus and newborn tissues maturity from their hestational age with persistence of embrional structures, became a cause of congenital developmental defects formation and make difficult adaptation of the organism to extra uterine life. Aim of the research: to study the structure of mortality and pathological morphology of kidneys of fetus and newborns at complicated pregnancy. The analysis 566 still-born children and 516 newborns, dead in perinatal periodwas done. There were allocated groups of research - control group, with anemia, and chronic pyelonephrotis. Object of research was both kidneys. Common histological methods, immune fluorescent investigation and morphometric research were made. Results of the research: The basic cause of death is asphyxia (35,6±2,3% against 54,4±1,9%),second place have respiratory disorders of lungs. Congenital developmental defects are on third place, their frequency is increased in two times till 17,6±1,8%, against 7,93±1,0%. Increase of cases of intra uterine infection till 16,7±1,8%, against 3,6±0,7% is also visible. Morphometric criteria of hestational and pathological immaturity of kidneys: number of layers and volumetric part of renal flomeruli, number of embryonic nephrons, and square of tubular surface. Hestosis of pregnant women is a high risk factor of renal dysembriogenesis. Pathological immaturity of kidney tissue at normal duration pregnancy and hestosis is diagnosed at 74,0±5% cases, focal and cystic cortical dysplasia of kidney structure was revealed at 13,0±4%. At histological research of kidneys of fetus and newborns in group with hestosis of pregnant women in 11,0±3% membranous and membranous –proliferative variants of glomerulopathies was revealed. At immune fluorescent investigation bright granular glow of IgM on basal membranes with blade changes of vascular glomeruli and line glow of C3 component of compliment were marked, these are testify about possible participation of anti renal immune factors of pregnant women in the development of congenital immune complex glomerulopathy. In the group of investigation pathological immaturity was revealed in 38,7±5,3% at presence of chronic pyelonephritis at mothers. Analysis of data of pathological morphological research of kidneys of fetus and newborns at complicated pregnancy describes some pathogenic mechanisms of congenital glomerulopathies development and causes, leading nephrogenesis disorders.

PP4-63

THE INFLUENCE OF TUBULAR PHENOTYPIC CHANGES ON THE DEVELOPMENT OF DIFFUSE INTERSTITIAL FIBROSIS IN RENAL ALLOGRAFTS

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It has been reported that myofibroblasts are the major cells in the development of interstitial fibrosis (IF) and therefore chronic graft dysfunction in renal allografts. In normal human kidney, tubular cells do not have myofibroblast differentiation and they don't have alpha-smooth muscle actin (alpha-SMA) expression. In this study we aimed to show that tubular cells can undergo phenotypic changes toward myofibroblasts and induce early IF and poor graft outcome in renal allografts. The expression of alpha-SMA and the formation of vinculin and paxillin containing adhesion complexes are the primary criteria for determining the differentiation of non-muscle cells such as renal tubule cells into contractile myofibroblasts. For this reason we immunostained first year renal allograft biopsies of 74 patients with alpha-SMA, Vinculin and Paxillin primary antibodies and the expression of tubules and glomerular cells were evaluated. Myofibroblast differentiation of renal tubules (alpha-SMA, vinculin and paxillin positive tubules) was found only 30 of 74 patients. In addition glomerular cells of 36 patients showed positive alpha-SMA, vinculin and paxillin staining. The development of diffuse IF was found significantly early in cases with tubules showing myofibroblast differentiation compared to cases with tubules that did not have myofibroblast differentiation ($p<0.01$). The presence of proteinuria in first year showed significant positive correlation with the glomerular alpha-SMA, vinculin and paxillin staining ($p<0.001$). Cases whom showed tubular and glomerular alpha-SMA, vinculin and paxillin staining showed worse graft outcome compared to cases that did not show tubular and glomerular staining ($p<0.001$). In conclusion our results showed that renal tubular and glomerular cells can show myofibroblastic differentiation and these cells have a role in the development of diffuse interstitial fibrosis and early proteinuria in renal allografts.

PP4-64

PERITUBULAR CAPILLARY AND VASCULAR MACROPHAGE INFILTRATION CORRELATES WITH MICROVASCULAR DESTRUCTION AND WORSENS STEORID RESPONSE AND RENAL ALLOGRAFT OUTCOMES FOLLOWING C4d NEGATIVE ACUTE REJECTION EPISODES

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We aimed to understand the influence of peritubular capillary (PTC) and vascular macrophage infiltration on steroid response and renal allograft outcomes after acute rejection (AR) episodes. Seventy-nine patients with biopsy-proven AR in their first year after transplantation were included in the study. Thirty patients with normal first year renal allograft biopsies were also included in the study and used as a control group. All biopsies were C4d negative. Immunohistochemically we assessed the degree of macrophages (CD68) and HLA-DR-positive infiltrating cells in PTC's, glomeruli, and on vascular walls and tubules. In addition HLA-DR expression of PTC's was also evaluated. The decreasing intensity of peritubular capillary HLA-DR (PTC-DR) expression was accepted as the increasing degree of the destruction of PTC. Compared to control group AR cases showed significantly higher degree of macrophage and HLA-DR positive inflammatory cell infiltration in PTC's, glomeruli, and on vascular walls and tubules ($P<0.001$ for all). PTC destruction was significantly higher in AR cases than control group ($p<0.001$). PTC, glomerular and vascular macrophage infiltration showed significant correlation with PTC destruction and steroid response ($p<0.001$ for all). Severity of PTC destruction with accompanying higher degrees of macrophage infiltration in PTC's, glomeruli and on vascular walls caused unresponsiveness to steroid therapy ($p<0.001$) and poor graft outcome ($p<0.001$). Five-year graft survival was 95%, 37% and 22% for cases with grade 0, 1 and 2 PTC macrophage infiltration respectively ($P<0.001$). In addition five-year survival was 80%, 36% and 1% for cases with grade 0, 1 and 2 vascular macrophage infiltration respectively ($p<0.001$). In conclusion peritubular capillary and vascular macrophage infiltration are important predictors of steroid response and renal outcome following acute rejection in cases whom especially had negative C4d.

PP4-65

ROLE OF POLY (ADP-RIBOSE) POLYMERASE ON MICROVASCULAR INJURY AND INFLAMMATION IN RENAL ALLOGRAFT REJECTION AND ITS INFLUENCE ON RENAL GRAFT SURVIVAL

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Introduction: The activation of poly (ADP-Ribose) polymerase (PARP) is well considered to play an augmenting role in inflammation and cell death. The aims of this study were to investigate the role of PARP in acute rejection (AR) and to assess the influence of PARP on renal survival. Methods: Study compromised 81cases and 55 of them had AR. Twenty-six cases had no pathology and used as a control group. PARP and HLA-DR expression of tubules, interstitium, arteries and peritubular capillaries (PTC's) were studied immunohistochemically and CD68 positive macrophage infiltration of tubules, interstitium, PTC's and arterial walls were evaluated. The decreasing intensity of PTC HLA-DR (PTC-DR) expression was accepted as the increasing degree of the destruction of PTC's. Results: AR cases showed higher degrees of tubular, interstitial and vascular PARP and HLA-DR expression compared to control group ($p<0.01$ for all). PTC-DR expression was lower and PTC-PARP expression was higher in AR cases compared to control group ($p<0.001$). Increasing of AR grade with the high level of PTC-PARP expression, caused decrease of PTC-DR expression and increase of PTC destruction ($p<0.01$). Tubular and interstitial HLA-DR expression, interstitial, tubular, vascular and PTC macrophage infiltration showed positive correlation with tubular, interstitial, PTC and vascular PARP expression ($p<0.01$ for all). In contrast PTC-DR expression showed negative correlation with all these parameters ($p<0.01$). Severity of PTC destruction with accompanying higher degrees of PARP expression on tubules, interstitium, arteries and PTC's caused unresponsiveness of steroid therapy ($p<0.01$) and poor graft outcome ($p<0.01$). Conclusion: Increased PARP activation leads to higher degrees of cell death and inflammation that AR cases with high renal PARP expression showed significant PTC destruction and renal inflammation. Therefore we suggest that PARP inhibitor drugs can combine with immunosuppressive therapy in order to control PTC destruction and renal inflammation

PP4-66

EFFECTS OF SULPHITES ON KIDNEY HISTOLOGY IN YOUNG AND ELDERLY RATS

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BACKGROUND:The human body is constantly generated from the metabolism of the sulfur containing amino acids. Sodium metabisulphite is used as an antioxidant and antimicrobial agent in a variety of drugs and functions as preservative in many food preparations. There is no knowledge about the histological effects of sulphites on kidneys. The aim of this study was to investigate the possible toxic effects of sulphites on kidneys by assessing histological findings in young and elderly rats. MATERIALS AND METHODS: Rats were assigned to four groups: Two sulphite administrated young and elderly groups, and two young and elderly control groups. Sulphite was administrated in the form of sodium metabisulphite 70mg/kg/day via drinking water. The kidney tissues were fixed on buffer formalin and then they were stained with H&E, methenamine silver, PAS and Masson's thricrome. RESULTS:There were no glomerular changes (i.e. thickness of basement membrane, focal segmental sclerosis), tubular changes, tubular interstitial changes or vascular changes. No differences between young and elderly sulphite administrated groups were observed histologically. CONCLUSIONS:In this study, the specimens were examined by light microscopy. Further studies done by electron microscopy are needed to support these findings.

PP4-67
HISTOPATHOLOGICAL STRUCTURE OF THE KIDNEY IN HIGH FAT DIET FED RATS: A LIGHT MICROSCOPICAL STUDY

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Background: To characterize kidney in high fat induced obesity model we have examined renal structure in adult Sprague-Dawley rats fed a control diet (CD) or fat-rich diet (HFD) during three months. Methods: Ten adult female Sprague Dawley rats were fed a diet constituted highly of fat (%30) for duration of 3 months. Ten control rats were maintained with a standard rat chow. All animals were weighed per 10-day for 3 months. At the end of the experiment, the naso-anal length of the anesthetized rats was measured to calculate the body mass index (BMI), and subsequently, whole kidneys of the intracardially formalin-perfused animals were removed. Qualitative features of kidney were examined histologically. Results: Light microscopic investigation showed a dilatation in blood vessels and bowman capsule, mononuclear cell infiltration, degeneration in nephrons including glomerulosclerosis and tubular defects and an increase in the connective tissue in the kidneys from the treatment group. Conclusion: Eventually, we have thought that fatty diet causes obesity and may lead to renal failure as a result of histopathological changes.

PP4-68
EXPRESSION OF INSULIN-LIKE GROWTH FACTOR IN PROLIFERATIVE GLOMERULONEPHRITIS

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Background Insulin-like growth factor-1 (IGF-1), a peptide growth factor produced by collecting ducts, and its receptor, IGF-1 receptor are present in the glomeruli and basolateral membrane of renal proximal tubular cells. Animal studies showed that IGF restores nephron growth and contributes to tissue growth by causing arterioler dilatation, increasing the glomerular filtration rate and accelerating tubular regeneration.The present study aimed that analyse the expression of IGF-1 in proliferative glomerulonephritis(PGN) and focused on its biologic significance in glomerulonephritis. Methods.IGF -1 expression was studied in 34 non diabetic specimens: normal kidney (6), proliferative glomerulonephritis (GN,14), nonproliferative GN (14). All slides were immunohistochemistry stained with IGF-1(G-17; SC-1422; Santa Cruz Biotechnology; California). In all cases, IGF-1 expression was scored semiquantative by two pathologists (0-3) in glomeruli and tubulointerstitial. Routine histochemical and immunofloresanse stains were performed and evaluated all cases. Activity index, chronicity, interstitial injury and sclerotic glomeruli were examined in all cases. Results.In normal kidney there was strong IGF-1 immunreactivity in the proximal tubules and minimal IGF -1 expression in the glomeruli.We found increased IGF-1 immunexpression in glomeruli at Proliferative GN groups compared with nonproliferative groups. IGF-1 expression was correlated with the activity index for Proliferative GN groups. Tubulointerstitial IGF-1 expression was similar in proliferative and non proliferative GN groups. We did not found a statistically significant correlation between fibrosis and glomerul IGF-1 expression in two groups. Only significant correlation was between fibrosis and tubulointerstitial IGF-1 expression. Both groups had similar sclerotic glomeruli proportions. Conclusion: These data indicate that IGF-1 indicates the activity in proliferative glomerulonephritis, but larger group studies are needed to confirm our results

PP4-69
EFFECT OF FK506 IN EXPERIMENTAL GLOMERULONEPHRITIS: LIGHT MICROSCOPIC AND ULTRASTRUCTURAL STUDY

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Background The anti-Thy1.1 model is a rat model of mesangial proliferative glomerulonephritis characterized by mesangial cell proliferation and accumulation of mesangial matrix expansion with subsequent resolution and return to almost normal histology. FK506 is an immunosuppresive drug used for treatment of autoimmune disease and after transplantation.In present study, we investigate the effect of FK506 on experimental glomerulonephritis model. Method We studied the expression of α-Smooth Muscle Actin(α-SMA), Proliferative Cell Nuclear Antigen(PCNA),Macrophage Calprotectin MAC), aminine, ascular Endothelial Growth Factor(VEGF) in paraffin embedded tissue section from kidney by immunhistochemical method and apoptosis with TUNEL.We studied kidney tissue with fluorescein isothiocyanate(FITC).IgG,IgM,IgA,C3,C1q and fibronogen. We examined ultrastructural finding with electron microscopy. Twenty male Wistar Albino rats were divided into four groups:GroupI Control(C):5 rats received of 0.1ml/100g normal saline(NS) intravenously(i.v) injection for 4 weeks;GroupII Glomerulonephritis(GN):5 rats received i.v injection of anti-Thy1.1(0.25µg/100g) at zero day. GroupIII Glomerulonephritis+FK506 (GFK):5 rats received i.v injection of anti-Thy1.1(0.25µg/100g) and then FK506(1mg/kg) for two weeks.GroupIV Control+FK506 (CFK):5 rats received i.v.injection of NS (0.1mg/100mg) and then FK506(1mg/kg) for two weeks.Serum creatinine,creatinine clearance and proteinuria were performed at the end of the study period.Renal tissue were assessed for light microscopic findings glomerul and tubulointerstitial injury.PCNA,α-SMA,VEGF,Laminin,MAC and

apoptosis were semiquantatively scored on glomerul and tubulointerstitial area.We examined renal tissue by electron and immunofluorecence microscopy. Results At the end of the study period,glomerular cell proliferation and tubulointerstitial injury were significantly increased in GN compared to G group. Glomerular immunohistochemical expression of PCNA, SMA, VEGF, MAC,apoptosis significantly increased in GN compared to C group.Also we found the electron dense deposit on glomerular capillary walls and mesangium with IgG and C3 by immunofluorecence microscopy.The expression of the PCNA,α-SMA and apoptosis were significantly decreased in GFK group compared to GN.But glomerular immunoexpression of MAC and VEGF were similar in GN and GFK groups.Also we found less proteinuria in GFK group compare to GN. Conclusion The present study suggests that FK506 may be useful in drug for the treatment of mesangial proliferative glomerulonephritis.

PP4-70

INVESTIGATION OF PROTECTIVE EFFECT OF PREDNISOLONE IN NEPHROPATHY INDUCED BY CO-TRIMOXAZOLE IN RAT

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Co-trimoxazole is a sulfonamide derivative, which is used as an antibacterial drug. Prednisolone is a dermo-corticostroid derivative, which is widely used as an anti-inflammatory and in the treatment of allergic reaction and collagen vascular diseases. Methods and Materials: The aim of this study was to find out the effect of co-trimoxazole on the renal interstitum and protective effect of predenisolone in rat. Four groups of animal were selected, namely A, B, C and control group. Groups A and B were treated with co-trimoxazole (150mg/Kg sulfa methoxazole + 30mg/Kg trimethoprim) while group C was treated with Co-trimoxazole and prednisolone (4mg/Kg) for 10 days. The reference group was only received some dose of water. The blood samples were collected from groups A and C 24 hours and from group B 14 days after the last dose of drug were administrated. Microscopic samples from kidney tissue were prepared for histopathological study. Results: The creatinine level in serum of group A (1.54) showed a significant increase as compared with the control group (1.04). In the group that received co-trimoxazole the histopathological study showed acute interstitial nephritis (AIN). In the group that treated with co-trimoxazole + prednisolone were not seen any histopathological changes . Conclusion: These results support the theory that AIN is drugs allergic reaction when co-trimoxazole is used.

PP4-71

COX 2 AND CD 34 EXPRESSION IN WILMS TUMOR (NEPHROBLASTOMA)

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BACKGROUND Wilms tumor (nephroblastoma) is the most common primary malignant renal tumor of childhood. It occurs in approximately 10 children per million under the age of 15 years and is usually diagnosed between the ages of 2 and 5 years. Approximately 90–95% of Wilms tumors involve one kidney. There has been much recent interest in the role of cyclooxygenase-2 (COX-2), one of the three enzyme isoforms that convert arachidonic acid to prostaglandins, in tumor development and progression. COX-2 expression is nearly ubiquitous in human cancers and has been correlated with poor prognosis [e.g., in tumors of the breast, cervix, colon, brain, and ovary]. Intriguingly, COX-2 seems to have multiple functions in tumor pathogenesis and thus represents an attractive therapeutic target. Among these, COX-2 seems to play a significant role as a positive regulator of tumor angiogenesis. METHOD Wilms tumor cases included to this study were obtained from archives of Akdeniz University Department of Pathology between 1997 and 2007. Immunohistochemical expression of COX -2 and CD 34 were independently evaluated and scored by two surgical pathologists. 17 patients (9 male / 7 female) were included in our group and their ages ranged from 1- 6 (Mean age 3.2). RESULTS In our study there was a significant negative correlation between angiogenesis, as measured by CD 34 expression and COX-2 expression ($p=0.018$, $r=-0.567$). There was no significant statistical correlation between gender, age and immunohistochemical results (COX -2 and CD 34 expression). There was also no difference between genders according to age. CONCLUSION In our study there was a significant negative correlation between angiogenesis, as measured by CD34 expression, and COX-2 expression. This would contradict the hypothesis that COX-2 acts as a pro-angiogenic stimulant, at least in Wilms tumor, and could suggest that COX-2 inhibits new blood vessel formation. Angiogenesis is regulated by a complex series of molecular pathways which, whilst they include the modulation of VEGF via PGE2 produced by COX-2, are subject to many other modulatory factors. Our study raises the possibility that COX-2 may influence tumor progression in Wilms tumor through mechanisms other than the promotion of angiogenesis.

PP4-72

SEVERE RENAL VASCULITIS IN A PATIENT WITH DRESS

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Introduction: Drug Rash with Eosinophilia and Systemic Symptoms (DRESS), is a severe generalized hypersensitivity reaction, characterised by fever, rash and internal organ involvement, such as liver and less frequently kidneys. It may be lethal in 10% of cases. Herein, we report on a case of a patient, who developed DRESS with renal insufficiency and proteinuria after allopurinol therapy, with the presence of a severe unexpected vasculitis at renal biopsy. Case Report : A 55 years-old woman, with a known impaired renal function due to nephroangiosclerosis, was admitted in 02/2006 for a gout crisis. Her serum uric acid, and creatinine levels were respectively 149 mg/l and 180 µmol/l. Allopurinol was introduced, but rapidly stopped because of myalgia, headache, and gastro-intestinal disorders. At that time, serum creatinine level was 188 µmol/l, with no proteinuria. Because of persistant hyperuricemia, allopurinol was re-introduced one month later. Eight weeks later, she presented with a diffuse maculopapular rash, fever (39°C), myalgia, and mild hypertension. Laboratory studies yielded the following results: serum creatinin level at 389 µmol/l, proteinuria at 0.8 g/d without hematuria, eosinophil count was 1192 / mm3, with mild cholestasis and C-reactive protein was 291 mg/l. The kidney biopsy revealed the presence of a severe leucocytoclasic necrotizing vasculitis with gigantocellular granulomas. There was no glomerulitis and no immune deposits. The diagnosis of DRESS with immuno-allergical vasculitis related to allopurinol was evocated. All immunological, bacterial and virological causes were ruled out. Allopurinol was stopped, and steroids initiated at the daily dose of 1 mg/k. Seven days later, serum creatinin level decreased to 220 µmol/l and cutaneous lesions

disappeared, but proteinuria remained elevated (1.6 g/d). Two months later, the patient presented with a nephrotic syndrom and severe hypertension. A second renal biopsy revealed interstitial fibrosis and tubular atrophy, with regression of vasculitis lesions. Unfortunately, 4 months later, hemodialysis had to be started. Conclusion: DRESS must be promptly recognized and all potential culprit drugs whithdrawn, such as aromatic anticonvulsants, sulfonamides, calcium channel blockers, ranitidine, thalidomide and allopurinol. A kidney biopsy should be performed in patients suspected to have DRESS with impaired renal function and/or proteinuria. It may reveal the presence of immuno-allergic vasculitis that may evolve rapidly to end stage renal disease despite steroid therapy.

PP4-73
RENAL AMYLOIDOSIS–CORRELATION MORPHOLOGY AND CLINICAL FEATURES

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BACKGROUND Amyloidosis was defined as the deposition of proteins that have the capacity to form beta-pleated sheets.The most common presentation of AA amyloidosis is that of renal diease.AL amyloid also frequently involved the kidney.In these study,we investigated whether different patterns of amyloid deposition occur in the kidney and these patterns can be related to the clinical findings. METHOD:29 cases of systemic amyloidosis with renal biopsies processed and examined histopathologically at the Department of Pathology, Faculty of Medicine University of Marmara were reviewed.Also all cases were stained with Amyloid A component(Ab-1;MS-1219-R7;Neomarkers;Fremont CA;USA)and Amyloid P protein(Ab-1;Neomarkers;RB-1786-R7 Fremont CA,USA)using the Standard avidin–biotin–complex immunoperoxidase method.The chemical types of amyloidosis were categorized according to the following staining reactions:AA amyloid was permanganate–sensitive and immunoreactive for AA protein.AL amyloid was permanganate resistant and not immunoreactive for AA protein.Renal amyloidosis were categorized glomerular,vascular and tubulointerstitial by semiquantitive.We analyzed renal histologic findings,type of renal amyloidosis and clinical data. RESULTS.The age range of patients was 25-75 years.The male-female ratio was 19:11.3cases were categorized as AL and 26 were AA amyl.There was a predominence of the glomerular pattern in all cases.Also glomerular amyloid deposition was divided on the basis of morphological characteristics,into 4 types:a mesangial nodular type showing nodular mesangial deposits with sparse capillary wall involvement(16cases),a mesangio-capillary type disclosing diffuse amyloid in the mesangium(2cases), perimembranous type(6cases)and a hilar type showing amyloid deposits almost exclusively in hilar arterioles(3cases).14 cases of mesangial nodular type showed amyloid protein of AA type.Also we were compared glomerular amyloid deposition type and fibrosis.We found statistically significant correlation between hiler type deposition and fibrosis.We were showed that if patients had glomerular and tubulointerstitial deposition fibrosis would be worse.Proteinuria was the main clinical manifestation,present in all cases.Chronic renal failure and renal death appeared more common in hilar type of glomerular deposition with that of tubulointerstitial amyloid deposition. CONCLUSIONS.The results obtained suggest that the chemical type of glomeular amyloid protein is associated with significant differences in the morphological and prognostic features of the renal involvement.

PP4-74
MORPHOLOGICAL CHANGES AFTER LONG USAGE OF HEROIN THROUGH INJECTION

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For the period 2003-2006 in the Centers of Forensic Medicine and Deontology and Clinical pathology at “Alaxandrovska” MBAL - Ltd., Sofia we had examined 33 patients (21 male and 11 female) that had died after a longtime intravenous usage of heroin and 23 patients with heroin dependency and clinical evidence of kidney damage who had undergone puncture biopsy (duration of the heroin dependency – 6 to 96 months). In order to determine the type of damages in the organism done directly by the toxic substance we studied macro- and microscopical (including electronic microscopical) morphological changes in different organs and systems – skin, underskin and underlying blood vessels – in the areas of the needle punctures (recent and old); lungs; heart; liver; kidneys and spleen. In all of the examined cases we discovered focused inflammatory changes on the skin and the underlying soft tissues in the areas most often used for the injection application of the narcotic. The most often morphological change seen was the fatty dystrophy. More rarely we encountered hepatitis with toxic origin, without the presence of fibrose changes in the liver parenchyma. The histological changed of these toxic liver damages are presented by vacuolization in the cytoplasm of the hepatocytes (fatty dystrophy), inflammatory infiltration of mononuclear cells and segment-nuclear leucocytes in the portal spaces of the liver (toxic hepatitis). In some cases there were inflammatory changes in the lungs, most often nonspecific (bronchitis, brohiolitis and focused pneumonia). In a particular case the morphological examination proved the presence of pneumonia caused by Pneumocystis carinii, damage of the lungs described in specialized literature by many leading authors. We succeeded in proving the agent of pneumocystis pneumonia not only by histochemical examinations (silver impregnation by Gomori-Grocott and PAS-reaction) but also at ultrastructural level by means of electronic microscopical examination. Kidneys are often affected in cases of longtime heroin usage. The percentage of the sclerotic glomerules of drug addicts, including those examined by us, is much higher than the one of people not addicted to drugs. The damage of the tubulo-interstitial apparatus (fibrosis, inflammatory interstitial infiltration and atrophic channels) is also a frequent morphological discovery in such individuals. Rarer are the kidney changes of the type of the glomerulopathies.

PP4-75
PHENOTYPIC ABERRATIONS IN MUCINOUS TUBULAR AND SPINDLE CELL CARCINOMA

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Mucinous tubular and spindle cell carcinoma (MTSCC) is a rare recently described variant of renal cell carcinoma with unique histological patterns which are sufficient to be designated by the World Health Organization as a distinct entity. We present a case of 55 year-old male who underwent a radical nephrectomy due to a tumor confined within the lower pole of the kidney. In gross examination the tumor was fairly circumscribed, measured 8.5 cm in greatest diameter and was tan-white in appearance. Microscopically the carcinoma was composed of variable proportions of tubular, tubulocystic, cord-like structures and

spindle cells together with rare compact areas in the presence of extracellular blue gray mucinous background (Alcian blue +). The tumor cells were cuboidal to columnar with focally atypical nuclear features with either no or rare mitoses. No tumor necrosis was found. Apart from the predominant components, unusual growth patterns and features were focally identified such as pseudo-papillae, well formed papillae with psammoma calcifications, multinuclear neoplastic cells, glomeruloid structures, hemangiopericytoma, oncocytoma and clear cell carcinoma – like areas. The tumor cells showed a diffuse cytoplasmic staining for vimentin, Cam5.2, CK7, CK18, CK19 and racemase. Both tubular and spindle cell areas were negative to Keratin 34BE12, CD10, CD15, and chromogranin. The diagnosis of MTSCC was based principally on the predominant morphologic features along with the immunohistochemical profile, consistent with the 2004 WHO classification pathologic criteria. Since there is a relatively small number of published cases of MTSCC, neither the morphologic variabilities nor the histogenesis have been elucidated. The current case highlights the aberrant phenotype of the tumor which may contribute to the establishment of the diagnostic pathologic criteria.

PP4-76
PRIMARY SYNOVIAL SARCOMA OF THE KIDNEY. PRESENTATION OF TWO CASES IN CHILDREN
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Background: Renal primary sarcomas represent 5 % of kidney infantile tumors. Clear cell sarcoma (CCSK) is the most common in children. In 1995 Beckwith described a series of primary embryonal renal sarcomas with mesenchymal monophasic embryonal cells. This tumor was morphologically consistent with monophasic spindle synovial sarcoma (SS) and harbored the same SYT-SSX gene fusion. These tumors are very rare in children with only 5 cases reported in the literature. METHODS: Two cases of renal pediatric SS were identified in the files of the Department of Pathology in our Hospital. Histological, immunohistochemical and molecular analysis were performed in both patients. RESULTS: Patient nº 1: An 8-year-old boy had a history of abdominal pain and hematuria. Abdominal CT revealed a right renal mass with necrosis and vena cava thrombosis. Under the impression of nephroblastoma he started systemic chemotherapy and afterwards a radical nephrectomy with vena cava thrombectomy. The specimen weighted 292 grs and had a multicystic tumor that measured 8×7cm. Histologically the tumor showed a mesenchymal proliferation of short monomorphous cells. This proliferation entrapped tubules; some were cystically dilated and had cells with a hobnail morphology. After the surgery the patient had intensive systemic chemotherapy and radiotherapy. He remains free of disease 6 years after. Patient nº 2: A 2-year-old girl with a mass that arose from the inferior side of the right kidney. Systemic chemotherapy and nephrectomy were performed. The mass weighted 568 grs and measured 11×8,5cm, was well circumscribed, white-tan with small cysts and had less than 20% of necrosis. Histologically, the tumor showed a hypercellular mesenchymal proliferation. The nuclei of the cells were round with an average mitotic rate of 20/10hpf. Immunohistochemical studies showed diffuse positivity with vimentin, CD99 and bcl-2. Both cases were confirmed by molecular analysis of the characteristical t(X; 18) that results in the fusion of SYT gene to the SSX1 or SSX2 genes. CONCLUSSION: The differential diagnosis of SS in children includes CCSK, mesenchymal nephroblastoma, congenital mesoblastic nephroma and cystic nephroma. The differential histological diagnosis in the case of CCSK is sometimes difficult. The positivity of CD99 and bcl-2 is helpful in distinguishing SS from other tumors. However confirmation of this entity is done by molecular analysis. These renal tumors have been described in young adults, but exceptionally in pediatric patients, with only 5 cases described in the literature.

PP4-77
COLOR CLASSIFICATION AND AUTOMATIC REGION IDENTIFICATION IN GLOMERULAR PATHOLOGY
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Backgroud: The paper presents a new computerized technique for region identification in microscopic fields. It is based on the automatic separation of several chromaticity classes that are associated with different types of entities. Method: The method has been implemented by software modules developed in the Matlab environment and operates with indexed images. The material consisted of specimens from renal corpuscle biopsies, stained for light microscopy with haematoxylin-eosin and light green trichrome (Merck, Germany). The resulting microscopic fields exhibit a wide variety of colors, which have to be exploited in order to recognize the renal corpuscle elements: nuclei, connective tissue corresponding to the mesangium and fibrous crescent, Bowman space and capillaries (possibly containing blood cells). Results: The key issue of the proposed method is the iterative construction of a color map, whose entries are grouped in chromaticity classes, corresponding to the types of elements to be identified. Initially, each class contains a small number of representative colors selected by the user. Then, the classes are expanded, each iteration adding at least one new color to one or several of the existing classes. This step-by-step augmentation of the color map is automatically guided by a reciprocal validation of the included color(s) in the sense of the minimal distance computed in both the red-blue-green (RGB) and luminance-hue-saturation (YIQ) spaces. A number of images are considered for illustrating the correctness of the identified regions. These regions can be further used for quantitative analysis. Conclusions: The applicability of the technique is not limited to the renal corpuscle elements, but our focus on renal corpuscles is motivated by the difficult classification of the colors typical to such microscopic fields. It is worth mentioning that before elaborating this technique, we unsuccessfully tried to adapt segmentation procedures reported in literature, including the thresholding approaches experienced by us in some previous works. All these procedures failed because of the complexity of analyzed color images.

PP4-78
CLASSIFICATION AND SCORING SYSTEM OF RENAL AMYLOIDOSIS; EXPERIENCE WITH 288 CASES
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Amyloidosis refers to the extracellular deposition of fibrils composed of a variety of proteins, is multisystemic disease, associated with a different conditions. 25 different precursor fibrils are known up to date, of which seven fibrils were important for kidney. Although a variety of deposition patterns of amyloid have been described there are no universal renal classification and scoring system. This is necessary for predicting patient outcome, organ failure and also to establish an objective interpretation for clinical trials. In the present study, renal amyloidosis classification and scoring system were developed and applied. Cases of renal biopsy-proven amyloidosis were retrospectively evaluated (between 1990 and 2007). Amyloid was diagnosed using the stringent, alkaline, alcoholic CR staining method of Puchtler et al. The characteristic green birefringent polarization was taken as proof of the presence of amyloid. Glomerular amyloid depositions were classified similar to SLE 2003 ISN/RPS classification (class I to VI). Renal amyloid depositions (glomerular pattern and percent, vascular and interstitial) interstitial fibrosis, inflammatory infiltration and glomerular sclerosis were also scored. Renal amyloid prognostic score (RAPS) was found calculation of these scores. RAPS was divided into three stage (early, moderate and advanced

amyloidosis). Total 305 renal (294 native, 11 transplant) biopsies from 288 patients were reevaluated. AA was detected in 90% of the patients. Biopsies from nine cases are inadequate for classification and staging. Depositions were evaluated as 10%, 18%, 22%, 38%, 1%, and 8% respectively for class I to VI. RAPS stages were found as 14%, 51% and 31% respectively for stage I –III. Clinical validation of this system has not been completed. We believed that renal biopsy is most suitable tissue for assessing of amyloid deposition intensity. Our classification and scoring system was applied successfully. Clinical validation studies are required to confirm importance and reliability.

PP4-79

IMMUNOHISTOCHEMICAL AND MORPHOMETRICAL ANALYSIS OF THE TUBULOINTERSTITAL CHANGES IN PRIMARY GLOMERULOPATHIES

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Alterations in the tubulointerstitial renal compartment were found in almost all glomerular diseases. The aim of this study was to analyse the tubulointerstitial changes in primary glomerulopathies and correlate them with the clinical data. Methods: We made a study on 50 renal biopsies with primary glomerulopathies and 20 control cases. For morphometric analysis we made a software color extraction of the interstitial area on tissue sections stained with trichrom Masson and expressed the results as percentage of the total scanned area. We made immunohistochemical analysis with Cytokeratin, HLA-DR, Vimentin, SMA and E-cadherin. We also applied double staining method, coupling Cytokeratin with Vimentin and SMA. Results: We found fibrosis, mainly focal, occupying more than 9% of the cortical tubulointerstitial surface (mean 18,75%) in 98% of the biopsies. In these areas the tubules showed marked atrophy. The tubular epithelial cells showed diminished positivity for Cytokeratin and E-cadherin in the areas of fibrosis, and enhanced expression of HLA-DR, unlike the control cases. The epithelial cells in the atrophic tubules, especially in the foci with inflammatory infiltrate, showed positivity for Vimentin and SMA, which was not visible in the control tissue sections. This was better visualized on the double stainings where both epithelial and mesenchymal markers were present in same tubular section. The morphometric analysis showed that the percentage of tubules with cells positive for Vimentin and SMA did not exceed 10% and 5%, respectively, of the total scanned area. The analysis showed statistically significant ($p<0.01$) positive correlation between the extent of fibrosis and the serum creatinine concentration. The extent of fibrosis was also positively correlated with the same statistical significance to the percentage of tubules with cells positive for Vimentin, SMA and HLA-DR. These parameters for tubular injury also show strong correlation with the serum creatinine concentration. Only the percentage of tubules containg cells positive for SMA was found to be correlated to the degree of proteinuria at the time of the biopsy. Conclusion: The tubular epithelial cells undergo epithelial-mesenchymal transition during various glomerulopathies, thus contributing to the interstitial fibrosis. The changes in the tubulointerstitial compartment influence the course of the disease. The quantitative histological analyses should be included in the process of nephropathological diagnosis in order to evaluate the histological risk factors in glomerular diseases.

PP4-80

AN ELECTRON-MICROSCOPIC (EM) AND LIGHT-MICROSCOPIC (LM) MORPHOMETRIC STUDY IN CHILDREN WITH GLOMERULAR IMMATURITY AND NEPHROTIC SYNDROME (NS)

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Introduction: Very little information is available in the literature about the glomeruli maturation. It is thought that above 2 years of age the number of immature glomeruli should not exceed 10%. In children with NS glomerulopathies we encountered a small number of cases in whom immature glomeruli outnumber the percentage allowed to the given age. The aim of the study was: to compare the results of LM and EM evaluation and to carry out a morphometry. MATERIAL AND METHODS: The study group consisted of 66 children with NS. The diagnosis was established in LM and EM. The control group consisted of 30 children in the same age (with the same glomerulopathies MCD, DMH), but without glomerular immaturity. The morphometric study was performed. RESULTS: EM confirmed the diagnosis of MCD in 32, DMH in 6 and FSGS in 2 cases. The EM study did not confirm the LM diagnosis of MCD in 6 cases (5 DMH, 1 membranous glomerulopathy) and DMH in 1 case (MCD). EM revealed features of immaturity. There were differences in the intensity of foot processes effacement and microvillous transformation (between MCD and DMH). Morphometric studies revealed significant differences regarding the area involved by capillary loops (CLA) and glomerular volume. These values were significantly lower in the group with immature glomeruli, when the number of cells per 1000 μm2 of CLA was higher. There were no correlations between the size of glomeruli and the age of children with glomerular immaturity (such correlations were evident in the control groups).

PP4-81

PROGNOSTIC FACTORS IN DIFFERENT MORPHOLOGICAL VARIANTS OF RENAL CELL CARCINOMA (RCC) (ESPECIALLY IN RCC WITH SARCOMATOID FEATURES)

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Introduction: There is increasing incidence of RCC, when the biological behaviour may be different, thus commonly used prognostic factors are not always sufficient in predicting prognosis. Because of many possibilities of treatment, the choice of the best therapy is of importance and searching for better prognostic markers is necessary. Abnormal expression of E-cadherin and MUC1 has been associated with penetration by neoplastic cells. Inhibitors of cycline kinase (p21, p27) are responsible for antiproliferative properties. Morphological subtypes of RCC may contain sarcomatoid areas related with poor prognosis. The aim was to evaluate the expression of above mentioned markers with respect to histological and clinical features. MATERIAL AND METHODS: We investigated the expression of E-cadherin, MUC1, p21, p27 in 64 cases of RCC (5-7 years of follow-up): 32 conventional RCC (CRCC), 12 papillary (PRCC), 10 chromophobe (CHRCC) oraz 10 with sarcomatoid areas. RESULTS: The expression of E-cadherin and MUC1 substantially differed between studied groups. The strongest E-cadherin immunoreactivity was observed in CHRCC, less intense in PRCC and in CRCC. It was only focal in sarcomatoid areas. A diffuse cytoplasmic staining pattern for MUC1 was noted in CHRCC, whereas PRCC and CRCC showed predominantly membranous reaction. The appearance of strong cytoplasmic immunoreactivity for MUC1 in CRCC and

sarcomatoid areas was related with higher grade of malignancy and poor prognosis. Differences with regard to p21 and p27 immunoreactivity were also observed. The lowest number of positively-stained nuclei was noted in CRCC and in sarcomatoid areas. Higher percentages were counted in PRCC and the highest in CHRCC.

PP4-82
IMMUNOHISTOCHEMICAL STUDY OF TUBULOINTERSTITIAL INFLAMMATORY CELL INFILTRATION IN HUMAN GLOMERULONEPHRITIDES

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The importance of the interstitial aspects of primary glomerular disease to overall renal function is continuously increasing. The aims of this study were to characterize the mononuclear inflammatory cells in the tubulointerstitium of the renal biopsies affected by glomerulonephritic lesions using a panel of monoclonal antibodies specific for leukocytic subpopulations. Correlations with tubulointerstitial changes and main clinical parameters of renal function were done. Methods: Renal biopsies from 50 patients with glomerulonephritides were studied. Ten control normal sections were obtained from kidneys removed for localized tumors. Paraffin sections were stained with HE, PAS, trichrom Masson, silvermethenamine Jones, as well as immunohistochemically with the following monoclonal antibodies: LCA, CD43, CD20, CD68, HLA-DR, E-cadherin. The morphometric analyses were done on sequentially taken images avoiding glomeruli and large vessels with an image analyzing system LUCIA M–NIKON. The extent of interstitial fibrosis was examined on trichrom staining. Results: 98% of the cases showed interstitial fibrosis higher than 9% of the surface of the total cross section, with a mean value of 18,75%. Mononuclear inflammatory infiltrate in the interstitium, mainly with focal distribution, was noticed in 90% of the cases. T lymphocytes were predominant (mean value 58,26%), the B cells were 18,62% and the macrophages were present with mean value of 22,92%. Positivity for HLA-DR showed 40,86% of the total inflammatory cells, a significantly higher value than the control group (23,5%). The tubular epithelial cells also showed higher expression for HLA-DR than the control group. The tubular cells, especially in cases with abundant inflammatory infiltrate manifested decreased expression for E-cadherin in comparison to the control group. Each of the inflammatory cells subpopulations, as well as the total inflammatory infiltrate showed strong correlations with the interstitial fibrosis. The clinical data showed strong association of the serum creatinine concentration with the number of cells from each of the leukocytic populations, as well as the total inflammatory cell count (Spearman R ranged between 0.44 and 0.47) and no correlation with the proteinuria. Conclusion: We can conclude that the interstitial inflammatory substrate may influence tubulointerstitial changes in the primary glomerulonephritides, as well as to determine the long-term prognosis of the disease, which implies further investigations in this field.

PP4-83
PRIMARY NON-HODGKIN LYMPHOMA OF URINARY BLADDER WITH RENAL INVOLVEMENT NINE YEARS LATER AND ABSENCE OF SYSTEMIC LYMPHOMA. A CASE REPORT

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Background. Primary bladder non-Hodgkin lymphoma (PBNHL) is very rare, especially as extranodal B-small lymphocytic lymphoma (B-SLL). Also, late isolated renal manifestation of PBNHL is extremely unusual. Case report. A 56-year-old woman was presented with a solitary tumor of bladder wall, with history of dysuria and night sweating. She underwent ultrasound, computed tomography (CT) and laparatomy which showed a solitary round tumor situated at the left lateral bladder wall. A transvaginal needle biopsy of the tumor was performed and diagnosis of primary extranodal B-SLL was made in the absence of bone marrow, lymph node or blood involvement. She was treated for 6 months with chemotherapy (LOP protocol) until achievement of complete remission, confirmed by CT and cystoscopy. Nine years later, she developed nephrotic syndrome and impairment of renal function. The renal tissue, obtained by percutaneous biopsy, was also diffusely infiltrated by small lymphoma cells with widespread tubulointerstitial destruction. Biopsy specimen showed a few glomeruli with focal segmental mesangial sclerosis and capsular adhesion, associated with segmental glomerular depositions of IgM detected by immunofluorescent microscopy. Thus, typical lesions for focal segmental glomerulosclerosis were observed. Immunohistochemical analysis of lymphoma cells infiltrating renal parenchyma and bladder wall revealed the same immunophenotype of lymphoma cell: LCA+, CD79a+, CD20+, CD5+, CD23+/−, CD43+/−, bcl-2+, CD3−, CD45RO−, bcl-6−, Cyclin D1−, with very low proliferative activity (less than 5% Ki-67+ cells). A diagnosis of B-SLL was confirmed. Conclusion. Here we present a case of PBNHL with late isolated renal involvement, manifested with nephrotic syndrome. Widespread destruction of tubulointerstitium led to decrease of renal function. Glomerular changes typical for focal segmental glomerulosclerosis caused nephrotic syndrome. Glomerular lesions could be due to glomerular overloading phenomena because of large destruction of renal parenchyma with lymphoma cells.

PP4-84
DIABETIC NEPHROPATHY ASSOCIATED WITH CRESCENTIC PROLIFERATION

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The purpose of this study was to investigate the cell types involved in the extra-capillary proliferation occurred in two cases of diabetic nephropathies. During the last couple of years, 14 renal biopsies with diabetic nephropathy have been thoroughly investigated for nephropathologic diagnosis. Two cases of already known diabetic patients have been found to develop extra-capillary proliferation featuring cellular crescents, segmental or circumferential, here and there developing epithelial, tubular profiles. Since the literature does not mention such an association, the problem raised is if this extra-capillary proliferation belongs to the diabetic pathology, or it is a

consequence of some superimposed lesions. In one case the proliferation can be connected with some moderate IgA deposits, while in the second we could not find any other explanation. The crescentic proliferations have been thoroughly investigated in immunofluorescence, light and electron microscopy. One first remark was that all glomeruli with crescents had an intact basement membrane of Bowman capsule. The crescents contained only epithelial cells and fibrin in some places. This comes in agreement with the hypothesis that all monocytic cells penetrate the crescents from the periglomerular area through capsular breaks. A second remark concerns the glycogen content of proliferated parietal epithelial cells. While usually parietal epithelial cells are reach in glycogen rosettes, our samples showed crescents of clear cytoplasm cells with little glycogenic granules. The general conclusion was that in some conditions of diabetic nephropathy, injured glomeruli may develop extra-capillary proliferation with some distinct characteristics.

PP4-85

COMPLEMENT C3 AND HYALINE VASCULAR DEPOSITS IN IgA NEPHROPATHY

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The aim of the study was to examine adult patients with IgA nephropathy (IgAN)and to analyze the effect of the clinical data and vascular lesions on renal failure progression. The clinical and pathologic materials of 25 IgAN patients were analyzed. The clinical data and histological features were recorded for each patient. The semiquantitative quantification was done for complement C3c and C3d vascular deposits evaluation. Clinical parameters were assessed at the known onset of the disease, at the time of renal biopsy, at the last outpatient check-up. Statistical analysis was done by segmentation, using the CHAID algorithm, included in the SPSS software. Hypertension was identified as the risk factor with the greatest impact upon risk of progression to renal failure among these patients ($p<0.0001$). Severe vascular lesions were significantly correlated ($p<0.005$) with a renal failure progression. Arteriolar C3c deposits were observed in 86.6% biopsies with IgAN, even in the biopsies from normotensive patients. Perinuclear vacuoles were observed in the smooth muscle cells in cases with C3c deposits in periglomerular arterioles in the biopsies from normotensive patients. The C3c and C3d deposition along the interstitial vascular wall was more severe in those with vascular lesions than in those without vascular lesions. C3d deposits were observed in arteries, arterioles and peritubular capillaries in all cases with hyaline vascular deposits. We have found focal arteriolar hyaline deposits, mostly in a nodular form, at the junction between arteriole and glomerular hilus in 48% of IgAN. The glomerular hilus was thickened and mesangium was enlarged at the vascular pole. Arteriolar hyaline deposits were correlated with development of the interstitial fibrosis and the percentage of C3d deposits area (morphometry – ImageJ). Vascular and tubulointerstitial lesions were identified as the most accurate histological prognostic factors regarding future evolution of IgA nephropathy. We suggest that complement activation at vascular level precede vascular lesions and subsequent hypertension. C3d vascular deposits and vascular hyaline reflect the disease degree and can be used as an important histological prognostic indicators.

PP4-86

SIGNIFICANCE OF CLINICAL PARAMETERS AND MORPHOLOGICAL ALTERATIONS IN ADULT-ONSET MINIMAL CHANGE DISEASE

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BACKGROUND: Minimal change disease (MCD) in children is the major cause of nephrotic syndrome with good prognosis, while adult-onset MCD has some differences such as slower response to corticosteroids and subsequent relapses. It has been reported that light microscopical and immunofluorescense may affect the response to steroid therapy and the long-term course. METHODS: Among the glomerular pathologies diagnosed by kidney biopsy between 1981 and 2006, we selected 18 adult patients whose biopsy result was the MCD. Clinical information was obtained from the hospital charts. The histologic sections were re-evaluated and morphological features were noted. RESULTS: The median age at diagnosis was 32.5 years, %66 of the patients were younger than 40. The male to female ratio was 1.25. Swelling of legs and periobital edema were the most common presenting symptom. Six patients had hypertension. The mean daily proteinuria was 3.95 g/day and serum albumin level was 2.5 mg/dl. Serum creatinine was high in 24% of cases. Urinalysis showed microscopic hematuria in 38% of patients, granular/hyaline casts in 57%. Hypertriglyceridemia and hypercholesterolemia were detected in 81% at presentation. In one patient, NSAID use was a suspect in etiology. The mean number of glomeruli per biopsy was 27 with 11% average ratio of global sclerosis. There were mesangial widening in 6 cases, trace amounts of immune deposits (C3, IgM or IgA) in 8. Arterial and/or arteriolar nephrosclerosis was noted in 9 patients. Five biopsies revealed mild interstitial inflammation. Clinical follow-up data were available on 12 cases. Mean follow-up period was 66 months (range, 8-204 months). Four patients experienced complete remission with no relapse. 5 patients had multiple relapses, 3 of which responded cyclosporine therapy. Time to relapse varied between 6 to 80 months. Presence of microscopic hematuria was the only parameter that is likely to correlate with the development of relapse ($p=0.07$). In all patients renal function was preserved after treatment. CONCLUSIONS: MCD is typically expected to show no abnormalities in light microscopy. However, there may be minor light microscopic alterations in some cases. In our study group composed of adult patients, neither the presence of these changes nor trace positivity of immune reactants predict the patient's clinical course or responsiveness to steroid therapy. Hematuria is the only clinical parameter which was likely to associate with the occurrence of relapse. Adult onset MCD has a good long term outcome with well-preserved renal function.

PP4-87

HYDATIC CYST OF THE KIDNEY: A SERIES OF 39 CASES

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Introduction: Kidney's hydatic cyst was rare and characterised by its clinical characteristics and the therapeutic problems. The aim of this study was to identify the epidemilogic particularities, the diagnosis and therapeutic modalities of hydatic cyst in this exceptional localisation. Materials and methods We report a retrospective study relating to 39 cases of hydatic cyst of the kidney diagnosed and treated between 1982-2005 in Habib Bourguiba's hospital, during the same period 2377 cases of hydatic cyst were operated in the same hospital. Results The male/femaleratio was 0,42. The ages ranged from 6 to 69 years (mean age 42,5). Clinically, the flank pain represented the most

frequent symptomatology (86,5%), and cystic mass in 25 cases (62%), the hydaturia was present in 14% of cases. The intravenous urography highlighted a tumoral syndrome in 66,6% of the cases. Ultrasound evoked the diagnosis in 72,2% of the cases and classify the hydatic cyst according to Gharbi & coll's classification in type I (18 cases), type II (5 cases), type III (8 cases), type IV (6 cases) type V (2 cases). Computed tomography was practised among 6 patients. The treatment was surgical in all the case and often conservative (85%), the nephrectomy was practised in 6 cases (15%). The diagnosis was confirmed by pathologic exam. Discussion The renal's hydatic cyst accounts for 1.6% to 4% of all localizations in the human hydatidose. The clinical symptoms are variable. Imagery associated to serology allow to make the diagnosis. The treatment is surgical.

PP4-88

VASCULAR ENDOTHELIAL GROWTH FACTOR IN RELATION TO NUCLEAR FACTOR – KAPPA B IN RENAL CELL CARCINOMA

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Objective: Renal cell carcinoma (RCC) is a malignancy with variable clinical course, partly attributable to specific genetic alterations of the different RCC types. Angiogenesis is important for tumor progression and metastatic spread. VEGF is a major factor which regulates angiogenesis while the nuclear factor-kappaB (NF-kB), a family of transcription factors; regulate a wide variety of cellular processes including cell growth, differentiation and apoptosis. The aim of this study was to evaluate the expression level of VEGF and to compare their values with the subcellular localization of NF-kB, proliferative rate of tumor cells and clinicopathological characteristics of RCC. Material and method: Immunohistochemical evaluation included VEGF expression, the subcellular localization of p65 member of NF-kB and Ki67 for proliferative rate of tumor cells in series of 16 RCC. Total RNA was extracted from the same tumor tissue samples, previously snap-frozen. Expression of VEGF was analyzed using quantitative Real-time PCR. Results: The difference in VEGF mRNA levels between conventional (11 clear cell RCC) and other histological types of RCC was not significant. Preliminary results indicate a more pronounce heterogeneity in VEGF expression within the group of clear cell RCC. VEGF mRNA levels show some positive association with VEGF protein expression evaluated as the percentage of positive cells and intensity of staining within the cells, while there was no association with NF-kB and Ki67. Larger tumors were characterized with higher value of VEGF expression, NF-kB and Ki67 and, moreover, the proliferation rate of tumor cells was higher in tumors with higher VEGF expression. Conclusion: Preliminary results indicate the heterogeneity in VEGF expression in conventional type of RCC. The association with tumor progression (tumor size and proliferation rate) indicates that VEGF is an important angiogenic factor in RCC. Although no association between NF-kB activity, VEGF expression and Ki67 was found on a small number of analyzed samples, our preliminary data suggest that the NF-kB may be important factor in renal carcinogensis by controlling cells proliferation. These findings need further validation of usefulness of NF-kB as a prognostic factor in RCC.

PP4-89

COLLOIDAL IRON STAINING PATTERNS IN RENAL CARCINOMA TYPES AND CORRELATION WITH ELECTRON MICROSCOPIC FINDINGS

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Background: Morphologically overlapping features of granular cytoplasm in clear cell carcinomas, chromophobe cell carcinomas and renal oncocytoma may cause difficulties in definitive diagnosis on light microscopic examination. Colloidal iron staining is considered to be characteristic for chromophobe renal cell carcinomas. In this study, our aim was to evaluate the colloidal iron staining patterns systematically in a spectrum of renal neoplasms and compare the findings with ultrastructural features. Method: We studied 116 cases of renal tumors which consisted of 93 clear cell carcinomas with different growth patterns and Fuhrman grades, 6 chromophobe cell carcinomas, 3 angiomyolipomas, 5 pelvic urothelial carcinomas, 5 papillary carcinomas, 2 oncocytomas and 2 collecting ductus carcinomas. One diagnostic paraffine section from each case was stained with colloidal iron by Hale's method. Following determination of the staining patterns, the sites of sections with spesific staining were marked on the paraffine embedded tissue blocks and these areas were evaluated by electron microscopy after de-paraffinization and re-prosessing for electron microscopy. Results: We observed that other renal cell carcinomas apart from chromophobe cell carcinomas showed colloidal iron positivity. Four types of staining patterns were identified as follows: diffuse, strong, reticular staining, diffuse but weak, homogeneous cytoplasmic staining, focal, apical cytoplasmic, finely granular cytoplasmic staining and finally coarse granular cytoplasmic staining. Strong reticular staining was the only pattern observed in chromophobe cell carcinomas. On the other hand, other 3 patterns were seen in clear cell and papillary carcinomas together with renal oncocytomas. Urothelial carcinomas, angiomyolipomas and collecting ductus carcinomas were all negative. The ultrastructural features, particularly regarding the mitochondrial and microvesicular distribution were correlated with the light microscopic findings. Conclusion: Hale's colloidal iron and the presence of numerous microvesicles on ultrastructural examination have been considered characteristic for chromophobe renal cell carcinomas. However, this study clearly demonstrated that clear cell renal carcinomas, oncocytomas and even papillary carcinomas could be diffusely positive for colloidal iron. Although staining pattern significantly differ in these tumors, light microscopic and histochemical findings sometimes require an ultrastructural confimation for definitive diagnosis.

PP4-90

PATHOLOGICAL CHANGES IN KIDNEY BIOPSIES FROM PATIENTS WITH PLASMA CELL DYSCRASIA

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Background: Renal impairment/insufficiency are common systemic complications of many hematological malignancies and they also occur in the majority of patients with plasma cell dyscrasia. To analyze incidence and clinico-morphological presentation of kidney involvement during plasma cell dyscrasia, retrospective analysis was performed. Methods: Patients with diagnosis of plasma cell dyscrasia with kidney involvement were identified from Renal non-tumor pathology Registry at Department of Pathology, University Hospital Dubrava. All the light microscopy glass slides (H&E, PAS, Masson, Jones and Congo special stain) were reviewed, along with material used for immunofluorescence and EM analysis. Results: During period

from October 2003 to March 2007, 397 native kidney biopsies were analyzed. Kidney biopsies from 20 (5.04%) patients showed histological features consistent with diagnosis of plasma cell dyscrasia. This group included 7 male and 13 female patients. Patient age ranged from 38 to 76 years, median 60.9 years. All of them had proteinuria, which in 5 patients progressed to the nephrotic syndrome. An acute renal insufficiency were noted in 3 and chronic renal insufficiency in 4 patients. The most common histological patterns of renal involvement were AL amyloidosis (8 patients, 40 %) and cast nephropathy (7 patients, 35 %). In the remaining 5 patients, an acute tubular damage (3 patients, 15 %) and tubulointerstitial nephritis (2 patients, 10 %) were noted. Previous diagnosis of AL amyloidosis or immunoproliferative disorder/plasmocytoma had 4 and 3 patients respectively, while in the majority of patients, hematological evaluation and confirmation of diagnosis were completed after kidney biopsy. Discussion and conclusion: The plasma cell dyscrasia is frequently used as generic term for description of three distinct clinical entities: multiple myeloma, monoclonal gammopathy of unknown significance and dysproteinemia. They all have frequent renal involvement in common which can either present the first manifestation, or can occur later, during the course of disease. Morphological patterns of involvement vary, depending on which renal compartment is predominantly targeted. Commoner tubulopathic manifestation include Fanconi's syndrome and cast nephropathy, while glomerular damage can be manifested as AL amyloidosis or monoclonal immunoglobulin deposition disease. Using combination of light microscopy, immunofluorescence and EM, all of them can be properly diagnosed in kidney biopsy, which is important for later clinical management and therapy.

PP4-91

PRIMARY EXTRASKELETAL MESENCHYMAL CHONDROSARCOMA OF THE KIDNEY OF A YOUNG WOMAN: MORPHOLOGIC, IMMUNOPHENOTYPIC AND ULTRASTRUCTURAL ANALYSES: A CASE REPORT AND REVIEW OF THE LITERATURE

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Background: Extraskeletal mesenchymal chondrosarcoma is a rare tumor that has been reported mostly in neurosurgical and orthopedic literature. Five cases of primary renal extraskeletal mesenchymal chondrosarcoma are reported in the English literature with a variable, nonspecific presentation and relatively an aggressive behaviour. We present a case of primary extraskeletal mesenchymal chondrosarcoma of the kidney in a 28-year-old woman. We describe the radiologic, light microscopic, immunohistochemical and ultrastructural features of the case and discuss the differential diagnosis of the ekstraskeletal mesenchymal chondrosarcoma at this unusual site. Case: Following an episode of macroscopic hematuria, a 28-year-old woman underwent a radical nephrectomy for a radiologically confirmed right-sided renal mass. A radical nephrectomy was performed. Since the computed tomograms of the chest, abdomen, and pelvis showed no other lesions or evidence of metastatic disease, the histopathologic diagnosis is primary renal ekstraskeletal mesenchymal chondrosarcoma with its typical combined undifferentiated mesenchymal cells and cartilage islands. Urinary system CT revealed a relatively well-demarcated 3-cm tumor with calcifications and peripheral enhancement associated with foci of hypodensity within enhancing solid components. Microscopically, the tumor was composed of primitive undifferentiated mesenchymal cells and well-defined islands of hyaline cartilage. The immunohistochemical study revealed diffuse vimentin positivity in the cytoplasm of the neoplastic cells. Pancytokeratin, epithelial membrane antigen, CD99, desmin and SMA were negative. S100 protein staining revealed dispersed positivity in cartilaginous areas. An electron microscopic study showed primitive precartilaginous mesenchyme displaying focal cartilaginous differentiation. Conclusion: The radiologic and the macroscopic aspect, together with the dual morphologic characteristics of differentiated cartilage islands interspersed within vascular undifferentiated mesenchyme, confirmed the diagnosis of primary extraskeletal mesenchymal chondrosarcoma of the kidney. In spite of its rarity, it is important to diagnose primary mesenchymal chondrosarcoma in kidney because its biological behaviour may be different from that of tumors of similar morphology. The differential diagnosis includes PNET/Ewing sarcoma and other rare sarcomas of kidney. The patient is free of disease nearly 13 months after its initial presentation.

PP4-92

ULTRASTRUCTURE OF GLOMERULAR DEPOSITS IN CRYOGLOBULINEMIA

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Aims: Cryoglobulins are serum immunoglobulins precipitating at lower temperatures. They have been described as having a crystalline/fibrillar configuration. Our experience is not in line with such a description. Therefore, the aim of this study was to reevaluate systemically our kidney biopsy material with a particular emphasis to the ultrastructure of glomerular immune deposits in patients with primary and secundary cryoglobulinemia. Methods: In our archived bioptic material of 1992 kidney biopsies there were 18 biopsies of 14 patients with primary cryoglobulinemia (210-3600 mg/l) and 30 biopsies of 19 patients with autoimmune systemic connective tissue diseases, mostly systemic lupus erythematosus (SLE), and secondary mixed cryoglobulinemia (124-3300 mg/l). All biopsies were examind by light, immunofluorescence and electron microscopy (EM). For EM tissue samples were fixed in OsO4 and in some cases in formaldehyde, embedded in Epon 812 and stained with uranyl acetate and lead citrate. EM analysis of immune deposits was performed on highly magnified photos. Results: Deposits of various electron density were observed in glomeruli of all biopsies. In primary and in SLE associated cryoglobulinemia glomerular capillary wall deposits were found to be homogeneous or finely granular, with no evidence of any fibrillar structure. In 4 patients with SLE besides homogeneous, fingerprint deposits were also demonstrated. Glomerular and extraglomerular fingerprint deposits were demonstrated in two patients with low cryoglobulin levels, one with primary Sjögren's disease and the other with non-classified autoimmune systemic disease. In a patient with glomerular monoclonal IgG kappa deposits showing an ultrastructure of randomly oriented bundles of parallel fibrils described as characteristic for cryoglobulinemia type I, no detectable cryoglobulins were found in the serum. Conclusion: Our detailed EM study on kidney biopsy specimens reveals that glomerular immune deposits in patients with primary and secondary mixed cryoglobulinemia can be of various electron density, but usually homogeneous or finely granular. Furthermore, our study suggests that cryoglobulin deposits display an organised substructure with crystalline/fibrillar configuration, described originally in 1977 by Feiner and Gallo and frequently cited as characteristic, only occasionally in a minority of patients with cryoglobulinemia.

PP4-93
FSGS VARIANTS: CORRELATION OF HISTOPATHOLOGICAL FEATURES AND CLINICAL PRESENTATION
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Background: Focal segmental glomerulosclerosis (FSGS) is a clinicopathologic syndrome characterized by nephrotic syndrome and glomerular sclerotic lesions. Recently, several morphologic variants of primary FSGS which have different prognostic and therapeutic implications were defined. These variants are (1) FSGS-Not Otherwise Specified (NOS), (2) FSGS-Cellular variant (CeV), (3) FSGS-with diffuse mesangial hypercellularity (DMH), (4) FSGS-Collapsing variant (CoV), (5) Glomerular tip lesion (GTL), and (6) C1q nephropathy. We have, therefore, decided to categorize the FSGS cases diagnosed in our Department and evaluate their clinical significance. Method: Renal biopsy samples of 48 cases of primary FSGS, which are stained with Hematoxylin and Eosin, periodic-acid Schiff, methenamin silver and trichrom, were revised and categorized according to the morphologic variant grouping. All cases were also examined by IFM and EM. Clinical data was retrieved from the patient files. Besides FSGS, the CeV is characterised by endocapillary hypercellularity occluding lumens while DMH has generalisad mesangial hypercellularity. The CoV has the wrinkled and collapsed glomerular basement membrane. GTL is defined by the presence of segmental lesions in the periphery of the glomerular tuft and synechia formation between the glomerular tuft and Bowman's capsule at the tubular pole. C1q nephropathy is defined by FSGS with variable mesangial hypercellularity and dominant paramesangial deposits of C1q. Results: Among these 48 cases, 11 (%22.9) were categorised as GTL, 5 (%10.4) as FSGS-CeV, 2 (%4.2) as FSGS-DMH, 2 (%4.2) as FSGS-CoV, 3 (%6.2) as mixed form of GTL and FSGS-DMH, 25 (%52.1) as FSGS-NOS. None of the cases was categorised as C1q nephropathy. IFM and EM did not show any difference between the variants of FSGS. Retrieving the clinical data, it was found that collapsing variant presented with proteinuria alone, and FSGS-DMH with both proteinuria and hematuria. FSGS-NOS, GTL and mixed form presented with either proteinuria alone or with hematuria. FSGS-CeV, usually presented with proteinuria alone, but in one patient it was associated by hematuria. In one patient the clinical data was lacking. Conclusion: As recent studies showed that identifying the morphologic variants of primary FSGS may provide further prognostic and therapeutic implications clinically, the renal pathologists should try to evaluate the morphologic variants of FSGS. IFM and EM do not seem to be useful in the differential diagnosis of FSGS variants.

Pulmonary Pathology

PP4-94
THE EXPRESSION OF E-CADHERIN IN NON SMALL CELL LUNG CANCER
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Background: Several diagnostic and prognostic markers are being used in recent years, in order to investigate the pathogenesis of lung cancer. E-cadherin is a related new, very promising marker in cancer research, which is not widely investigated in lung cancer. E-cadherin is important for cell-cell adhesion. Reduction and/or loss of E-cadherin expression in non-small cell lung cancer correlates positively with malignant transformation of tumor and with the potential of these tumors for invasion and metastasis. The aim of the present study was to examine immunohistochemically the expression of E-cadherin in non-small cell lung cancer (NSCLC) and their relationship with clinicopathologic parameters such as histological type, grade, tumor size, sex ad age of the patients. Material and Methods: Adenocarcinomas, squamous cell carcinomas and large cell carcinomas fall within the spectrum of NSCLC. 42 patients (19 women and 23 men) with NSCLC treated surgically were examined retrospectively. The tumor specimens have been immunostained for E-cadherin. The tumors were squamous cell carcinomas (12 cases), adenocarcinomas (18 cases) and large cell carcinomas (14 cases). The age of the patients ranged from 44 to 78 (mean age 65 years). Immunohistochemistry was performed using an avidin-biotin complex method and monoclonal antibody against E-Cadherin. Membrane and cytoplasmic staining in >50% of tumors cells was considered as a positive expression of E-Cadherin. Results: Impaired E-cadherin expression (loss or cytoplasmic delocalization <50% of cells) was observed in 36 (85,7%) of 42 samples. Absent or reduced expression of E-cadherin was observed in 10(83,3%) squamous cell carcinomas , 13(72,2%) adenocarcinomas and 13(92,8%) large cell carcinomas. The Impaired expression of the E-cadherin was observed with a higher frequency in high- grade (squamous cell carcinomas and adenocarcinomas) than in low grade tumors (squamous cell carcinomas, adenocarcinomas and large cell carcinomas) 82% and 48% respectively, ($p<0.0001$). No relationship was found between E-cadherin impaired expression and tumor size or sex of the patient. Absent or reduced expression was seen in advanced age of the patients. Conclusion: Impaired E-cadherin expression was fount more frequently in high grade and more aggressive histological type tumors and as well as in advanced age of the patients.

PP4-95
EXPRESSION OF CD44 AND MMP-2: POSSIBLE ASSOCIATION WITH HISTOPATHOLOGICAL FEATURES OF INTRATHORACIC SOLITARY FIBROUS TUMORS
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Background: Recent researches had showed that tumor cell adhesion molecular CD44 and matrix metalloproteinases (MMP-2) were expressed strongly in many tumors, and was associated closely with invasion and metastasis of the tumors. Although solitary fibrous tumors (SFT) have a good prognosis , a minority

bahave malignant. The aim of this study was to analyze CD44 and MMP-2 expression to question histopathological parameters. Method: We reviewed 10 patients who had undergone surgical resections for benign or malign SFT . Clinical findings and follow–up informations were obtained for all patients. Haemotoxylen-Eosin stained sections were reexamined for evalution of histopathological parameters (degree of cellularity, keloidal type collagen deposition, perivascular hyalinization, hemangiopericytoma-like area, nuclear pleomorphism, number of mitoses, necrosis, cyst formation, myxoid areas). All cases were positive for CD34. Immunostaining of CD44 and MMP-2 were performed by using the streptavidin-biotin method with mouse monoclonal antibody. The intensity of CD44 and MMP-2 were evaluated by light microscopy. Results: 10 patients underwent surgical resection . 8 SFT were benign and 2 SFT were malign. There were 3 men (%30) and 7 women (%70) of a mean age of 54.5 years (range 30 to 71 years). One patient had a history of asbestosis exposure . Complete resection was performed in all cases. The tumor arising from pleura was excised with pleurectomy. In one embedded intraparenchimal tumor, pneumonectomy was performed. According to England criteria, 2 malign SFT were contained multiple masses. 3 cases originated in the right hemithorax and 7 in the left. Tumor size ranged from 4.5-27 cm. Coagulative necrosis was observed in 2 benign SFT and 2 malign SFT. All cases expressed strong CD44. However only 2 malign SFT expressed focal MMP-2. Conclusion: Although MMP-2 positivity was observed in 2 malign cases, CD44 positivity was not associated with malignant criteria in solitary fibrous tumors.

PP4-96

MUC-1 EXPRESSION IN BENIGN PULMONARY LESIONS AND NORMAL LUNG TISSUES

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Background: The mucin MUC-1, encoded on chromosome 1q21, is a high molecular weight transmembrane glycoprotein and is expressed normally at the apical borders of epithelial cells in the breast , colon, and lung. Expression of MUC-1 has several effects in cells, including inactivation of integrin mediated cell to matrix adhesion and E-cadherin mediated cell to cell adhesion as well as protection from cytotoxic lymphocyte. Our aims were to study association between the expression of MUC-1 and biologic nature benign pulmonary lesions and normal lung tissues. Method: We selected 4 intrapulmonary bronchogenic cyst, 2 sclerosing haemangioma, 2 usual interstitial pneumonia , 1 adenoid cystic malphormation and 16 normal lung tissues. Normal tissues were obtained from histologically normal lung tissue adjacent to carcinoma. Among the neoplatic tissues 9 were adenocarcinomas, 5 squamous cell carcinomas, 1 adenosquamous carcinoma and 1 mucinous type bronchioloalveolar carcinoma. Two normal lung tissues contained atypical adenomatous hyperplasia. MUC-1 expression was assessed by immunohistochemistry with monoclonal antibody against MUC-1 mucin. Membranous staining for MUC-1 was divided into complete and incomplete staining. Results: Apical surface of upper respiratory epitelium in all cases were stained completely with MUC-1. The surface cells in sclerosing haemangioma and atypical adenomatous hyperplasia were strong complete positive for MUC-1. The positivity with MUC-1 in normal tissues around the adenocarcinomas was stronger than squamous cell carcinoma. Conclusion: Complete MUC-1 positivity showed type II pneumocyte lineage. This feature will facilitate differential diagnosis of based type II pneumocyte pulmonary lesions.

PP4-97

HISTOLOGICAL FINDINGS IN CARDIOGENIC ALVEOLAR-CAPILLARY BARRIER DAMAGE

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Background: Chronic heart failure increases resistance to gas transfer across the alveolar-capillary interface. Acute pressure or volume overload can injure the alveolar blood-gas barrier. These alterations are generally reversible due to the reparative properties of the alveolar surface. However, when alveolar-capillary membrane is chronically challenged, remodeling of pathophysiologic and clinical parameters may take place. The resulting pulmonary histological changes in surgical lung biopsy specimens have been barely exploited. Method: We examined medical records of all cardiac patients with chronic heart failure, presenting lung diffuse infiltrates, who underwent surgical lung biopsy, from January 1982 to December 2005. Patients with congenital cardiopathy, infectious manifestations or under immunosuppressive treatment were excluded. Twenty-four patients met our study criteria. Histological features including alveolar collapse, edema, protein deposition, hemossiderin-laden macrophages accumulation and hemorrhage, venous and lymphatic ectasia, vascular sclerosis, capillary congestion and fibroblast foci were studied. Results: Twenty patients were men and 4 women, with a median age of 54 years (range 16 to 79 years). The main chronic heart failure causes were myocardial ischemia, heart valve diseases and dilated hypertensive and Chagas cardiomyopathies. Based on alveolar-capillary barrier changes three main groups were created. Group I comprised acute cardiogenic pulmonary edema, ranging from minimal "cuff" around of bronchovascular axis to severe alveolar fluid accumulation. The second group comprised chronic capillary congestion presenting different degrees of septal thickening, capillary dilatation and hemossiderin-laden macrophages alveolar accumulation. Group III were histologically characterized by diffuse alveolar damage, in focal or segmental distribution. In 3 of 5 patients belonging to group I, acute cardiogenic pulmonary edema was the main change due to myocardial ischemia. Among the 11 patients belonging to group II, 4 had myocardial ischemia whereas 3 had heart valve disease. Diffuse alveolar damage, found in 7 patients, didn't show any clinical picture predominance. Conclusion: Alveolar-capillary barrier injury due to chronic pressure or volume overload is manifested through different patterns of histological changes which recognition can influence prognosis and treatment. Patients with chronic heart failure should be evaluated to establish treatment protocols for acute pulmonary edema, chronic passive congestion and diffuse alveolar damage.

PP4-98

INTENSITY OF LUMINAL ALVEOLITIS IN PULMONARY TUBERCULOSIS

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Material and methods: Material of the study included the transbronchial biopsy samples obtained from 30 tuberculous patients. The quantity of inflammatory and immunocompetent cells was estimated by stereometric method numerical density. The term "perigranulomatous alveoli" is used to denote the alveoli surrounding a granuloma at the distance of 100 microns from its external edge. The remaining alveoli are refered to as "the alveoli far away from a granuloma". Results: Numerical density of all cells found in the lumen of the alveoli far away from a granuloma ranges between 18.327/mm^3 and 96.120/mm^3,

the mean value being 43.042/mm^3. Lymphocytes predominate - 21.778/mm^3 (50.6%), followed by macrophages 19.916/mm^3 (46.3%). Numerical density of all cells present in the lumen of perigranulomatous alveoli is found to range from 14.639/mm^3 to 197.628/mm^3, the mean value being 74.662/mm^3. Lymphocytes predominate 42.460/mm^3 (56.9%), followed by macrophages 29.555/mm^3 (39.6%). Discussion: The presence of inflammatory and immunocompetent cells on the surface and in the lumen of the alveoli accounts for luminal alveolitis. The presence of these cells in the lumen of the perigranulomatous alveoli accounts for perigranulomatous luminal alveolitis which has a significantly higher intensity than the luminal alveolitis in the alveoli far away from a granuloma. Thus the intensity of perigranulomatous luminal alveolitis is found to be 74.662 cells/mm^3, while the intensity of the luminal alveolitis in the alveoli far away from a granuloma is 43.042 cells/mm^3. Conclusion: Perigranulomatous luminal alveolitis in tuberculosis is 1.7 times as intense as alveolitis in the alveoli far away of a granuloma.

PP4-99

COMPARISON OF OSTEOPONTIN, β-CATENIN AND HNRNP B1 EXPRESSION IN LUNG CARCINOMAS

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BACKGROUND: This study was performed to compare osteopontin, β-catenin and hnRNP B1 immunoreactivities in small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC). Correlation of these three antibodies with grade and stage in NSCLC was also investigated. METHODS: Twenty-nine SCLC, 6 large cell carcinoma, 36 adenocarcinoma and 30 squamous cell carcinoma (SCC), totally 101 cases, were included in this study. Osteopontin, beta-catenin and hnRNP B1 expressions were immunohistochemically evaluated. In addition, these immunoreactivities were compared with grade and clinicopathologic stage in NSCLC. RESULTS: Osteopontin positivity was 6.9% in SCLC and 67% in NSCLC. When NSCLC types were individually considered, osteopontin positivity was 66.7% in large cell carcinoma, 80% in SCC and 55.6% in adenocarcinomas. β-catenin positivity was observed in 49.3% of NSCLC and none of SCLC cases. These results were statistically significant ($p<0.05$). No statistically significant difference was found in RNP immunostaning between SCLC and NSCLC ($p>0.05$). Neither grade nor stage of NSCLC was correlated with osteopontin, β-catenin or hnRNP B1 immunoreactivity. CONCLUSION: We observed that osteopontin and β-catenin are useful in differentiating SCLC from NSCLC. In this study, grade and stage of NSCLC were not found to be correlated with any of three antibodies. We propose that osteopontin and β-catenin may be useful in discriminating NSCLC with neuroendocrine differentiation and high-grade neuroendocrine tumors of the lung.

PP4-100

GLUCOSE TRANSPORTER-1 EXPRESSION IN PULMONARY NEUROENDOCRINE CARCINOMA

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Background: Malignant cells are known to have accelerated metabolism, high glucose requirement and increased glucose uptake. Transport of glucose across the cellular membrane is mediated by facilitative glucose transporter proteins. Glucose transporter-1 (GLUT-1) is one of 14 members of this family and is typically not detectable in normal tissue or benign lesions. Elevated levels of GLUT-1 expression and/or activation have been shown to be associated with malignancy. Since only limited studies address the issue of GLUT-1 expression in lung carcinoma, we sought to investigate its expression in pulmonary neuroendocrine carcinomas. Method: Tissue microarray based samples of 178 neuroendocrine carcinomas, including 48 typical carcinoids (TC), 31 atypical carcinoids (AC), 27 large cell neuroendocrine carcinomas (LCNEC), and 72 small cell carcinomas (SCLC) from different patients were studied immunohistochemically for GLUT-1 (Polyclonal, 1/300, Dako, Carpinteria, CA) expression. A composite score including distribution and intensity of labeling was compiled from each core. Correlation of GLUT-1 expression with clinical-pathological variables was performed utilizing SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Results: Forty seven percent (75/161) of pulmonary neuroendocrine carcinomas were immunoreactive with GLUT-1. Distinct membranous staining was observed in neoplastic cells with concentration along the luminal border of tumor islands. Normal bronchial epithelium and alveolar pneumocytes were negative. GLUT-1 expression correlated with tumor differentiation/type, $p<0.001$: 6.5% (3/46) cases of TC, 20.7% (6/29) of AC, 73.9% (17/23) of LCNEC, and 77.8% (49/63) of SCLC were positive for GLUT-1. Five year survival of GLUT-1 negative neuroendocrine carcinomas was 61% versus 21% GLUT-1 positive cases ($p<0.001$); however, this correlation was not independent of tumor type/grade. There was no correlation between GLUT-1 expression and gender, tumor size or stage. Conclusion: GLUT-1 is expressed in approximately half of pulmonary neuroendocrine carcinomas and displays membranous staining with predilection for the luminal surface of tumor islands. GLUT-1 shows strong correlation with neuroendocrine carcinomas differentiation/grade, but not with other clinicopathologic variables.

PP4-101

NUCLEAR ROUNDNESS IN NEUROENDOCRINE TUMORS OF THE LUNG

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INTRODUCTION: The most recent WHO classification of pulmonary neuroendocrine tumors recognizes four entities: typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma and small cell carcinoma. AIM: The aim of this study was to estimate nuclear size and roundness in carcinoid tumors and small cell carcinomas of the lung. MATERIAL AND METHODS: At Institute of Pathology, University of Nis five cases of typical carcinoid tumor and ten cases of small cell carcinoma of the lung were analyzed on biopsy samples obtained by fiberoptic bronchoscopy. After formaline fixation and paraffin embedding, serial histologic sections were routinely stained with H&E. The nuclear size et roundness were estimated using image analyzer LUCIA M 3.51 ab (Nikon) at objective 40x, after binary image editing. In each case a hundred nuclei were measured. A statistical analysis was performed using Mann-Whitney test. RESULTS: The roundness of nuclei in typical carcinoid tumor (0.963±0.007) was significantly larger than in small cell carcinoma of the lung (0.908±0.021), $p<0.01$. No significant differences in nucler size were found. CONCLUSIONS: The

authors conclude that nuclear shape is more rounded in neuroendocrine lung tumors of low-grade -typical carcinoids in comparison to high-grade tumors -small cell carcinomas. Further studies on a larger number of patients are required to confirm these findings. Key words: roundness, carcinoid, small cell carcinoma.

PP4-102
COEXISTING PULMONARY TUBERCULOSIS AND MULTIFOCAL PULMONARY CARCINOID IN THE SAME LOBE: A CASE REPORT

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The synchronous occurance of lung carcinoma and tuberculosis is well known , but coexistens with carcinoid tumor is very rare. One case was mentioned in the literature. In our Institute between 2003-2006 among 96 cases was one patient. A 38 years old woman has observed on screening chest X-ray multiplex nodules in lower left lobes. TTB was taken but cytological examination reveal necrosis.Bronchoscopy was negativ too.The patient underwent surgery for lobectomy. Intraoperativ cytology shows necrosis. Mediastinal lymph node dissection was performed simultanously. The histology showed tuberculosis acino-nodosa caseosa and multifocal appearence of typical carcinoid.With Zeel-Nielsen stain can be found some acid stain rod shape bacilli in granulomatous nodules. Immunhistochemical reaction in tumor cells was positiv with NSE,Chromogranin-A, Synapthophysin and panCytokeratin. Gatrointestinal examination looking for primary site for tumor was taken with negativ findings, serum chromogranin was in normal range. The patient has got antituberculotic therapy.Apropos of this case authors would like to present the diagnostic difficulties of multiplex nodules of lung. Which nodules must be investigated?

PP4-103
EXPRESSION OF p63, K903, TTF-1 AND CK-7 IN LUNG CARCINOMAS AND DIFFERENTIAL DIAGNOSIS

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Background: Lung carcinomas are the leading cause of death by cancer in the world both males and females.Accurate morphologic dictinction between small cell (SCLC) and non-small cell lung cancer (NSCLC) has therapeutic significance. However evaluating morphology can be limited by crush artifact, tumor necrosis, limited tumor representation and overlapping morphologic features in small biopsies. Method:In this study, we evaluated a panel of antibodies , containing p63, K903, TTF-1 and CK7, for their efficiancy in distinguishing between morphologic types of lung carcinoma by immunohistochemical method. Formalin fixed parafin embedded tissue section of 23 squamous cell carcinoma (SCC),12 adenocarcinoma (AC), 5 large cell carcinoma(LCC) and 6 small cell lung carcinoma(SCLC), that belong 46 patients underwent surgery and bronchoscobic biopsy (for only SCLC cases) were used. Results:High proportion of SCC were positive with p63(% 94) and K903 (%88), whole SCLC were positive with TTF-1 (100) and most of AK were positive with CK7 (%76). Staining patern in tumor cells especially for p63 changed with tumor differantiation . Conclusion: Accurate histologic typing of lung tumors can be evaluate with a panel of immunohistochemistry, especially in the little biopsies that have limited morphologic features.

PP4-104
IMMUNOHISTOCHEMICAL ANALYSIS OF COX-2 EXPRESSION PATTERN IN SPORADIC LUNG CARCINOMAS OF NEUROENDOCRINE ORIGIN

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BACKGROUND-OBJECTIVE: Cyclooxygenases are proteins that mediate the conversion of arachidonic acid to prostaglandins and other biolactive lipids.Prostaglandins are involved in cell growth regulation, angiogenesis and metastatic process. Two COX isoforms have been identified COX-1 and COX-2. In normal cells the COX-1 is expressed constitutively whereas COX-2 is usually undetectable but rapidly inducible by a variety of stimuli. COX2 overexpression has been detected in various tumors such as colon, gastric, breast, prostate and lung carcinomas. Up-regulation of COX-2 has been associated with resistance to apoptosis, angiogenesis, tumor invasiveness and poor prognosis. Deletion of COX-2 and selective COX-2 inhibitors can prevent lung cancer growth suggesting a promising therapeutic approach. Although a number of studies on COX-2 expression in NSCLC have been published, little is known on its expression in other types of lung carcinomas such as small cell carcinoma (SCLC) which is characterized by aggressiveness and chemoresistance. In this context we investigated the expression of COX-2 and its cellular and subcellular distribution in carcinomas of neuroendocrine origin such as SCLC and LCNEC (large cell neuroendocrine carcinoma). We also compared the COX-2 tumor expression levels to those of normal lung tissue obtained from the same patients.MATERIALS AND METHODS: Retrospective study of 90 cases of sporadic lung carcinomas of neuroendocrine origin (63 SCLC, 1 SCLC combined with adenocarcinoma component, 26 large cell neuroendocrine carcinoma LCNEC) obtained by bronchoscopic biopsies or FNBs and tumor or lung lobe resections. IHC detection of COX-2 protein expression using anti-COX-2 monoclonal antibody (clone COX 229, 1:100 dilution) and semiquantitative assessment of the expression levels.RESULTS AND CONCLUSIONS: A. In normal lung cells COX-2 expression was absent and rarely weak B. COX-2 expression in carcinomas was mainly cytoplasmic as follows : SCLC (N=59, ND=4) 28.8% with 3+ immunostaining, 25.42% with 2+, 30.5% with 1+ and 15.25% absence of expression, SCLC combined 1/1 with 3+ expression and LCNEC 30.77% displayed 3+ immunoreactivity, 11.54% moderate, 46.15% weak and 11.54% no expression. No significant tumor cell COX-2 expression heterogeneity was observed. In conclusion COX-2 expression was higher in tumor cells compared to normal ones suggesting a putative role of COX-2 in SCLC and LCNEC tumor progression and a promising therapeutic intervention by using selective COX-2 inhibitors against neuroendocrine lung tumors.

PP4-105
IMMUNOHISTOCHEMICAL ANALYSIS OF VASCULAR ENDOTHELIAL GROWTH FACTOR SECRETIVE ISOFORMS VEGF121, VEGF165 AND VEGF189 EXPRESSION PATTERN IN SPORADIC SMALL CELL AND LARGE CELL NEUROENDOCRINE LUNG CARCINOMAS

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BACKGROUND-OBJECTIVE: Angiogenesis is an essential process in tumor progression A number of interacting factors, such as growth factors, receptor tyrosine kinases, matrix metalloproteinases and integrins are involved in this

phenomenon. VEGF is an important regulator of angiogenesis and its gene gives rise in six protein isoforms through alternative splicing. VEGF exhibits proliferatve action mainly restricted to endothelial cells, stimulates microvascular leakage and induces proteases expression in endothelial cells contributing to tumor invasion and metastasis. High levels of VEGF expression correlate with poor prognosis in a variety of cancers including lung cancer especially NSCLC. However, information on VEGF expression in other lung tumor types such as SCLC is limited and needs further investigation taking into account the high metastatic potential of this carcinoma. In this regard we examined the expression of VEGF isoforms 121, 165 and 189 and their cellular and subcellular distribution in sporadic small cell (SCLC) and large cell neuroendocrine (LCNEC) lung carcinomas.We also compared the VEGF tumor expression levels to those of normal lung tissue obtained from the same patients. MATERIALS AND METHODS: Retrospective study of 90 cases of sporadic lung carcinomas of neuroendocrine origin (63 SCLC, 1 SCLC combined with adenocarcinoma component, 26 large cell neuroendocrine carcinoma LCNEC) obtained by bronchoscopic biopsies or FNBs and tumor or lung lobe resections. Simultaneous immunohistochemical detection of three VEGF isoforms expression using anti-human VEGF monoclonal antibody (clone SP28 1:100 dilution) that reacts with the VEGF121, VEGF165 and VEGF189 splice variants of VEGF. Semiquantitative assessment of the expression levels. RESULTS AND CONCLUSIONS: A. In normal lung cells VEGF expression was absent and rarely very weak . B. VEGF expression in carcinomas was cytoplasmic as follows : SCLC (N=56, ND=6) 3.57% with 3+ immunostaining , 8.93% with 2+, 30.35% with 1+ and 57.14% absence of expression, SCLC combined 1/1 with moderate 2+ expression and LCNEC (N=24, ND=2) 4.17% displayed 3+ cytoplasmic immunoreactivity, 12.5% moderate, 37.5% weak and 45.83% no expression. No significant tumor cell VEGF expression heterogeneity was observed. In conclusion VEGF expression levels in SCLC and LCNEC tumor cells were low or negative and demonstrated no significant difference from normal lung epithelia. This finding may suggest that other angiogenic factors than VEGF are responsible for the high microvessel density observed in these neoplasms.

PP4-106

IMMUNOHISTOCHEMICAL STUDY OF EMBRYONIC-LETHAL ABNORMAL VISION-LIKE ELAV/HUR PROTEIN EXPRESSION PATTERN IN SPORADIC SMALL CELL AND LARGE CELL NEUROENDOCRINE LUNG CARCINOMAS

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BACKGROUND-OBJECTIVE: Embryonic lethal abnormal vision (ELAV)-like protein HuR belongs to the RNA recognition motif (RRM) protein superfamily and is involved in cell growth and differentiation through posttranscriptional regulation of RNA transcripts. HuR is implicated in the stability and translation of various mRNAs, such as those encoding oncogenes, angiogenic factors and immunosuppressive cytokines. This transcripts stabilization occur via HuR binding to AU-rich elements (ARE) in the 3′ untranslated regions (UTR) of mRNAs. Therefore, dysregulation of HuR expression through putative genetic alterations or posttranscriptional and/or translational changes might contribute to malignant tumor progression . Indeed, there is a growing body of evidence that HuR is overexpressed in brain tumors, breast, ovary, NSCLC and colon carcinomas. Thus, HuR could provide a target for new therapeutic approaches by altering post-transcriptional events of tumor cells. In this context we investigated the expression of HuR and its cellular and subcellular distribution in carcinomas of neuroendocrine origin. We also compared the HuR tumor expression levels to those of normal lung tissue obtained from the same patients. MATERIALS AND METHODS: Retrospective study of 90 cases of sporadic lung carcinomas of neuroendocrine origin (63 SCLC, 1 SCLC combined with adenocarcinoma component, 26 large cell neuroendocrine carcinoma LCNEC) obtained by bronchoscopic biopsies or FNBs and tumor or lung lobe resections. Immunohistochemical detection of HuR protein expression using anti-HuR monoclonal antibody (1:500 dilution) and semiquantitative evaluation of the expression levels. RESULTS AND CONCLUSIONS: A. In normal lung cells HuR expression was both nuclear 2+ and cytoplasmic 1+ immunostaining. B. HuR expression in carcinomas showed : SCLC 87.3%, SCLC combined 1/1 and LCNEC 92.3% displayed 3+ nuclear and 2+ cytoplasmic immunoreactivity . All tumor types were characterized by significant tumor cell HuR expression heterogeneity (almost 30-40% of the tumor cells had no expression at all). In conclusion HuR expression levels were higher in tumor cells compared to normal ones suggesting a putative role of HuR in carcinogenesis and tumor progression via oncogene mRNAs stabilization.An important feature was the high tumor cell percentage (30-40%) with no HuR expression at all suggesting a dysregulation of post-transcriptional machinery leading to putative higher mRNAs degradation rates of other important molecules such as oncosuppressive gene transcripts.

PP4-107

PRIMARY PULMONARY MENINGIOMA: REPORT OF A CASE AND REVIEW OF THE LITERATURE

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Background: Meningiomas are well recognized in the central nervous system and comprise 15% of all intracranial tumors. Primary ectopic meningiomas are very rare neoplasms which are usually found in the head and neck region (scalp, orbit of the eye, temporal bone, sinonasal area, mandible and ear, paraspinal soft tissues) and less frequently in the mediastinum or retroperitoneum. Primary pulmonary meningioma is very rare. According to the recent literature, there are around 30 cases reported. Gross, histological and immunohistochemical features are similar to their intracranial counterparts. Although in some reports there is slight female predominance, other authors proposed that there is no predilection for sex, age and any particular lobe or segment of lung. These tumors usually present as a solitary pulmonary nodule, grow slowly, are benign in nature and have excellent prognosis. Clinically they can mimic any other pulmonary tumor including metastasis. Case report: A 34 year old woman was accepted to the Thoracic Surgery Department of our hospital with a four months history of cough, dyspnea and occasional hemoptysis. Flexible bronchoscopy revealed a mass lesion in the right lower truncus intermedius blocking bronchial lumen. Atelectasis at the right basal area was discovered with the chest x-ray. With the computed tomography of thorax, right lower atalectasis and intrabronchial solid mass lesion 5.3x3cm in diameters in the right medial-lower lobe was found. Right lung- medial and lower bilobectomy was performed with the clinical diagnoses of bronchial carcinoma or carcinoid

tumor. Gross specimen of lung tissue revealed a 2.5x2x2cm, grey-white, relatively well circumscribed, hard tumoral lesion blocking the intermediary bronchus lumen. Histologically the tumor was composed of cells with eosinophilic cytoplasm and round to oval nuclei in syncytial arrangement. Occasionally nuclear inclusions, rare mitosis and atypia were observed. Immunohistochemical examination showed positive staining for EMA (epithelial membrane antigen) and Vimentin. Stains for PanCytokeratin, Chromogranin, Synaptophysin, Neuron Spesific Enolase, HHF-35, S-100, HMB45, Desmin, CD68 , GFAP, TTF-1 and CD34 were negative. Conclusion: We present a case of primary pulmonary meningioma by reviewing the literature and discussing the differential diagnosis of this rare neoplasm.

PP4-108
THE ROLE OF IMMUNOHISTOCHEMISTRY IN DISTINGUISHING MALIGNANT MESOTHELIOMA AND ADENOCARCINOMA

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Background: Histological diagnosis of malignant mesothelioma (MM) and differentiation from adenocarcinoma (AC) is often difficult. In recent years, a large number of immunohistochemical markers have been increasingly applied for the differential diagnosis for these tumors. The aim of this study is to compare the value of MOC 31, HBME-1 and calretinin for discriminating between these malignancy. Materials and Methods: Eighty six cases of MM and twenty six cases of AC retrieved from the files of pathology department of Cukurova University Hospital were included in this study. Immunohistochemistry was performed on each case using avidin-biotin-peroxidese tecnique with antibodies to keratin, EMA, CEA, MOC-31, HBME-1 and calretinin. Results: Keratin was positive in all cases. EMA stained 76 of 86 MM (88.3%) and 23 of 26 AC (88.4%). CEA was positive in 23 of 26 AC (88.4%) and stained 17 MM cases (19.7%). MOC 31 stained 19 of 26 AC cases (73%) as well as 8 of 86 MM (9.3%). HBME-1 was positive 75 of 86 MM (87.2%), thick and membranous staining was prominent. HBME-1 stained 14 of 26 AC (53.8%). Calretinin was positive in %86.3 MM cases, both nuclear and cytoplasmic staining were noted. None of AC cases reacted for calretinin. Conclusion: This study confirms the importance of an immunohistochemical panel in the diagnosis of MM, and the important role of calretinin in this panel. A panel of four markers (two positive and two negative) usally allows for the distinction to be made between MM and AC. Calretinin, MOC 31 and CEA should be included in this panel.

PP4-109
ASSESSMENT OF THE PROTECTIVE EFFECT OF VITAMIN D ON RADIATION-INDUCED LUNG INJURY

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BACKGROUND: Vitamin D, especially its most active metabolite 1,25-dihydroxyvitamin D3 (calcitriol) is essential in regulating a wide variety of biologic processes, such as blocking mesangial cell activation. Recent studies indicate that calcitriol induces anti-fibrotic hepatocyte growth factor expression, which in turn blocks the myofibroblastic activation and matrix production in interstitial fibroblast. Myofibroblasts express alpha-smooth muscle actin, are present in the early phase of acute lung injury. The objective of this study was to assess the histopathological evaluation of the effectiveness of vitamin D as a protectant against radiation-induced lung injury. METHODS: The study included 18 Wistar female albino rats with an average weight of 250-300gr. All animals were divided into tree groups. Group 1: control (n=5), Group 2: irradiation alone (n=7), Group 3:irradiation and vitamin D via IM (n=7). Rats were exposed to 20 Gy radiation to the right lung in a Co60 radiotherapy machine where group 3 animals were treated with single dose of vitamin D3 (0.2mgr) injected I.M. for 2 hours before exposure of irradiation. Fifty days after post-irradiation rats were sacrified. The lungs were dissected and blinded histopathological evaluation was performed to asses for lung injury. Immunohistochemically we were stained alpha-smooth muscle actin to highlighted myofibroblasts. RESULT: Light microscopy of right lung sections fifty days after radiation revealed widespread distortion of architecture, with areas that showed markedly thickened alveolar walls. There was abundant vasculitic changes and extravasated neutrophils. Irradiation and vitamin D group of right lung sections also showed thickened alveolar walls but these finding focal rather than diffuse. No vasculer change determined of this group. Histopathologically the lung injury score was significantly lower for irradiation alone when vitamin D was l be needed to prove its safety. administered ($p \leq 0.05$). Also myofibroblastic differantiation score was significantly lower for the irrradiation alone than radiation and vitamin D group ($p \leq 0.05$). CONCLUSION: This study indicates that administration of vitamin D3 has an histological evidence of effectiveness as a protectant againts acute lung injury through blocking myofibroblastic activation. Further trials will be needed to prove its safety.

PP4-110
ADRENALINE ATTENUATES THE ACUTE LUNG INJURY AFTER INTRATRACHEAL LIPOPOLYSACCHARIDE INSTILLATION: AN EXPERIMENTAL STUDY

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Background Endotoxin is a major cause of endotoxinemia, sepsis and pneumonia due to gram-negative bacteria as well. Experimental endotoxin administration via the tracheal route has been extensively used to study the biological and pathophysiologic pathways of inflammation. In particular, experimental endotoxin instillation in the respiratory tree has allowed an extended research with regard to the local response of the lungs to the pathogenic stimulus. Method This study aims to examine the effects of adrenaline subcutaneous infusion in experimental lung inflammation by administration of intratracheal lipopolysaccharide (LPS) in an in vivo animal model. Two groups of animals were used for that purpose, a control group (single LPS administration) and a study group (subcutaneous adrenaline infusion following LPS administration). Results We found that in both groups, a mixed monocytic and lymphocytic infiltrate is an early event during the course of inflammation, occurring approximately two hours post-endotoxin instillation. In the study group, we determined that adrenaline mediated the lung inflammation in a statistically significant degree. By the use of immunohistochemistry, we also identified an increased population of CD4 T-lymphocytes in the inflammatory infiltrate, further endorsing the hypothesis that T-helper lymphocytes, along with monocytes, secrete cytokines

which amplify the inflammatory response. Conclusion Our study establishes that systemic adrenaline administration after LPS instillation may ameliorate the inflammatory lung response in vivo.

PP4-111
LARGE CELL LUNG CARCINOMA –(LCLC)– APPROACH TO RECLASSIFICATION ACCORDING TO THE DEFINITION AND CRITERIA CHANGES IN 1999 AND 2004 WHO CLASSIFICATIONS

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In the Pathology Department of Military Institute of Health Services in the years 1996 to 2007 911 cases of Lung Non Small Cells Cancers have been observed. In this group 89 have been primarily diagnosed as Large Cells Lung Cancers according to definitions and criteria described in previously published WHO Lung Tumors Classifications. The new WHO Classification of Lung Tumors published in 2004 changes some of the morphological criteria and definitions especially in that group of Lung Cancers. Aim: The aim of this study is to reclassify observed and primarily diagnosed Large Cell Lung Cancers according to the changes in current classification using standard microscopy evaluation and histochemical or immunohistochemical methods. Methods: All 89 LCLC cases identified (54 postoperative, 28 from oligobiopsy and 6 interoperative frozen sections) have been reclassified independently by 3 pathologists experienced in pulmonary pathology – selected slides from all cases have been stained with mucicarmin, PaS and alcian blue, CKAE1-3, CK7, CK20, TTF-1, ChromograninA, Synaptophisin, Calretinin, Vimentin and Ki-67/MIB-1 antibodies. Results: From 89 selected LCLC 79,5% cases have been classified as variants of Large Cell Cancer (classic type 44,3%; basocellular 11,3%, neuroendocrine 12,5%, other subtypes 11,4%). 18 (20,5%) observed cases have been classified according to the new WHO criteria and definitions as: Adenocarcinoma – 8%, squamous carcinoma – 10% and sarcomatoid carcinoma – 2,5%. Statistical analysis of Clinical Stage Tumor size, Nodal involvement (pTNM) and some of epidemiological data have also been performed. There were no statistical differences in tumor size (pT), nodal involvement (pN) and observed Pathological Stage between newly classified LCLC and other cancers. Conclusions: New definitions and criteria of 2004 WHO classification change about 20% of previously made diagnoses of LCLC based on earlier editions of WHO Lung Cancer Classifications. It should be emphasised that in our own material about 12% of reclassified LCLC have an neuroendocrine differentiation identified by immunohistochemical methods. For exact diagnosis based on contemporary classification of the LCLC immunohistochemical methods are necessary.

PP4-112
EXPRESSION OF SP-B (M-PR) AND TTF-1 IN ATYPICAL ADENOMATOUS HYPERPLASIA (AAH), BRONCHIOLOALVEOLAR CARCINOMA (BAC) AND PULMONARY ADENOCARCINOMA

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BACKGROUND: Atypical adenomatous hyperplasia (AAH) is reported as a focal premalignant change accompanying carcinomas in lungs that are not affected by fibrosis. The tumor that AAH most often accompanies is a peripheral adenocarcinoma. AAH may be also found in association with multiple adenocarcinomas. AIM OF THE STUDY: We examined the expression of surfactant protein B (mature and precursor/SP-BM, SP-BPr) and thyroid trancription factor-1(TTF-1) in two (2) cases of invasive parenchymal type lung adenocarcinomas develops probably from AAH through an intermediate stage of bronchiolalveolar carcinoma (BAC). PATIENTS: CASE 1: A woman patient aged 59ys with clinical history of bronchitis and asthma. For 20ys she has been on ''lediol'' treatment. Symptoms: cough – dyspnoea. Macroscopically: Surgical specimen of upper lobe of left lung with a tumor mass dim 3x2,8x2,5 cm localized 3 cm from the surgical bronchial edge. Microscopically: Mucin-producing invasive adenocarcinoma with multiple foci of AAH and lesion with BAC growth pattern. CASE 2: A woman patient aged 66ys with cough and dyspnoea has been treated with antibiotics after a recent episode of pneumonia with fever. CT and HRCT findings: Mass dim 4X6X2,5 cm on the upper lobe of right lung. FNB: Well differentiated adenocarcinoma type BAC. Macroscopically: Surgical specimen of upper lobe of right lung with a tumorous mass dim 3X2X3 cm. Microscopically: Peripheral BAC with foci of AAH in surrounding lung parenchyma. METHODS: Immunohistochemistry (IMC) was performed using a panel of primary antibodies such as: epithelial markers (CK 18, 19, 7), CEA, surfactant – protein B (M-Pr), thyroid transcription factor (TTF-1). RESULTS: Case 1: CK 18(+), CK 19(+), CK 7(+), CEA focally (+), SP-BM (-), SP-BPr(+), TTF-1(+). Case 2: CK 18(+), CK 19(+), CEA focally (+), SP-BM (-), SP-BPr(+), TTF-1(+). A variability of expression of SP-BM/SP-BPr was detected on cellular level in foci of AAH comparing with lesions of BAC or invasive adenocarcinoma. CONCLUSIONS: (1) The variability of differences in the expression of SP-B (M and Pr) in foci of AAH near BAC or invasive adenocarcinoma, may be helpful in early diagnosis of this type of lung cancer and in the future the use of these markers is expected to have prognostic value. (2) It is not clear whether multiple foci of AAH and adenocarcinomas (BAC or invasive adenocarcinoma) in the same patient represent independent neoplastic foci or probably clonally related to each other.

PP4-113
ENDOGLIN (CD105) EXPRESSION IN NON-SMALL CELL LUNG CANCER AND ITS CORRELATION WITH EGFR, CD34 AND OTHER HISTOPATHOLOGICAL PARAMETERS: PILOT STUDY

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Introduction: Endoglin (CD105), a cell-surface glycoprotein, recently has been identified as an optimal indicator of the proliferation of human endothelial cells. In this pilot study, we wanted to explore its presence in non-small cell lung cancer (NSCLC) and its correlation with epidermal growth factor receptor (EGFR), CD34 as well as with other standard histopathological parameters. Material and methods: Thirty-eight samples of NSCLC, diagnosed in period 2005-2006 at the Department of Pathology, Clinical Center of the University of Sarajevo, were randomly selected and retrospectively analysed. 19 squamous cell carcinomas (50%), 15 adenocarcinomas (39.4%) and 2 samples of adenosquamous and large cell carcinomas (5.3%) were stained for endoglin, CD34 and EGFR using monoclonal antibodies. All immunohistochemical results were captured by Olympus Digital Camera and scored by three researchers independently. Mean value was taken as a final score of immunohistochemical analysis. Statistical Package for Social Sciences (Version 11.5, SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Results: Endoglin expression was negative in 22 samples (57.9%) whereas positive expression was detected

in 16 cases (42.1%). The mean number of blood vessels stained by endoglin was 17.18 per field (magnification 200x). It was slightly higher in squamous cell compared with adenocarcinomas whereas CD34 expression had the opposite distribution but without significant association in both cases. Endoglin significantly correlated with another vascular endothelial marker, CD34 (p=0.024, Spearman's Correlation Rank 0.364). Also, significant positive correlation was detected with vascular invasion, previously confirmed on routine HE samples (p=0.008, Spearman's Correlation Rank 0.423). Endoglin did not correlate with tumor size whereas CD34 inversely did (p=0.039, Spearman's Correlation Rank –2.076). CD34 inversely correlated with tumor grade (p=0.05, Spearman's Correlation Rank –0.289). Vascular endothelial markers did not correlate with EGFR whose expression was slightly higher among squamous cell carcinomas compared with other subtypes. Conclusion: Endoglin is reliable marker of neoangiogenesis and its presence was frequently found in NSCLC. It significantly correlated with vascular invasion, important marker of tumor aggressiveness.

PP4-114

DIAGNOSTIC CHALLENGE ON FROZEN SECTION: PULMONARY PAPILLARY PNEUMOCYTOMA (SCLEROSING HEMANGIOMA) AND IMPORTANCE OF CLINICAL DATA

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We report here a rare case of primary pulmonary papillary pneumocytoma (sclerosing hemangioma), diagnosed in a 45-year old Bosnian female. Well-demarcated, 1.2x1 cm large, soft, partially calcified, multinodular tumor located in right middle lobe was received for intraoperative analysis. The initial diagnosis was metastatic papillary carcinoma. The tumor was composed of polygonal cells with eosinophilic cytoplasm arranged in papillary pattern (CK7 and EMA positive). The stroma was mainly composed of small, round cells (TTF positive). In between the network of small blood vessels was present (CD31 and CD34 positive). Ki-67 staining (MIB1 antibody) revealed low percentage of positive cells (less than 5%). In consultation with the surgeon we found out that the lesion persisted for several years. This information enabled the final diagnosis as well as second opinion since the sample was consulted in another institution. Although this entity is very care, careful examination and clinical history are of critical importance for accurate diagnosis.

PP4-115

INFLAMMATORY MYOFIBROBLASTIC TUMOR (IMT) WITH ABNORMAL OCTREOSCAN® POSITIVITY: A MISLEADING CASE

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Background: Inflammatory myofibroblastic tumor (IMT) is a relatively uncommon lesion, which has been reported to occur in nearly every site in the body. We present an unusual clinical presentation of pulmonary IMT, clinically misdiagnosed as carcinoid due to Octreoscan® abnormal positivity. Case Report: In August 2005 a 52 year-old woman, former smoker of 10 cigarettes/day, was admitted in hospital with thoracic pain. At HRCT the nodule appeared as a focal parenchymal solitary lesion, sharply circumscribed. The 2-Deoxy-2-[18F]Fluoro-D-Glucose (FDG)-PET/CT scan showed increase in tracer activity of the lung mass, suggestive of malignant lesion. The Octreoscan® showed abnormal accumulation of radioactivity in the left hilar region of the lung. A clinical/radiological diagnosis of carcinoid was postulated. An intra-operative frozen section was done, however the confirmed diagnosis of carcinoid could not be achieved. The tumor was composed of spindle cells with a bland cytology, low nuclear atypia and absence of mitosis. The lesion was regarded as low-grade malignancy. The chosen therapeutic procedure was surgical excision (lobectomy). At histology, the lesion consisted of spindle cells arranged in storiform pattern and an admixture of inflammatory cells, with a predominance of hystiocites and lymphocytes. The tumor was negative for ALK1, cytokeratin, CD34, S100 protein and EMA. The inflammatory cells infiltrating showed a strong staining for somatostatin receptors (SSTRs; subtypes 2A and 5). A final diagnosis of IMT was made. Six months after the surgical excision, the patient is alive and well. Conclusion: SSTRs have been identified on human immune cells, including mononuclear leucocytes and peripheral blood lymphocytes. The positive finding of the Octreoscan in our case was due to large intra-tumoral infiltration by lymphocytes and monocyte which expressed SSTRs. To our knowledge, this is the first case of IMT documented with Octreoscan and with immunohistochemistry for SSTRs.

PP4-116

ACUTE REJECTION IN HUMAN LUNG TRANSPLANTATION: A POSSIBLE SYNERGIC ROLE OF RHINOVIRAL-CYTOMEGALOVIRUS CO-INFECTION

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Background: Lung transplantation offers a chance of an improved quality of life and survival for many patients with advanced lung disease. Cytomegalovirus (CMV) and non-CMV viral respiratory infections often involve the lower respiratory tract and are associated with significant morbidity and mortality. The aim of the study was to assess the frequency of different viruses in bronchoalveolar lavage (BAL) specimens and to investigate a potential impact of the most frequent infections on allograft function. Methods: 240 monitoring BAL samples from 48 consecutive lung-transplant patients were analysed by polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR for Cytomegalovirus (CMV), Adenovirus, Rhinovirus (RN), Herpes Simplex Virus, Influenza Virus and Epstein-Barr Virus. Different clinical and morphological parameters, including acute rejection (AR), were recorded for a mean follow up time of 2.86 1.24 years. Results: High frequency of viral infections was detected in our BAL samples (160/240, 67%): particularly CMV and RN (122/160, 76% and 39/160, 24% respectively). Viral co-infections were found in 43/160 (27%) with frequent occurrence of CMV/RN (23/43, 53%). Patients with RN and RN/CMV positive BALs (group 1) were compared with patients with only CMV positive BALs (group 2) and with patients with CMV/other viruses (group 3). Group 1 showed a significant higher rejection index (n ARs/ n total biopsies) than group 2 (p=0.04). Recurrent infective episodes were more frequently detected in group 1 than in group 2 (p=0.006). No significant correlations were observed between group 2 and group 3. Conclusion: 1) CMV/RN is a frequent co-infection in lung transplant patients; 2) recurrent episodes of CMV/RN co-infection and the synergic action of the two viruses could play a crucial role in the induction of AR.

PP4-117
OXALOSIS AND NECROTIZING PULMONARY ASPERGILLOSIS: TWO CASES
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Introduction: The pulmonary oxalosis is a very rare pseudotumoral lesion; it is often secondary to an infection by Aspergillus niger and more rarely by Aspergillus flavus, Aspergillus fumigatus, Aspergillus luchurensis or by Beauveria bassiana. The diagnosis was based on histological examination Our aim is to discuss the diagnosis and the histogenesis of this pathologic association. Observations: We report two cases of chronic necrotizing pulmonary aspergillosis associated with oxalosis among two 17 and 69 year old men with a history of tuberculosis. The thoracic radiography revealed an hydroaeric level in the leg. The computed tomodensitometry revealed a pyopneumothorax and a collapses of the homolateral leg with tuberculosis lesions probably associated to an aspergillosis greff. The serology of aspergillosis was positive in the two cases. The histological analyse of the surgical specimans highlighting the birefringent calcium oxalate crystals by polarisation associated with branching septate hyphae. The culture yielded Aspergillus niger for the young patient. Conclusion: The oxalosis indicates evry intra-tissue oxalate crystal precipitation of calcium hereditary or acquired. Several authors described a brocho-pulmonary deposits of oxalate of calcium associated to an aspergillosis, the oxalic acid is a mycotoxin released especially by A.niger The diagnosis is based on mycologic examination with culture. The treatment is surgical in case of serious hemoptysy or a resistant surinfection to the medical treatment or to the drainage. The evolution is in general favourable.

PP4-118
EFFECTS OF PROPOLIS IN THE PROGRESSION OF URETHANE-INDUCED LUNG EXPERIMENTAL CANCER
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Background : Propolis, a natural beehive product has been known for centuries for a variety of beneficial traditional medicinal properties. The present study was conducted to ascertain the antineoplastic potential of propolis Design: 3mg/kg urethane was injected in male Balb-c mice, with 7 to 13 life weeks. One group with 13 mice was the control, and another group with 18 mice were submitted to 1.5 mg of Propolis diluted in 350 ml of drinking water . Food and water were given ad libitum. After 16 weeks, all animals were sacrificed and lung nodules and hyperplasias were counted in histological analysis. Results: There was no difference in the number of nodules and hyperplasias between the groups and the control (p= 0.69 and p=0.92, respectively). The final animal weight and food and water ingestion was similar in all groups. Conclusion: Propolis seems to have no effect in the progression of lung cancer in the dose used. It should be interesting to test this substance in higher doses.

PP4-119
SCLEROSING THYMOMA: CASE REPORT OF A RARE SUBTYPE
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Sclerosing thymoma is classified as a rare subtype of thymoma in the WHO classification. Few reports of this entity have been made since its first description by Kuo in 1994. Here, we report a case of sclerosing thymoma in a myasthenia gravis patient. A 41 year-old male presented with dysphagia, ptosis and muscle weakness. Computerized tomography showed a 3,5x1,5 cm mass in the anterior mediastinum. With a preliminary diagnosis of thymoma, the patient underwent maximal thymectomy. The specimen was 18x6,5x3 cm in diameter and weighed 175 grams together with the surrounding adipose tissue. On cut surface of the thymectomy specimen a hard, homogenous, spicular mass measuring 4,5x3,5x1 cm and gray-tan in color was detected. Microscopically, the lesion was mostly composed of hyalinized collagen-rich stroma in which aggregates of spindle and polygonal epithelial cells admixed with lymphocytes were noticed. The epithelial cells showed neither atypia nor mitosis. No hemorrhage or necrosis was present. The neoplastic cells were found to be positive for pancytokeratin and negative for LCA and chromogranin. A diagnosis of sclerosing thymoma was made. It is claimed that sclerosing thymoma is occuring as the result of ancient/regressive changes in conventional thymomas. It is a rare and contentious entity which should be considered in the differential diagnosis of small mediastinal biopsies composed of fibrous tissue.

PP4-120
INVOLVEMENT OF APOPTOSIS IN AMIODARONE INDUCED PULMONARY TOXICITY AND FIBROSIS
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Background Amiodarone (AMD) is a potent anti-arrhythmic drug frequently used for treating ventricular and supraventricular arrhythmias. Despite its effective antiarrhythmical properties, the use of AMD is often limited by its toxic side effects. The most severe adverse effect of the drug is the Amiodarone-Induced Pulmonary Toxicity (AIPT), which includes alveolitis, phospholipidosis and irreversible fibrosis. However, the mechanism underlying the involvement of AMD to the pathogenic molecular machinery of the disease remains unclear. Recent studies suggest that apoptosis of alveolar epithelial cells (AECs) plays an important role in AIPT. In the present study we investigate the level of involvement of AMD to the triggering of apoptosis. Method The immunohistochemical expression of pro-apoptotic proteins, activated caspase-3 and apoptosis inducing factor (AIF), and anti-apoptotic proteins Bcl-2, survivin and c-Flip, was studied in 33 samples of pulmonary tissue from Wistar rats after oral administration of AMD for 14 days at a dose of 30 mg/kg/day (minimum therapeutic dose). Tissue samples from rats where the drug had not been administered served as controls. Apoptosis was detected by the TUNEL technique and the apoptotic index (AI) was calculated. Results Administration of AMD was correlated with increased apoptosis, increased expression of activated caspase-3, and decreased expression of Bcl-2, survivin and c-Flip. Specifically, we found that AMD had been administered to the 66.7% and 63.2% of samples that presented increased AI and increased expression of activated caspase-3, respectively. On the contrary, administration of AMD was significantly correlated with decreased expression of Bcl-2, survivin, and c-Flip ($p \leq 0{,}001$, $p \leq 0{,}001$, and $p=0{,}023$ respectively). AIF protein showed consistent expression (58.1% of samples) especially among the cellular pulmonary population located far from lymphocyte infiltrations, however did not exhibit any significant correlations. A significant correlation was also found between immunoexpression of activated caspase-3 and TUNEL positive AECs ($p=0.040$). The immunoexpression

pattern of Bcl-2, survivin, and c-Flip were also found to be significant correlated (Bcl-2 vs survivin and c-Flip p≤0,001 and p≤0,001 respectively, survivin vs c-Flip p≤0,001). Conclusion Apoptosis plays a crucial role in AIPT. In contrast to AIF protein, activated caspase-3 acts as the effector molecule of apoptosis in AIPT. Inhibition of the anti-apoptotic molecules Bcl-2, survivin, and c-Flip is also involved in AIPT.

PP4-121

EXPRESSION OF SMAC/DIABLO AND CASPASE-3 IN NON SMALL CELL LUNG CARCINOMA (NSCLC)

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Background: The second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO) was recently identified as a protein that is released from mitochondria in response to apoptotic stimuli and promotes apoptosis by antagonizing inhibitor of apoptosis proteins. Caspase-3 is a cysteine protease that plays an important role in the process of apoptotic cell death. However, the involvement of Smac/DIABLO and caspase-3 in NSCLC is not fully understood. Method: Expressions of Smac/DIABLO and caspase-3 were investigated immunohistochemically in 112 non-small cell lung carcinoma patients. Staining patterns were correlated with tumor histological type, TNM stage and prognosis. Results: Smac/DIABLO was detected in 12 (11%) and caspase-3 in 60 (53%) of 112 NSCLC cases. There was no significant relationship between Smac/DIABLO and caspase-3 expression and age, gender, histological type, TNM stage and prognosis. In addition, no significant correlation was found between Smac/DIABLO and caspase-3 expression. Conclusion: Our data demonstrate that expression of Smac/DIABLO is rare among NSCLC's, suggesting that Smac/DIABLO or its agonists is unlikely to be an appropriate target for therapeutic options in patients with these tumors.

PP4-122

MYXOID TYPE OF INFLAMMATORY MYOFIBROBLASTIC TUMOR OF THE LUNG: REPORT OF A CASE WITH AN UNUSUAL PRESENTATION

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BACKGROUND Inflammatory myofibroblastic tumor (IMT) is a subgroup of inflammatory pseudotumors and has been reported in multiple anatomic locations including lung. It is composed of a variable mixture of collagen, inflammatory cells and a spindle cell proliferation showing myofibroblastic differentiation. IMT occurs mostly in patients less than 40 years. The clinical findings depend on the site of the involvement. Some authors believe that IMT is a reactive process rather than a tumoral lesion, while some support the theory of IMT as being a low grade mesenchymal malignancy. METHODS We report of a case of IMT with an unusual clinical presentation. A 28 year-old man was referred to the hospital with massive hemoptysis. A mass, which was suspicious for arteriovenous malformation, aneurysm or malignancy, was detected in the right upper-lobe adjacent to the interlobar fissur, radiologically. Angiography was in normal limits. Because of the high risk for hemorrhage, a transthoracic aspiration biopsy was done peroperatively. The sample was generally hypocellular, however there were some cellular areas consisted of mesenchymal cells with vesicular nuclei bearing a centrally located nucleoli. In some cells binucleation was seen. The clinician was warned for a possible mesenchymal lesion which malignant potential could not be predicted. Because the borders of the tumor could not be differentiated from the adjacent parenchyma and the extensive hemorrhagic appearance of the lung, the patient underwent a right upper-lobectomy. RESULTS On macroscopic examination a peripheric mass, that had irregular borders and hemorrhagic cut surface, and measured 3 cm in diameter, was detected in the hilus of the lobe. Histology revealed a well-demarcated tumoral lesion composed of myxoid stroma, neovascularization and bland appearing spindle cells. Binucleated cells, atypia and pleomorphism, rare mitoses were also seen. Immunohistochemistry displayed positive reaction for vimentin, smooth muscle actin, CD31 and p53, whereas no staining was detected for S-100, CD34, desmin, cytokeratin, ALK-1 and HMB-45. The possibility of a metastatic tumor was excluded by PET screening. CONCLUSION IMT is a rare tumor with various histologic patterns that can be confused with other malignant and benign tumors. The diagnosis can be very difficult, especially at the time of surgery in the frozen room. The appropriate choice of treatment is the total excision of the lesion, besides IMT is believed to be a low-grade malignant tumor. Although most of the patients are asymptomatic, clinical presentations differ according to the localization and the components of the tumor.

PP4-123

ENDOBRONCHIAL HAMARTOMA: SHOULD THE DIAGNOSIS BE MADE ON A BRONCHOSCOPIC BIOPSY?

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BACKGROUND Pulmonary hamartomas (PHs) are rare benign tumors composed of varying proportions of mesenchymal tissues and combined with entrapped respiratory epithelium. PHs are usually peripheric, however 3-20% of tumors are endobronchial. Endobronchial lesions clinically cause obstructive symptoms and are localized within the larger airways as broad-based polyps. Histological examination reveals mature cartilage tissue, surrounded by other mesenchymal elements and clefts of respiratory epithelium. In endobronchial hamartomas fat tissue may predominate and epithelial component may be shallow or absent. METHODS We report a case of an endobronchial hamartoma occuring in a 35 year-old man, who was admitted to the hospital with a 2 years of history of recurrent pneumonia and cough. Radiology demonstrated postobstructive pneumonia findings. A bronchoscopy was done with suspicion of a malignancy. Endoscopically, a polypoid lesion measured 0.5 cm in diameter was detected within one of the segment bronchi of right lower-lobe. RESULTS Histologically, bronchoscopic biopsy displayed structures of irregular chondroid and other mesenchymal elements in small tissue fragments . The possibility of a chondroid hamartoma was reported. However a malignant tumor could not be excluded. The patient underwent a segmentectomy procedure. In the frozen section room the mass defined by bronchoscopy could not be detected macroscopically. The bronchoscopy was repeated peroperatively and the mass was seen within an abnormally branching segmental bronchus. The segment carrying the mass was resected. Histologic examination

of the lesion confirmed the initial diagnosis with chondroid, myxoid mesenchymal and adipose tissue elements containing mass within the abnormal superior segmental bronchus. CONCLUSION PHs are benign lesions which have similar radiological and clinical features with malignant tumors. A biopsy is necessary for the differential diagnosis. Preoperative or peroperative diagnosis is important, since the definitive diagnostic procedure is segmentectomy or lobectomy. The presence of small, irregular chondroid and mesenchymal tissues can be seen in both mesenchymal and hamartomatous tumors. The pathologist and the bronchoscopist have to keep in touch to make the diagnosis.

PP4-124
LYMPHOVASCULAR INVASION PREDICTS RECURRENCE IN RESECTED STAGE I NON-SMALL CELL LUNG CARCINOMA

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Introduction: Prognosis of patients with non-small cell lung carcinoma (NSCLC) still remains a major problem. Numerous biological markers have been evaluated for prognostic significance but the results have been conflicting. In NSCLC, the prognostic value of lymphovascular invasion (LVI) has been evaluated in early stage patients. Recently, LVI has been reported to be a prognostic factor in patients with resected NSCLC with intrapulmonary metastases. Perineural invasion (PNI) has already been regarded as one of the prognostic parameters to be included for the next staging revision. Materials and Methods: Intratumoral LVI and PNI were investigated in 58 patients who had Stage I (T1N0M0) NSCLC by hematoxylin and eosin stained sections. LVI was determined by the presence of tumor cells either in lymphatic or blood vessels. Survival curves were estimated using the Kaplan-Meier method. The prognostic value of individual clinical and pathological variables was analyzed by the log-rank test. Results: LVI was detected in 50% and PNI was detected in 20.7% of NSCLC cases. The median follow-up for the entire group was 35.5 months. In univariate analysis, neither LVI nor PNI was predictor of overall survival. However, LVI was found to be significantly predictive of recurrence. The estimated relative risk of recurrence in patients with LVI was 3 compared with patients without LVI. Conclusion: LVI predicted recurrence in resected TIN0M0 NSCLC cases.

PP4-307
DIAGNOSIS OF DIFFUSE PULMONARY INTERSTITIAL DISEASE BY TRANSBRONSCHIAL BIOPSY (TBB): OUR EXPERIENCE (322 CASES).

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Introduction: Transbronchial pulmonary biopsy (TBB) is a good recognized method in the diagnosis of the Lung Interstitial Diseases and its utility has been proven throughout the years. Current technologies (immunohistochemical, molecular and other types of studies) allow a more precise diagnosis. The conditions that are necessary to assess these samples have been simplified in the last years, as well as the technical behavior of them. Also permit to obtain multiples samples of Interstitial Diffuse Diseases in different categories of hospitals. Material and methods: From 1985 to the present moment 322 TBBs have been made in our hospital to patients with suspicion of interstitial Disease, 307 of which were immunocompetent patients; a meticulous protocol has been followed with all of them for the complete use of the sample: initial seriation of minimal specimens (20 preparations, numbered and occasionally mounted in slides for immunohistochemical techniques) and a later staning, alternatively, of 8 of them: 4 H.E, 1 Masson, 1 PAS, 1 Perls, 1 Blue Alcián; the samples not stained initially, are stained afterwards, if they are necessary; and if not, they are filed. All the cases have been reviewed, in Clinical-Pathological Conferences, and the results of the biopsy have been assesed. They have been always valued with the bronchoalveolar lavage (BAL). Results: The diagnosed pathology has been: A) Sarcoidosis (23), Tuberculosis (4), Hypersensitivity Pneumonitis (30), Eosinophilic Chronic Pneumonia (9), “BOOP pattern” (27), Respiratory Bronchiolitis-Interstitial lung Disease (DIP pattern) (32), Constrictive Bronchiolitis (3), Lung hemorrhage (7, one of them a Goodpasture's Syndrome and other vasculitis), Usual Intersititial Pneumonia (33), Pneumoconiosis (11), Acute lesion (14) (Diffuse Alveolar Damage, Infectious Organizing Pneumonia, Iatrogenic damage,…), Infarct (2), Lymphangioleiomyomatosis. (2), Alveolar Proteinosis (2), Pneumocystis (1), Herpes virus (2), Aspergillosis (2), Bronchoalveolar Carcinoma (9), Lymphoma-Leukemia (4), Carcinomatous lymphangitis (5); B) The diagnosis was orientative in 34; C) Without alterations in 31; and D) The sample was insufficient in 34. Conclusions: 1.- The TBB was diagnostic or orientative, with a latter clinical confirmation in 80% of the cases. 2.- In 20% of the cases it the TBB was insufficient, or non valid (non representative of the process). 3.- The maximum use of the samples and the meticulous technical behavior, as well as the multidisciplinary team work, have contributed to it in a definitive way.

Skin Pathology

PP4-125
DEEP PENETRATING NEVUS, BLUE NEVUS AND SPITZ NEVUS – PROBLEMS OF DIFFERENTIAL DIAGNOSIS

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Background: Deep penetrating nevus (DPN) is a variant of melanocytic nevus that can be clinically mistaken for malignant melanoma (MM), pigmented nevus (PN), blue nevus (BN) and Spitz nevus (SN). Also, the histopathological features prone to confusion with MM, SN and BN. The final diagnosis is established by histopathologic/ immunohistochemical studies. BN is a benign lesion with two recognized variants: the common blue nevus and the cellular blue nevus. It is believed to represent dermal arrest of neural crest melanocytes during embryonal migration towards epidermis. SN (benign juvenile melanoma, spindle and epithelioid cell nevus) is a benign melanocytic nevus, which shares some histopathological features with malignant melanoma. Material and method: We analyzed the nevocelular tumors diagnosed in our department during two years (2005-2006): 574 nevocelular nevi and 54 MM. There were two DPN, two BN and two SN. We studied the histological features of all tumors (cellularity, cellular polymorphism, mitotic figures and lymphocytic infiltrate), the integrality of resection and the immunohistochemical markers for melanocytic tumors (S100, HMB 45, Melan-A, NSE). Results: Most of the patients were women (5 out of 6), with medium age 39.6 years. Most lesions occurred on the face (4 out of 6), one case on the foot (plantar side), the other having lumbar localization. All the tumors were composed of clusters and fascicles of polygonal and spindle shaped cells, with abundant cytoplasm and bland nuclei, sometimes with visible nucleoli. Cellular and nuclear polymorphism was noted in all cases. Evident diminution in cell size (maturation) towards the base of the lesion was present. Mitoses were present in one DPN (mitotic index 10/100 high power fields), none of them atypical. None of the tumors had pagetoid invasion of the epidermis. The DPNs had additional large, multinucleated tumoral cells in the superficial part. Conclusion: DPN, BN and SN are benign melanocytic tumors, difficult to differentiate both one from the other and especially from a MM. The correct diagnosis has the outmost importance for the patient's treatment and prognosis.

PP4-126
POSITIVE AND DIFFERENTIAL DIAGNOSIS IN TUMORS WITH SEBACEOUS DIFFERENTIATION

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Background: Benign sebaceous lesions are frequent, but malignant cutaneous tumors with sebaceous differentiation are relatively uncommon. In this group sebaceous carcinoma is a rare variant of cutaneous tumor with an aggressive biological potential showing a marked tendency for both local recurrence (9-36%) and distant metastasis (14-25%). Method: We studied 18 cases of sebaceous tumors diagnosed in our department in the last four years; 4 of them were sebaceous carcinoma SC, 6 were basal cell carcinomas with sebaceous differentiation (BCCSD), 4 were Jadassohn's nevi (JN) and 4 were benign sebaceous proliferations (1 sebaceous adenoma (SA), 3 sebaceomas), analyzing histological patterns, cytoplasmic and nuclear aspects, mitoses, necrosis, invasion and IHC features. Results: There was a significant predominance of male patients (61.1%); the average age differed within the five groups of lesions: 63 for SC, 49.2 for BCCSD, 38.9 for JN and 74.2 for sebaceoma. All the SC were located in the ocular area (3 in the eyelid and one on the conjunctiva); the tumors belonging to the other groups were both ocular and extra ocular (mainly on the scalp). All the SCs were diagnosed as grade II and showed lobular proliferation of atypical vacuolated cells with sebocityc differentiation, necrosis and, in one of the cases, pagetoid invasion of the epidermis; the well differentiated areas showed some similarities with benign sebaceous tumors that are analyzed. The BCCSDs are considered to be borderline tumors as they do not metastasize so that the identification of conventional BCC features associated with mature sebocytic elements were essential to separate them from SC. While the SA revealed proliferation of fully differentiated sebocytes and basaloid cells, the sebaceomas were composed of mixed immature and mature sebocytes. JN presented marked acanthosis, papillomatosis and sebaceous hyperplasia with apocrine proliferation. Histological aspects and IHC data are discussed. Conclusion: Tumors with sebaceous differentiation have various clinical presentations and histopathological aspects that make differential diagnosis both important and difficult. On one hand, well-differentiated sebocytic component of malignant tumors can be mistaken for a benign lesion especially in small bioptic specimens; on the other hand, the BCC component in a malignant tumor with sebocytic differentiation must be separated from SC. Careful examination of numerous areas of the tumor and IHC test must be performed in order to establish the correct diagnosis.

PP4-127
IMMUNOHISTOCHEMICAL EXPRESSION OF HUMAN HERPES VIRUS–8 IN THE DIAGNOSIS OF KAPOSI'S SARCOMAS

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BACKGROUND: Kaposi's sarcoma is a low-grade vascular neoplasm that has been shown by molecular analyse to uniformly express latent nuclear antigen-1 of human herpes virus 8. In the present study we assesed the sensitivity and specifity of human herpes virus 8 latent nuclear antigen-1 in the diagnosis of Kaposi's sarcoma and its potential usefulness for distinguishing it from various mimickers. METHOD: In total, 35 cases of Kaposi's sarcoma from 25 patients and with 18 mimickers (two cases of spindle cell hemangioendothelioma, three cases of kapiller hemangioma, a case of cavernous hemangioma, two cases of pyogenic granuloma, a case of stasis dermatitis, three cases of fibromatosis, two cases of dermatofibroma, a case of pilar leiomyoma, a case of fibroma and two cases of blue nevus) were included in this study. Fixed, paraffin embedded tissue sections were examined immunohistochemically using human herpes virus 8 latent nuclear antigen-1. Strong diffuse nuclear staining was considered as positive. Also the following variables for Kaposi's sarcoma cases were analysed; age, sex, localization and stage of the tumor. RESULTS: Of the 25 Kaposi sarcoma's cases there were 16 males and 9 females, giving an overall male:female ratio of 1.7.The age of the patients ranged from 37 to 80 (mean 66.36) years. The lesions occured more commonly in the lower extremity (54.29%), upper extremity (25.71%) head and neck region (17.14%) and trunk (2.86%). Eight biopsy specimens were in the patch or plaque stage that reflects the early

stage of Kaposi's sarcoma and 27 were in nodular stage. In 35/35 cases of the Kaposi's sarcoma showed positive nuclear staining for human herpes virus 8 latent nuclear antigen-1 (sensitivity, 100%), whereas 3/18 of the mimickers; a case of capillary hemangioma, a case of stasis dermatitis and a case of a dermatofibroma was positive for this antigen (specifity, 83.34%). CONCLUSION: The high sensitivity of human herpes virus 8 in our cases of Kaposi's sarcoma (100%) were similar with other investigations using immunohistochemistry or polymerase chain reaction. However, human herpes virus 8 specifity of 83.34% detected in this study was a little lower when compared to the literature. The result indicate that its use for diagnostic purpose must be carried out with caution.

PP4-128
C-KIT (CD117) EXPRESSION IN CLASSIC KAPOSI SARCOMA

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Background: Kaposi sarcoma (KS) is a multicentric angioproliferative neoplasm characterized histologically by the progressive proliferation of spindle-shaped tumor cells that progresses from patches to plaques, with eventual formation of nodule. Human herpesvirus 8 (HHV8) is associated with all epidemiologic forms of KS and has been shown in vitro to induce the tyrosine receptor kinase c-kit in HHV8- infected endothelial cells. The aim of this study was to evaluate c-kit expression by immunohistochemistry in different proliferative stages of classic Kaposi sarcoma. Method: In total, 30 cases of Kaposi sarcoma; at various histologic stages (4 patch, 2 plage, 24 nodular); from 18 patients were included in this study. Formalin-fixed, parafin-embedded tissue sections were stained using immunohistochemistry with antibodies to c-kit. Strong diffuse cytoplasmic staining was considered as positive. Also the following variables for Kaposi's sarcoma cases were analysed; age, sex, localization and stage of the tumor. Result: Of the 18 Kaposi sarcoma's cases there were 12 males and 6 females, giving an overall male:female ratio of 2/1.The age of the patients ranged from 37 to 80 (mean 66.36) years. The lesions occured more commonly in the lower extremity (54.29%), upper extremity (25.71%) and head and neck region (20%). C- kit immunoreactivity of lesional cells was demonstrated in 19 (63.33%) cases. There was no difference in the intensty of c-kit staining between age, sex, localization and histologic stages of KS. Conclusion: These findings indicate that c- kit expression in lesional cells can be detected by immunohistochemistry in different histologic stages of KS. The detection of c-kit expression in KS reveals a potential target for pharmacologic intervention that warrants further study.

PP4-129
MAST CELLS AND P53 EXPRESSION IN PSORIASIS VULGARIS

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Background: Psoriasis vulgaris is a chronic inflammatory T-cell mediated immune dermatosis, characterized by a high epidermal cell turnover, which results in a typical epidermal hyperplasia. As a result of the studies carried on by using antibody of "mast cell tryptase and toluidin blue stain", which are specific mast cell determiners, mast cells are found to be increased in psoriasis lesions. In addition, it is observed that there is an increase in cell proliferation of these lesions when p53 is used. The purpose of this study is to show the relationship between p53 protein accumulation that prevents epidermal hyperproliferation and mast cell number in its pathogenesis. Methods: In this study, punch biopsies of the 56 cases with diagnosis of psoriasis vulgaris have been investigated. The presence of mast cells in which the granules stain metachromatic with 1% toluidin blue solution has been investigated in cross-sections of formalin-fixed and parafin-embedded tissue samples. In addition, mast cells that show immunoreaction to mast cell tryptase antibody have been observed by streptavidin-biotin peroxidase technique. p53 proportion (number of positive nuclear cells/1000) in epidermal keratinocytes has been determined. Results: Fifty six cases with psoriasis vulgaris consisted of 31 (55.4%) females and 25 (44.6%) males. The ages of the cases range between 6 and 68. The average age is found 41.25 ± 14.14 (mean ± SD). In psoriasis cases, mast cells number (mean: 61.44± 25.98) and p53 proportion (mean: 454.93± 221.07) are found. In control group, mast cells number (mean: 74,61 ± 25,89) and p53 proportion (mean: 176,36 ± 109,18) were determined. A significant difference between psoriasis cases and control group in terms of mast cell number ($p<0.008$) and p53 proportion ($p<0.001$) was detected. No correlation has been found between mast cell number and p53 proportion. However, a moderate degree correlation ($r:0,30$, $p<0.05$) has been found between mast cell number and p53 proportion in the control group. Conclusion: Since the obtained correlation between mast cell number and p53 proportion of healthy skin is not valid for psoriasis cases, it is thought that these variables act differently in pathogenesis of psoriasis vulgaris.

PP4-130
EXPRESSIONS OF HEREGULIN IN MELANOCYTIC NEVUS, DISPLASTIC NEVUS, AND MELANOMA

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BACKGROUND. Heregulins are natural ligands for the growth factor family receptors called c-erbB-3 and c-erbB-4. Current studies suggest that thay can regulate a variety of responses including proliferation, differentiation, and survival. In addition, overexpressions were shown to be associated with stimulation of carcinogenesis. In this report, we study the expressions of heregulin in melanocytic, displastic nevi, melanoma and investigate their role in neoplastic transformation of melanocytes . METHOD. Using specific antibodies of heregulin, formalin-fixed paraffin embedded specimens of 13 melanoma, 8 displastic nevi, and 12 melanocytic nevi were examined for the expressions of heregulin. RESULTS. Nine of 13 (69%) melanoma, 7 of 8 (87%) displastic nevi, and 8 of 12 (66%) melanocytic nevi tissues showed moderate to strong immunoreactivity. Predominantly diffuse cytoplasmic staining was observed. Junctional melanocytic nests had stronger reactivity than dermal nests. Seventeen out of 33 (51 %) samples expressed heregulin in > 50 % of tumor cells. Additionally, keratinocytes at the upper layer of the epidermis also showed moderate to strong immunoreactivity in all samples. CONCLUSION. Although, heregulin expression were found in the majority of the tissues, we have demonstrated variable expression of heregulin in nevi and melanomas which do not differantiate between nevi and melanomas, but suggest an idea to investigate the heregulin as a new melanocyte marker. As the number of the tissues investigated in our study were taken into consideration, analysis of heregulin expression in larger series will enable us to identify as a useful marker.

PP4-131
MRNA VEGF-A EXPRESSION AND VEGF-A PROTEIN IN SKIN WOUND HEALING

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Introduction: Healing is a complex process of initial inflammation, granulation tissue formation and tissue remodeling. Angiogenesis is a necessary part of wound healing, and VEGF-A is a dominant mediator of physiological and pathological angiogenesis. VEGF-A is a mitogen for endothelial cells. Syntetic pentadecapeptide BPC 157 has so far demonstrated influence on healing process in morphological studies. To our knowledge most of healing studies involving VEGF were performed in vitro. Purpose of the study: to analyze in vivo wound healing by second intention through expression of mRNA VEGF- A and protein VEGF-A and attempt of compare them in control group and group treated with BPC 157. Methods: Full-thickness excisional wounds were made on the back of adults Wistar rats (3 animals / group). Animals were treated with BPC 157; 10 μg / kg, i.p. or eqivolume of saline.. Wounds were left uncovered and harvested 4, 6, 12, 24, 48, 72, 120, 168 hrs after injury. Half of the full thickness skin wound area was taken for histology/immunohistochemistry and molecular analysis respectively. For immunohistochemistry we used VEGF 147 antibody (Santa Cruz Biotechnology) following manufacturers protocol. For analysis mRNA VEGF expression analysis RT-PCR method was used. Results: In our study the expression of mRNA VEGF-A in control group followed the published data in wound healing by second intention (VEGF levels peaked at 6 and 48 hrs post-injury) and in group treated with BPC 157 mRNA VEGF showed a sinusoidal curve with three positive and two negative peaks tending toward basal level. Expression of VEGF-A protein was found in epidermis, dermis, hypodermis in both groups of animals, in vessels and in perivascular area. VEGF protein expression was significantly elevated 12 hrs post-injury in vessels of hypodermis in treated group and 48 hrs in perivascular area of hypodermis control group of animals. VEGF protein expression was significantly elevated 168 hrs post-injury in epidermis, and 12 hrs in vessels of dermis in control group. Conclusion: Our data suggest that BPC 157 has influence on mRNA VEGF and protein VEGF-A expression. Treatment with BPC 157 results with better controlled and prolonged expression of VEGF. Control group shows that in vivo wound healing by second intention confirms results of in vitro studies.

PP4-132
INFANTILE SYSTEMIC HYALINOSIS, A CASE REPORT

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BACKGROUND. Infantile systemic hyalinosis (ISH) is an autosomal recessive, rare and fatal disease. Hyaline deposition occurs in multiple organ systems, including skin. It is characterised by painful joint contractures, small pearly papules on the head, flesh nodules in the perianal region, gingival hypertrophy, generalized osteopenia, diarrhea, recurrent infections and usually thickened skin. The onset occurs within the first few weeks of life and death occurs by two years of age as a result of recurrent pulmonary infections and diarrhea. Unfortunately, treatment is primarily palliative as there is no cure currently available. METHOD AND RESULTS. Herein, we report an 8-month-old boy that was referred for a rash that appeared on his neck approximately 4 months earlier. He was born after a full-term pregnancy by caesarean section due to breech presentation. He was the sixth child of non-consanguineous parents who were from the same village. There was no family history of ISH. At 2 months of age, his mother noticed decreased upper and lower limb movements. His physical examination revealed gingival hypertrophy, extensive pearly erythematous papules all around his neck, a 4x4 cm purple plaque located over the sacral area, multiple flesh nodules around perianal region and purple patches located over his right 3th and 4th proximal interphalangeal joints and on both ankles. There was also extension contracture and stiffness in his cervical region, flexion deformity of his limbs with frog-leg position. Radiological evaluation showed generalized osteopenia and metaphysial enlargement. Histopathological examination of one of the papules showed compact amorphous eosinophilic hyaline deposits in the dermis. With the characteristic clinical presentation and histopathological findings, a diagnosis of ISH was made. Unfortunately, the patient died at 16 months of age. CONCLUSION. When encountering an infant with joint stiffness, purplish discoloration over the joints and papules around the neck, ISH should be a differentiative diagnostic consideration.

PP4-133
THE VALUE OF IMMUNOPHENOTYPING AND T-CELL RECEPTOR GAMMA GENE REARRENGEMENT IN THE DIAGNOSIS OF MYCOSIS FUNGOIDES

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Background: Mycosis fungoides is characterized with neoplastic infiltration of skin by dominant CD4 positive T lymphocytes. However, the diagnosis may be difficult for both dermatologist and pathologist in early lesions, hence clinical and histomorphological features may be nonspecific. Our aim was to evaluate the value of immunophenotyping and T cell receptor γ gene rearrengement studies as an adjunct to the histomorphological diagnosis of mycosis fungoides. Method: Histomorphological, immunohistochemical and polymerase chain reaction analysis were performed on formaline fixed, parafine embedded tissue sections of 73 cases (39 mycosis fungoides with classical histomorphology, 16 with suspicious histology for mycosis fungoides, 18 benign inflammatory dermatoses as a control group). Histomorphologically, three major (epidermotropism, Pautrier microabscess, atypical lymphocyte) and two minor (spongiosis, collagen increase) criterias were evaluated on H&E slides. Antibodies to CD3, CD4, CD8 by using immunohistochemistry and T cell receptor γ gene rearrengement analysis with polymerase chain reaction were performed. Results: Significant differences were found in epidermotropism (p=0,000), presence of Pautrier microabscess (p=0,004) and atypical lymphocyte (p=0,000), CD4 percentage (p=0,006), CD4/CD8 ratio (p=0,010), and clonality (p=0,000). Besides, CD8 percentage was not statistically different between three groups. In our study, we found out diagnostic cut-off values for CD4 percentage and CD4/CD8 ratio. That was 26% (sensitivity 82%, specifity 69%) for CD4 percentage and 0,65 (sensitivity 74%, specifity 69%) for CD4/CD8 ratio. Conclusion: Histomorphological features are still gold standart for diagnosis of mycosis fungoides. However, in early mycosis fungoides and the suspicious cases, immunophenotypical evaluation and T cell receptor γ gene rearrengement with polymerase chain reaction analysis -not certainly- but may help to the diagnosis of mycosis fungoides.

PP4-134
MELANOCYTIC LESIONS ARISING WITHIN SEBORRHEIC KERATOSES
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Background: Seborrheic keratoses are common benign epidermal neoplasms with considerable variety of histologic features. They are rarely associated with other skin lesions, such as basal and squamous cell carcinomas and solar keratoses. The precence of co-existing melanocytic lesions is extremely rare. The aim of the present study is to examine the frequency of the association between seborrheic keratosis and melanocytic lesions and the possible origin of the latter. Method: A retrospective analysis of 500 consecutive cases of histologically diagnosed seborrheic keratoses from the Department of Pathology, University Hospital of Ioannina, Greece was carried out. In all cases immunohistochemical staining for Mart-1 (Melan-A, DAKO) was performed using the EnVision system (DAKO) and DAB as chromogen. Results: In only one of the 500 cases (0.2%) an associated, arising within, malignant melanoma was identified (superficial spreading, radial and vertical growth phase), while no benign melanocytic lesions were noted. Non-neoplastic melanocytes were immunohistochemically identified within all the seborrheic keratoses, dispersed in variable numbers. However, their number never exceeded that of the adjacent normal epidermis. Conclusion: The frequency of melanocytic lesions arising within a seborrheic keratosis is extremely low, at least in our region. The reported case of malignant melanoma could be interpreted as a collision tumor, with the melanoma spreading in the seborrheic keratosis, or as a de novo melanoma development within the pre-existing seborrheic keratosis. Evidence supporting the latter is the intriguing presence of melanocytes amongst the squamous and basaloid cells of the seborrheic keratosis, as shown.

PP4-135
SQUAMOUS CELL CARCINOMA COMPLICATING NEVUS SEBACEUS OF JADASSOHN IN A CHILD
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Background: Sebaceus nevi are uncommon congenital skin lesions with a well recognized potential for neoplastic changes. Such change, however, is rare before puberty. Method- Results: We present a case of squamous cell carcinoma arising in a naevus sebaceous of Jadassohn (NSJ) in a 12-year-old girl. Both these events are rare. We review the literature and discuss the association of malignant tumors on a NSJ, and the need of prophylactic surgical excision of this lesion during childhood. Conclusion: this case is unusual for two reasons: first, because squamous cell carcinoma is an uncommon malignancy in NSJ; and, second, because of the patient's unusually young age.

PP4-136
PROLIFERATING TRICHILEMMAL TUMOR OF THE VULVA
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BACKGROUND: Proliferating trichilemmal tumors (PTTs) are rare cutaneous neoplasms that show features of typical pilar cysts but also show extensive epithelial proliferation, variable cytologic atypia and mitotic activity. PTT are benign lesions; however, the malignant potential of PTT is controversial, as only a small number of clinically or histologically malignant PTTs have been reported. We report an unusal case of PTT occuring on the vulva of a young woman. METHODS AND RESULTS: A 33-year-old woman presented with a gradually enlarging, painless nodule of the left labium majus. The excised tumor was well demarcated, measured 2.5 cm in greatest diameter and was covered by unremarkable skin. Based on the histopathologic findings, this tumor was diagnosed as PTT, despite the presence of moderate cytologic atypia and a florid mitotic activity. A squamous cell carcinoma was ruled out in the absence of infiltrative growth and tumoral necrosis. The patient is disease-free at last follow-up. CONCLUSION: To our knowledge, this is the third reported case of such a tumor occurring in this location. The range of the biological behaviour of this tumor is discussed. Complete surgical excision and accurate follow-up in all cases of PTT are recommended as local relapses and metastatic spread are possible and the biological behaviour of this neoplasm seems to be difficult to predict.

PP4-137
EXTRAMAMMARY PAGET'S DISEASE
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Extramammary Paget disease (EMP) is a scaly erythematous eruption affecting apocrine gland bearing areas of the skin. It usually occurs in the genital, axillary or perianal regions. The majority of cases represent an apocrine adenocarcinoma in situ that has a high recurrence rate and may invade the dermis and then possesses metastatic potential. Primary EMP is an apocrine adenocarcinoma in situ that most likely arises from intraepidermal cells of apocrine gland ducts. We present 4 cases of EMP that occurred in the axillary, perianal and vulvar regions. The clinical findings were suggestive of eczema or Bowen's disease. The treatment of EMP is essentially by surgical excision with clear margins.

PP4-138
BASAL AND SQUAMOUS CELL CARCINOMAS: DIFFERENCES IN THE VASCULATURE, VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), VEGF RECEPTOR-1 (VEGFR-1) AND VEGF RECEPTOR-2 (VEGFR-2) EXPRESSION
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BACKGROUND: Basal (BCC) and squamous cell (SCC) carcinomas are the most common skin carcinomas. It is well known that there is a significant difference in the behavior of these tumor types, with SCCs but not BCCs undergoing metastatic spread. Vascular endothelial growth factor (VEGF) is a homodimeric disulfide-linked glycoprotein involved in angiogenesis. Tumor angiogenesis is essential for tumor growth and appears to play an important role in tumor progression and development of metastases. The aim of the present study was to investigate whether the different biological behavior of BCCs and SCCs is associated with differences in their microvasculature and the molecules involved in neoangiogenesis. METHOD: We performed a retrospective study in 107 patients, 51 with BCCs (27 male, 24 female, average age 69.5 years, average maximal diameter 1.13cm) and 56 with SCCs (37 male, 19 female, average age 74.3 years, average maximal diameter 1.25cm). We

performed immunostaining on formalin-fixed, paraffin-embedded tissue sections using En Vision System (DAKO) and the monoclonal antibodies CD31 (Menarini Hellas), VEGF (VEGF Ab-3 JH121, Neomarkers, USA), VEGFR-1 (RP 077, DBS, USA) and VEGFR-2 (RP 076, DBS, USA). DAB was used as a chromogen. The percentage of the tumor cells that exhibited a positive immunoreactivity was determined using a ×40 objective lens. At least 1000 neoplastic cells were counted in each case and a semiquantitative method was used. RESULTS: Microvessel counts and VEGF and VEGFR-2 (but not VEGFR-1) expression differed in a statistically significant manner in the two tumor types, being more prominent in the SCCs. Furthermore, there were cases of BCCs with essentially no intratumoral blood vessels and no VEGF or VEGFR-2 expression. In SCCs, increased microvessel counts and VEGF, VEGFR-2 expression was significantly associated with low differentiation. CONCLUSION: The differences in the vasculature, VEGF and VEGFR-2 expression between SCCs and BCCs may, at least in part, account for their different biological behavior. Furthermore, SCC may represent a suitable neoplasm for antiangiogenic treatment in combination with conventional therapeutic approaches.

PP4-139

A PATIENT WITH SEBACEOUS CARCINOMA, SEBACEOUS EPITHELIOMA, KERATOACANTHOMA, BASAL CELL CARCINOMA, SEBORRHEIC KERATOSIS AND MULTIPLE ACTINIC KERATOSIS WITHOUT INTERNAL MALIGNANCY (MUIR-TORRE SYNDROME): A CASE REPORT

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Muir-Torre Sydrome represents the association of multiple sebaceous tumors, or keratoacanthoma with visceral malignancies. Cutaneous neoplasms can precede or follow a diagnosis of visceral malignancy. A 75 year old man was admitted the dermatology clinic for multiple skin lesions on his face. 14 lesions were excised.We reported one sebaceous carcinoma, one sebaceous epithelioma, one keratoacanthoma, one basal cell carcinoma, one seborrheic keratosis and nine actinic keratosis. We thought this case as a Muir-Torre syndrome and searched for internal malignancies. Colonoscopy, whole body computed tomography and prostate biopsy had made. But internal malignancy could not find. This man was worked in municipal worker for 37 years, so exposed sun light for a long time. 15 years ago and 10 years two lesions were excised, 5 years ago one more lesion was excised again. This year all of 14 lesions all appeared within 1 month. Crusted papulonodular new skin lesions are devoloped after the last exicisions. The patients with multiple sebaceous tumors and keratoacanthoma must be evaluated for the presence of visceral malingnancies. Recognition of multiple sebaseous neoplasms by pathologists and communication to clinicians of its strong association with Muir-Torre syndrome is of diagnostic importance. Our patient is followed up for skin lesions and for internal malingnancies.

PP4-140

PURELY CUTANEOUS ROSAI-DORFMAN DISEASE

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Background: Rosai-Dorfman disease (RDD) is a rare, benign, histiocytic disorder of unknown etiology. It's usually characterized by extensive painless lymphadenopathy often associated with many systemic manifestations. Extranodal involvement is seen in 25 to 43% of cases. Although the skin is the most common site of extranodal involvement, purely cutaneous RDD is very rare. We report a case of a 12-year-old girl who presented a particular purely cutaneous RDD. Methods: Among a rare case of purely cutaneous RDD, we discuss the epidemiological features, clinicopathological manifestations and treatment of this disease. Results: A12-year-old girl with a 3-month history of a widespread non pruriginous papulo-nodular eruption consulted in our department of dermatology. Physical examination revealed a profusion of firm erythematous papules and nodules, distributed mainly on cheeks, perioral region, the upper trunk, thighs and legs, mimicking a molluscum contagiosum eruption. No fever or lymphadenopathy was noted. Complete blood count was normal. Erythrocyte sedimentation rate was elevated to 60 mm/hr. Serum protein electrophoresis showed polyclonal hypergammaglobulinemia. Skin biopsy showed a reticular dermal nodular infiltration by lymphocytes, neutrophils and large vacuolated histiocytes with emperipolesis. Histiocytes were positive for PS-100 and CD68, and negative for CD1a. No systemic involvement was detected within a systemic work-up. These features were consistent with the diagnosis of purely cutaneous RDD. Because of the profusion of skin lesions, the patient was treated 20mg daily with acitretine for 4 months and surgical excision of large lesions. Skin lesions cleared progressively and continued to clear spontaneously after treatment interruption. Conclusion: Cutaneous variant of RDD (CRDD) has been rarely reported. About 3% of patients have disease detectable only in the skin. CRDD affects habitually older people with a preference for white females. Clinical features of this disease are nonspecific and there are no cutaneous sites of predilection. Typical histologic features are usually present in CRDD. Immunohistochemical studies are of great interest for the diagnosis.

PP4-141

HISTOPATHOLOGIC CLASSIFICATION IN BASAL CELL CARCINOMA AND COMPARISON OF P53 AND BCL-2 STAINING PATTERNS BETWEEN HISTOLOGIC GROUPS

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Basal cell carcinoma (BCC) is a skin tumor originating from pluripotential primordial germ cells in epidermis and skin appendage. It is usually local invasive and rarely makes metastasis. However, many studies were carried out with respect to this tumor due to its characteristics of considerable morbidity, risk of developing a secondary BCC in individuals having this malignity and rare but potential risk of causing metastasis. In several studies, it was presented that aggressive BCC's expressed more p53 and stained lesser with bcl-2. In our study, 75 patients, who were diagnosed as BCC between 2001-2005 at Pathology Department of Ankara Education and Research Hospital of the Ministry of Health were re-examined. Patterns were evaluated in respect of age, gender, localization, histological subtypes, staining patterns with p53 and bcl-2, ulcer, inflammation and presence of pigment. Although our findings do not indicate statistically satisfactory results, higher rate of staining by using bcl-2 and lower rate of staining by using p53 was noticed in non-aggressive histologic groups. However, there was no considerable difference between two markers in aggressive tumor sub-types. Our study pointed out that expression of bcl-2 and p53 may not be used as identifiers indicating aggressive or non-aggressive progress in BCC.

PP4-142
EPIDEMIC AND CLINICO-PATHOLOGIC PROFILES OF MELANOMA IN CENTRAL TUNISIA
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Background: Melanoma is a malignant cutaneous tumor characterised by an important metastatic potential. The aim of the present investigation was to report the epidemic and clinico-pathologic profiles of melanoma in Tunisia. Methods: A retrospective study was made recording all patients diagnosed as melanoma at the dermatology and the pathology departments of Farhat Hached hospital in Sousse, from January 1985 to December 2006. Results: Fifty nine patients were assessed with a mean age of 60,7 years (range: 5-92 years). Women were more commonly affected, the sex ratio being 1,36 (34F/25M). Consultation average was 16 months (range: 15 days- 8 years). The melanoma occurred in 66% (n=39) on a normal skin. It was localised on: extremities in 51,7 % (n=30), legs in19,1% (n=11), trunk in13,8% (n=8), face and hair in 13,8% (n=8). One case of nail's melanoma was noted. The most common clinico-pathologic variant was the acro-lentiginous melanoma (ALM) diagnosed in 30 cases (51,7%). The other variants were: nodular melanoma (NM) in 16 cases (28%), superficial spreading melanoma (SSM) in 9 cases (16%) and lentiginous malignant melanoma (LMM) in one case (1,8%). Histo-pronostic criteria showed Clark level higher than IV in 75,6% (n=34) of patients. The mean Breslow was 7,15 mm (range: 0,6-15 mm). Conclusion: The melanoma is considered as the most malignant cutaneous tumor. InTunisia, the most frequent clinico-pathologic variant is represented by the acro-lentiginous melanoma (ALM) estimated to 51,7 % in our study, whereas the SSM (superficial spreading melanoma) is the most frequent clinical variant (70%) reported in other countries. Unfortunately, this disease is frequently diagnosed late, that's why prognostic indicators are pejorative. Educational programs are important to be established in order to improve the early detection of this tumor.

PP4-143
FOLLICULAR MUCINOSIS AND HODGKIN DISEASE
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Follicular mucinosis is a peculiar cutaneous disorder, usually appearing as an incidental finding, but uncommonly associated with mycosis fungoides. Follicular mucinosis has been discovered besides in patients with malignant diseases, such as Kaposi sarcoma, chronic lymphocytic leukaemia, and Hodgkin's disease. We had this association developed in a 38-year-old male, who presented with lymph node enlargement in cervical, axillar, and inguinal regions, and simultaneously with cutaneous lesions in face and neck. A 2 cm axillar lymph node was excised, and also a cutaneous biopsy of the lesions was taken. The skin showed a normal epidermal layer, and a superficial and medium dermal inflammatory infiltrate. Lymphocytes and some eosinophils adopted and interstitial pattern, with focal extension to follicular epithelium. There were mucin deposits inside the follicles and dissecting epithelial cells. They stained with colloidal iron. Neither neoplastic lymphocytes nor atypical cells were seen. The lymph node showed on the histological study an architectural lack of structuration, with large nodules of small and medium round cells, and only scattered original follicles, with small germinal centres, entrapped among the rest of cellularity. Some cells had big nuclei, folded and multilobated, with pale chromatin, and small nucleoli, with the typical form of LH cells of lymphocyte predominance Hodgkin's lymphoma. Immunohistochemical study revealed B-cell markers expression in both small and LH cells, with minimum presence of reactive T CD3-positive cells. CD30 and EMA were negative, and bcl2 had a faint expression without nodular enfacement. The diagnosis was follicular mucinosis and nodular lymphocyte predominance Hodgkin's disease. Subsequent studies were done, and a bone marrow biopsy showed lymphomatous infiltration with nodular pattern similar to the one in lymph node. Although the association described is an uncommon event, there may have special significance based in the fact that several studies suggested a possible poor prognosis in patients with this two conditions. Our case seems to be in the same line, because the wide lymphomatous dissemination at the time of diagnosis, is not the usual presentation for lymphocyte predominance Hodgkin's disease.

PP4-144
SUPERFICIAL GRANULOMATOUS PYODERMA GANGRENOSUM
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Pyoderma gangrenosum (pg) is an idiopathic neutrophilic dermatosis described by brunsting et al, in 1930. Since its description, the pathogenesis of pg has remained obscure even as an ever-widening array of systemic diseases has been described in association with it. Classic pg can occur on any skin surface, but is most commonly seen on the legs, with one or more ulcers with raised edges. genitalia may be involved. Superficial granulomatous pyoderma is a variant of pg, important to recognize and differentiate from classic, with better prognosis and not associated with underlying diseases. there are approximately 55 cases in the literature. The most important histopahological characteristic is the granulomatous pattern and his diferential diagnosis with others granulomatous dermatosis. clinical history: A 78 year old male patient with type 2 diabetes mellitus developped a nodular lesion in his penis.clinical suspicion of carcinoma. after non-conclusive biopsies, a partial penectomy was performed. later he developed more lesions in scrotum. Histologically shows granulomatous formation with sinus tracts and numerous imflamatory cells and plasma cell infiltration. The differential diagnosis included fungal or mycobarcterial infection, bromoderma, ulcerative sarcoidosis and other types of cutaneous granulomatous disorders. We have to think in pgg in cases of granulomatous pattern even in atypical localizations.

PP4-145
IMMUNOHISTOCHEMISTRY HELPS DIFFERENTIATING HALO NEVUS FROM MELANOMAS WITH BRISK TUMOR INFILTRATING LYMPHOCYTES
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Background: Lymphocytic infiltration that accompanies melanocytic lesions causes difficulty in distinction between malignant and benign lesions on conventional histopathology. Although labeling of HMB-45 and expression of Ki-67 in benign and malignant melanocytic lesions has been investigated in many studies, little is known about the expression of these markers in melanocytic lesion with dense lymphocytic infiltrate. In this study; we aimed to investigate whether there is a possible differential expression of HMB-45 and Ki-67 in benign and malignant melanocytic lesions with or without intense lymphocytic infiltration. Methods: Immunohistochemistry was performed using formalin-fixed, paraffin-embedded tissue blocks from 68 melanocytic lesions; 13 halo nevi, 7 dysplastic nevi with

halo phenomenon, 17 dysplastic nevi without halo phenomenon, 15 invasive malignant melanoma with brisk lymphocytic infiltrate and 14 invasive malignant melanoma with nonbrisk lymphocytic infiltrate. Results: Both halo nevus and dysplastic nevus cases showed the same HMB-45 labeling pattern; strong labeling in the junctional component but only focal or weak in superficial dermal component. In melanomas, however, HMB-45 labeling was also evident in deep dermis as well superficial dermis. The number of Ki-67 positive cells in the melanoma cases with non-brisk infiltrate was significantly higher than that observed in any of the melanocytic lesions studied ($p<0.001$). Kaplan-Meier survival analysis revealed that neither Ki-67 expression nor HMB-45 labeling correlated with overall survival in melanomas. Conclusions: An immunohistochemical panel, including HMB-45 and Ki-67 might be useful in evaluating melanocytic lesions characterized by intense lymhpocytic infiltration.

PP4-146
TWO CASES WITH T PSEUDOLYMPHOMA

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Background: Cutaneous pseudolymphomas encompass a heterogeneous group of benign reactive lymphoproliferative diseases of T- and B-cell which is clinically and histopathologically resembling malignant lymphomas. The clinical appearance of pseudolymphoma is variable and usually characterized by single or multiple, and small or large red-purple doughy or firm bumps that are usually located on the head and neck, but it can involve any area of the body. Histopathologic apperance of T-cell pseudolymphomas are characterized by a superficial bandlike infiltrate or, rarely, by a nodular pattern located in the upper dermis. Cases: 18 year-old patient with erythematous, indurated nodules on the right appearance of chin and 35 year-old patient with hyperpigmented dome shaped nodul on the nose were taken biopsy. Histologically and immunologically, we detected T-cell pseudolymphoma in both cases. Conclusion: Classification of cutaneous lymphoproliferative disorders can only be achieved by a combination of clinical, histopathologic, immunophenotypic investigation. The purpose of this article is to summarize the criteria in the differential diagnosis of benign or malignant T-cell lymphoid infiltrates of the skin. We reported two patients who had been histopathologically diagnosed as T-cell pseudolymphoma. Key words: T-cell, pseudolymphoma, skin

PP4-147
EPIDERMAL LANGERHANS CELLS AND DERMAL MAST CELLS IN BENIGN AND MALIGNANT NEOPLASTIC SKIN LESIONS

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Immune cells of the epidermis and dermis participate in the tumor progression and development. Langerhans cells are epidermal antigen presenting dendritic cells. Mast cells represent a critical role in the immune system in the dermis. In the present study, we aimed to evaluate mast cells and Langerhans cells in some skin lesions including seborrheic keratosis, keratoacanthoma, actinic keratosis and squamous cell carcinoma. This study included 12 patients with seborrheic keratosis, 9 patients with actinic keratosis , 15 patients with keratoacanthoma and 35 patients with squamous cell carcinoma. Dermal mast cells and epidermal Langerhans cells were labeled with mast cell tryptase and S100 antibodies. In the data analysis, mast cells and Langerhans cells were counted and averaged in three representative area at x200 magnification. The number of the mast cells was significantly increased in the patients with squamous cell carcinoma in comparison with seborrheic keratosis (p:0.001). However, this parameter did not show significant difference between patients with seborrheic keratosis and actinic keratosis and between patients with seborrheic keratosis and keratoacanthoma ($p=0.733$, $p=0.417$). The number of the mast cells obtained from patients with squamous cell carcinoma did not show significant difference among patients with actinic keratosis and keratoacanthoma ($p=0.986$, $p=0.079$). The number of the Langerhans cells obtained from the patients with squamous cell carcinoma was significantly increased in comparison with seborrheic keratosis ($p=0.023$). Any difference was not observed between the patients with actinic keratosis and keratoacanthoma ($p=0.731$, $p=0.601$). Nevertheless, the number of the Langerhans cells was increased in the patients with squamous cell carcinoma in comparison with actinic keratosis ($p=0.002$). The numbers of the Langerhans cells and mast cells showed a lineer correlation only in the patients with actinic keratosis (r:0.8, p:0.008). Our data suggested that both epidermal Langerhans cells and dermal mast cells which have some contributions in the immune system, may have role in progression, development or regression of some skin tumors.

PP4-148
THE STAINING METHODS OF CHITOSAN AS A NATURAL BIOPOLYMER

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Background: Chitosan is a natural cationic linear polymer that has recently emerged as an alternative nonviral gene delivery system.It has been advantageous biological properties in the application as wound dressing, namely biocompatibiltiy, biodegradability, anti-infectional activity and a property to accelarate wound healing.In experimental animal models, chitosan was shown to influence all stages of wound repair.The hemostatic activity of chitosan can be seen in the inflammatory phase. It also interacts with and regulates the migration of neutrophils and macrophages acting on repairing process. During the inflammatory stage, chitosan accelerates the infiltration of inflammatory cells.At the new tissue formation period, formation of granulation tissue takes place within the wound space. We have researched the different methods for staining chitosan in the experimental skin wound model.Histological analyses that performed for assessment in wound healing of chitosan products are important.Therefore, it is necessity a simple and effective method for proper histological identification of chitosan materials. Method: For skin topical application, Wistar rats were first anesthetized, their backs shaved and full thickness skin wound was made at two individual sites on the dorsum of the back of each animal.Thereafter, 100µg LMW chitosan per wound sites were topically given.Skin samples were harvested 24,48,72hours (acute term) and 1,2weeks (chronic term) after application.Specimens were paraffin embedded, sectioned and able to observe specific staining of the chitosan particles with Cibacron-Brillant Red-3BA and Iron Hematoxylin and also Safranin-O/fast Green and Iron Hematoxylin staining methods.

Results: CBR-3BA iron hematoxylin was used for identifying the presence of chitosan incorporated with chitosan-given to rats.Chitosan was observed as red particles, iron hematoxylin was resulted blue coloration of nuclei.Cytoplasm and collagen fibrils were appeared pink color.The second method for specific staining of chitosan used the Safranin-O/Fast Green.Chitosan particles was appeared green color and with iron hematoxylin yielded stained nuclei. Conclusion. Chitosan that is an attractive material in biotechnology, used widespread fields as tissue engineering and drug delivery.Therefore, simple and specific staining methods are critical.This staining methods were highly successful in locating small quantities of chitosan in a dermal wound model.These methods may key a role progressing in the histological study of chitosan-based biotechnological products.

PP4-149

BCL-2 OVEREXPRESSION AT LYMPHOCYTES IN SKIN LESION AND PERIPHERAL BLOOD IN PATIENT WITH PSORIASIS VULGARIS

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Psoriasis is a chronic inflammatory skin disease which is characterized with healing and relapsing processes. Cellular immunity mediated by T cells is accused in pathogenesis. Bcl-2 is an anti-apoptotic gene related to the bcl-2 gene family, and its overexpression in lymphocytes has been shown in some chronic inflammatory and neoplastic diseases. 39 patient with psoriasis vulgaris were taken for study group and bcl-2 expression was investigated in skin biopsies by immunohistochemically which was taken from the active skin lesion and in peripheral blood by flowcytometric analysis. It was planned that to be done flowcytometric analysis before and after the topical steroid treatment. 10 pilonidal sinus cases were selected as negative control group for immunohistochemistry and 10 patient with active Systemic Lupus erythematosus (SLE) as positive control group for flowcytometric analysis. Bcl-2 overexpression was determined in dermal lymphocytes in different intensities in all the psoriatic skin lesions except one but not found in control groups. Flowcytometric analyses could be performed in 18 patients with psoriasis vulgaris before the treatment and in 10 of 18 patients after the treatment. Mean bcl-2 expression value was found 86.82 % at the beginig and 86.02 % after the treatment and 89,3 % in groups of active SLE patient. Significant correlation was found between psoriasis vulgaris and active SLE groups. The presence high levels of bcl-2 overexpression in dermal lymphocytes and in peripheral blood which was taken before the treatment thought that the bcl-2 overexpression may have an important role in the pathogenesis of psoriasis vulgaris. Patient with active lesion of psoriasis vulgaris exhibited levels of bcl-2 expression similar to those in active SLE, suggesting that raised bcl-2 levels may be characteristics of the inflammatory diseases. Although the skin lesions have improved with topical steroid therapy, the continuity of high levels of bcl-2 expression in peripheral blood indicate the insufficiency of the topical treatment. In conclusion, we speculated that systemic steroid therapy may be useful to reduce the bcl-2 expressing T lymphocytes levels in peripheral blood and that alternative theapy may be more effective in the treatment or in prevention of recurrences and systemic complications of psoriasis vulgaris. Further studies about the effects of systemic treatment on bcl-2 expression will be helpful.

PP4-150

EXTRAOCULAR SEBACEOUS CARCINOMA ARISING FROM THE SCALP

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Sebaceous carcinomas of the skin are rare tumors. Most of them often occur on the skin of the head and the neck. They are slow-growing, locally aggressive and capable of metastatic spread. They exhibit a variety of histologic growth patterns which may cause differential diagnosis hardship. We describe a case of grade II sebaceous carcinoma in a 67-year-old Tunisian woman localized on the scalp. It was associated with a metaplastic squamous component that has led initially to a diagnosis of degenerated trichilemmal cyst or squamous cell carcinoma. But, histochemical (ORO +: oil red O stain for lipid) and immunohistochemical (EMA+, ACE -, CD15-) studies using formalin-fixed and paraffin-embedded tissue specimens confirmed the the diagnosis of sebaceous carcinoma. We discuss clinical, histological, histochemical and immunohistochemical profiles of this aggressive neoplasm.

PP4-151

HAEMORRHOIDS AND GRANULOMA INGUINALE

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Background: To present an unusal case of granuloma inguinale presented on the surface of haemorrhoids. Method: The patient was male, 46 years old, presented to the surgical department of our hospital, due to enlarging of haemorroids. The physical examination confirmed that there was cirsoid ectasia of the haemorroid veins. There was also an extended skin rash of the perigenital and perianal area. The patient underwent haemorrhoidectomy. Results: Four reddish nodules, 2.2-3.4 cm in greatest diameter were received. On the surface small, whitish, elevated lesions were observed. Microscopic examination revealed the usual findings of the veins of the submucosal. The mucosa was infiltrated with many lymphocytes, plasma cells and neutrophills, sometimes in small aggregates. There were also many histiocytes. The squamous epithelium showed pseudoepitheliomatous hyperplasia and focal small ulcerations. The Giemsa stain revealed that the histiocytes contained small intracytoplasmatic inclusion bodies which considered to be Donovan bodies. The patient was then examined by a Dermatologist who agreed with the histological diagnosis and added Doxycyclin in the treatment. Conclusion: Donovanosis is a tropical endemic disease, rare in the West countries. It is a sexually transmitted disease caused by Calymmatobacterium granulomatis. It usually affects the perianal and perigenital area. To our knowledge is not usual on the surface of haemorrhoids.

PP4-152

A CASE OF MONOCYTIC LEUKAEMIA CUTIS IN A PATIENT WITH MYELODYPLASIC SYNDROME TRANSFORMING TO ACUTE MYELOID LEUKAEMIA

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Background: Leukaemias are a group of disorders characterized by the presence of white blood neoplasic cells in the blood stream and in bone marrow. Rarely infiltration of skin may be observed. Occasionally, skin lesions may precede haematological manifestations or may be concomitant with the diagnosis of systemic leukaemia; we report a new case of monocytic leukaeia cutis in a patient with myelodysplasic syndrome. Case report: A 66-year-old- women with a 3-year- history of myelodysplasic syndrome with abnormal bone marrow cytogenetics. Over the previous few months she had developed cutaneous lesions on the buttok and thigh; the skin biopsy showed a diffuse infiltrate of cells with bean-shaped nuclei in the upper dermis extending to the subcutis. Immunohistochemistry showed positivity for CD15, CD68 and CD 34. Immunostainingg for CD3 and CD20 was negative. These results were consistent with leukaemia cutis. Conclusion: Leukaemia cutis as the initial clinical presentation of a transforming myelodysplastic syndrome into acute myeloid leukaemia has been reported only very rarely. Our case underlines the need to look meticulously for skin changes and perform early skin biopsies in haematological patients. Skin lesions can represent the first clinical signs of an otherwise not evident bone marrow disorder.

PP4-153

BETA-CATENIN AND CYCLIN D1 EXPRESSION IN THE DETERMINATION OF THE BIOLOGICAL BEHAVIOUR OF KERATOACANTHOMA

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Background: Keratoacanthoma (KA) is a benign cutaneous tumor that has rapid growth phase similar to the malignant tumors. KA and squamous cell carcinoma (SCC) are sometimes difficult to distinguish by histopathological examination. The purpose of this study is to determine some histopathological criteria to make a distinction between SCC and KA and the pattern of β -catenin and cyclin D1 immunostaining to evaluate its potential value in difficult cases Material and methods: Immunoperoxidase staining of β -catenin, and cyclin D1 were performed at paraffin-embedded sections of 14 KAs taken from archival material. On reviewing the histology, seven of the 14 KAs were characterized as KA, and the rest as KA resembling SCC. Additionally, 16 well differentiated superficial spreading SCCs were examined. We evaluated macroscopical tumor characteristics (duration of the lesion, diameter, ulceration, symmetricity, central keratin crater) and microscopical findings at each group. Results: Ulceration was found more frequent in SCC(p=0,03) and the symetricity in KAs (p=0,005). Central keratin crater, duration of the lesion or intraepithelial polymorphonuclear abscesses were not significant parameters in the distinction between KA and SCC. Immunohistochemical analysis showed that; in KA group, 6/7 (%85,7) , in KA resembling SCC 1/6 SCCs (%16,7) and in SCC group 5/16 (%31,3) were membranous β -catenin positive in the deeper parts of the lesion . So; when the lesion became invasive, the deeper part of the lesion lost membranous localization of β -catenin(p=0,02). Cyclin-D1 expression was not significant between any groups. Conclusion: These findings suggest that macroscopical findings such as ulceration and symetricity of the lesion are more important than the central keratin crater, intraepithelial polymorphonuclear abscesses or duration of the lesion so as to distinguish KA from SCC. Additionally the loss of membraneous β -catenin expression at the deeper part of lesion may be helpful at the differential diagnosis between KA and KA resembling SCC.

PP4-154

CORRELATION OF EXPRESSION OF GROWTH HORMONE RECEPTOR AND GROWTH HORMONE ON DYSPLASTIC NAEVUS AND SKIN MALIGNANT MELANOMA CELLS

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AIM OF THE STUDY: To investigate possible differences in expression of growth hormone and its receptor among normal skin, dysplastic naevus and malignant melanoma group. MATERIAL AND METHODS: Twenty cases, ten males and ten females, from each of above mentioned groups were examined to analyse expression of growth hormone and its receptor. The results of immunohistochemistry were shown in semiquantitative manner and were analysed with help of statistical methods, including x2 – test and Kruskal-Wallis ANOVA test, with the level of importance <0,05. RESULTS AND CONCLUSION: The results showed that there was a higher expression of growth hormone receptor in malignant melanoma material, compared with dysplastic naevus and normal skin. Less impressive were results with growth hormone, although very similar pattern of immunohistochemical expression has been detected among mentioned groups. This was confirmed with both x2 – test and Kruskal-Wallis ANOVA test. Therefore we conclude that there might be a possibility to intervene the course of malignant melanoma and dysplastic naevus via growth hormone receptor antagonists. We strongly suggest studies on larger number of patients to be made.

PP4-156

RESEARCH OF THE ANTI-SCAR ACTIVITY OF THE PREPARATION ISONIDEZUM AS A REMEDY, INHIBITING THE CONNECTIVE TISSUE FORMATION

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The Preparation Isonidezum as a remedy for the preventive maintenance of the postoperative intraperetoneal adhesions is produced by the pharmaceutical enterprise "Farmako" (Moldova). We used Isonidezum in cosmetology surgery for aesthetic correction of the postoperative scars in comparison with the ointment Dermaxinab, containing 0,05% Clobetanzoli propionatum. The purpose of research - To use the preparation Isonidezum with a new purpose- for a primary preventive maintenance of postoperative scars which were considered as an non- aesthetic complication after removal the nevi. Material and methods of research. We carried out the clinical test for two groups of patients, each of which consisted of 50 individuals requiring the removal of skin benign formations. Their age was of 15-60 years and 11-55 years, in two groups respectively. For confirmation of the clinical diagnosis all the skin formations have been subjected to the morphological analysis in the respective

groups of research: - nevocellular nevi - 34 cases (68%); seborrhea keratosis - 8 cases (16%); papillomas - 3 cases (6%); angiomas - 2 cases (4%); keloids - 2 cases (4%); dermatofibroma - 1 case (2%). In group of comparison: - nevocellular nevi - 45 cases (90%); seborrhea keratosis - 4 cases (8%); keratoacanthoma - 1 case (2%). Results of research. In the patients of the experimental group surgical treatment was accompanied by the subsequent processing of a zone of surgical intervention by the preparation Isonidezum. The borders of the wound were sutured by in layers, with the subsequent application on the wound of a sterile tampon impregnated by solution of Isonidezum. The keeping of the tampon on the wound under light pressure within 2 hours after removal of the formations was recommended. Patients of control group underwent to a usual cosmetic operation without subsequent application Isonidezum. For definition of clinical aspects postoperative scar (color, consistence, smoothness of the surface) patients of both groups were surveyed in 3 and 6 months after surgical intervention. As a result of the researches the occurrence keloids: in research group was one case, in the control group was 6 cases ($p<0,05$). Hence, the application of Isonidezum with these purposes showed the maximum preventive effect, no cases of the repeated referrals of the patients to doctors and cosmeticians. The remote consequences of the application of the preparation Isonidezum in these pathological conditions were not observed.

PP4-157

EXPRESSION OF p14, p16 AND p53 IN RELATION TO HPV IN (PRE)MALIGNANT SQUAMOUS SKIN TUMORS

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Background: The tumor suppressor p14 is thought to play a key role in cell cycle control, since p14 provides a crosstalk between the two main pathways governing cell growth, namely the p14-MDM2-p53 and p16-CDK4/6-RB pathway. The expression of p14 in keratinocytic intraepidermal neoplasia (KIN) lesions has not been studied yet. Immunohistochemical studies in cervical dysplasia have reported over-expression of both p14 and p16 in high risk HPV-associated cases. Relations between HPV (human papilloma virus) and the tumor suppressors p14/p16 and p53 in skin (pre)cancer have not been clarified yet. Aims: To study 1) p14 expression, in relation with p16 and p53 expression, in (pre)malignant squamous skin tumors, and possible relations with risk factors for cutaneous carcinogenesis (sun exposure and immune status), and 2) a possible role of HPV in (pre)malignant squamous skin tumors. Materials and methods: Immunostaining for p14, p16 and p53 was performed on paraffin embedded sections of 22 low grade KIN (LKIN) lesions, 49 high grade KIN (HKIN) lesions, and 34 CSCCs from 52 renal transplant recipients (RTRs) and 53 immunocompetent individuals (ICIs). HPV detection was performed using a short PCR fragment (SPF-LiPA) assay, allowing for the detection and genotyping of 25 mucosal HPV genotypes. Beta papillomavirus detection and genotyping was carried out with the PM-PCR RHA method for identification of the 25 established beta-PV types. Results: P14 expression proved independent of the expression of both p53 and p16, irrespective of immune status and sun exposure. We found a significant association between the presence of beta-PV and sun exposure in the RTRs ($p=0.002$). There was no significant association between the overall presence of beta-PV and protein expression, in both the RTRs and ICIs. Conclusions: Relations between p14/p16/p53 expression and HPV presence in skin neoplasia, is different from findings in cervical neoplasia. Main causes are probably that in skin cancer, besides HPV, also sun exposure is implicated, and that in skin lesions, HPV integration in the genome is rare, in contrast to cervical cancer. Our data support the hypothesis that HPV and ultraviolet radiation play a synergetic role in promoting carcinogenesis, probably enhanced by immunosuppression, since we found a significant association between the presence of HPV and sun exposure in the RTRs.

PP4-158

PAPULAR MUCINOSIS: REPORT OF TWO CASES AND REVIEW OF THE LITERATURE

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BACKGROUND: Papular mucinosis (PM) is a rare disease characterized by a symmetric distribution of erythematous to yellowish papules and/or plaques, most commonly involving the face, neck, upper trunk and arms. Some cases are associated with systemic diseases such as paraproteinaemia, dermatomyositis, scleroderma, systemic lupus erythematosus, non-Hodgkin's lymphoma and hypothyroidism. We report herein two cases of PM. The first case was an example of PM in conjunction with Hashimoto's thyroiditis which regressed following thyroid hormone supplementation. The second case was an example to the idiopathic form, which was not associated with any other pathology. REPORT OF THE CASES: Case 1: A 48-year-old woman had erythematous to yellowish, symmetric, 2-5 mm papules and nodules on her forearms since two months. Clinically disseminated granuloma annulare or sarcoidosis were suspected and a punch biopsy was done. Histopathological examination revealed mucin deposition in the dermis. Clinical findings were reassessed and the patient was diagnosed as a dermal mucinosis compatible with PM. Routine blood tests were unremarkable except the level of serum-free T4 10.9 pmol/L (normal range: 11-25), TSH 16.8 mIU/L (normal range: 0.4-4) and anti-thyroglobulin antibodies 379 IU/ml (normal range: 0-60 U/ml). Laboratory findings revealed hypothyroidism associated with Hashimoto's thyroiditis. Lesions regressed frankly after nine months of thyroid hormone supplementation. Case 2: A 13-year-old girl had multiple yellowish and white, symmetric, pruritic, papular lesions on the dorsal part of fingers and hands, forearms, neck, face, and suprapubic region. Clinical diagnosis was PM. Two punch biopsies, from the dorsum of the hand and from the neck were taken. Histopathological examination revealed mucin deposition in the dermis. In the biopsy taken from the hand a fibroblastic proliferation in deep dermis accompanied mucin deposition, which was reminiscent of the scleromyxedema variant. Routine blood tests were unremarkable and paraproteinaemia was not found. Lesions regressed frankly following four months of methylprednisolone (16 mg/day) and isoretinoin (40 mg/day) therapy. CONCLUSION: It is not clear why mucin production increases in pathological conditions. Mucin is secreted in small amounts by fibroblasts in the dermis. The cause is probably multifactorial: cytokines, immunoglobulins and serum factors induce fibroblast proliferation and mucin production. It is important to remember that the diagnosis of mucinoses depends upon clinical features and biochemical investigations.

PP4-159

THE EXPRESSION OF P63 AND P53 IN INTRAEPIDERMAL AND INVASIVE NEOPLASMS OF THE SKIN

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Aim: p53 is a well-known tumor supressor gene and its mutation is a common event in intraepidermal and invasive neoplasms of the skin. p63 is a homologue of the tumor supressor gene p53, which is expressed in human basal squamous epithelium. p63 plays a role in the development of squamous epithelium and, despite its homology to p53, it is considered to act as an oncogene. We evaluated p63 expression in usual skin cancers and intraepidermal neoplasms including Bowen's Disease, actinic keratosis (AK), in situ malignant melanoma (MM) an Paget's Disease to clarify the putative role of p63 expression in the development and differantial diagnosis of these lesions. Material Metod: The pathology database of the Sisli Etfal Education and Research Hospital was retrospectively reviewed to identify cases between 1999-2005. There were 17 squamous cell carcinomas (SCC), 23 basal cell carcinomas (BCC), 16 keratoacanthomas, 26 AKs, 22 Bowen's Diseases, 7 in situ MMs and 3 Paget's Diseases. In addiation, nonsunexposed normal skin tissues resected due to benign lesions acted as controls. Immunohistochemistry using antibodies against p63 and p53 was performed.Statistical analysis was done using the Kruskal Wallis, Mann Whitney and Wilcoxan Rank tests. The Spearmen test used for correlation analysis and $p<.05$ was considered significant. Results: There was a significant p63 staining in Bowen's Disease, AK, BCC, SCC and keratoachantomas respectively. In contrast, none of the in situ MM and Paget's Disease was positive for p63. Conclusion: Based on our findings, analysis of p63 expression may help in the differential diagnosis of Bowen's Disease and AK vs in situ MM and Paget's Disease, especially in small biopsies.

PP4-160
DIAGNOSIS AND FOLLOW UP OF KERATOACANTHOMA-LIKE LESIONS: A CLINICAL-HISTOLOGIC STUDY OF 43 CASES

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Background: Keratoacanthoma (KA) is easily confused with Squamous Cell Carcinoma (SCC) either, on clinical- or histopathologic bases. However, KA undergoes spontaneous regression while SCC does not. In this way, to improve the histopathologic features that better discriminate the two tumors, clinical regression could be used as gold standard for KA. Our objective was to study the histopathologic features associated to clinical regression in KA-like lesions in which clinical course was assessed. Method: 43 biopsies of KA-like lesions were taken at patient admission. One month later, surgical excision was undergone in 18 growing lesions. Involuting lesions were left untreated even when histopathological reports of SCC were rendered. Classical histopathologic features and diagnosis were blindly recorded in both, biopsies- and surgical specimens. Results: Most lesions occured in elderly. Their settings were sun-exposed skin of head/neck and limbs. All tumors were described as having acute onset and rapid growth. No patient had recurrence following natural regression or excision. On clinical and histological basis, 32 lesions were assessed as KA and 11 as SCC. Features that indicated malignancy were observed in both groups (medium 1.63 in KAs and 6.18 in SCCs). Three out 32 KAs were assessed as KA-like undoubted SCC on blind histologic examination. No KA presented deep tumor extension beyond the level of the sweat glands. One KA showed perineural extension. Regressing stage KAs displayed less histologic signs indicative of malignancy. Conclusion: SCCs and KAs have more pathological similarities than differences. The differentiation of a KA in the proliferative stage from SCC may be impossible. The combination of the most useful features did not allow the nosologic diagnosis in difficult cases, but helped.

PP4-161
PHOTODAMAGE IN THE CAT SKIN: CLINICAL AND HISTOMORPHOMETRIC ANALYSIS

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Background: Photodamage (PD) is a term that describes the array of clinical and histologic changes caused by long-term exposure to solar radiation. In human beings, although the visible manifestations take years before become clinically evident, the underlying damage occurs from the earliest exposures. The dermatologic importance of PD lies in the considerable cosmetic and psychosocial distress it causes in the older persons and in its strong presumably etiologic relationship to skin cancer. Heavy ultraviolet (UV) exposure is also harmful to animals. On animal models, most of our knowledge in the effects of UV rays, is based on mice. Cats share the same environment as their owners, and may serve as sentinels for cancer development seen in human beings. The relatively high incidence of spontaneously occurring feline solar keratoses (SK) and the significant association between SK development and white coat make the cat a good model for photodamage research in natural populations. The purpose of the present study was to investigate clinical and histomorphometric features of cat skin under long term solar exposition. Methods: Ear skin of 34 domestic shorthair cats that were chronically exposed to sun were classified, on clinical basis, as follows: group 0: normal (n= 13); group 1: initial stage of photodamage (PD) (n= 10); group 2: advanced stage of PD (n= 11). Sex and age of the cat, as well as the color of the ear that had been submitted to biopsy were recorded. Histological sections were stained with haematoxylin-eosin and microscopic features were blindly and separately recorded by 2 examiners in 10 sequential linear high power fields (HPF). Dermal cellularity was studied through a cycloid morphometric grid positioned in ten sequential HPF. An objective assessment of the epidermal thickness and relative adnexal unit area was obtained in digitalized images, with the aid of an Axiophot photomicroscope (Carl Zeiss - KS300 system). Results: Through statistical methods, older age, white-colour ear, dermal edema and sclerosis, telangiectasis, reduced adnexal area and squamotized appearance of epidermal basal cells were significantly associated to advanced stage of photodamage. There was not dermal elastosis. The animals developed thickening of the epidermis since the initial phase. Conclusion: Our results indicate a high level of skin hypersensibility to sun rays in cats. The findings may be useful for clinical tests and in general veterinary pathology and dermatology.

Head and Neck Pathology

PP4-162
DOWNREGULATION OF CD9 PROTEIN, BUT NOT OTHER TETRASPANINS, IN ORAL SQUAMOUS CELL CARCINOMA: CORRELATION WITH POOR PROGNOSIS AND DECREASED DISEASE-FREE SURVIVAL

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Background: Squamous cell carcinoma of the oral cavity (OSCC) is a commom malignancy, characterized by a high degree of agression and metastasis to cervical lymph nodes. During the process of OSCC carcinogenesis, impaired function of metastase supressor genes appear to be crucial in the progression of cancer cells to a metastatic phenotype. Tetraspanins are proteins with functional roles in a wide array of cellular processes and have been reported to be associated with the biological behavior of solid tumors, especially with their metastatic potential. Other functions include cell adhesion, motility, differentiation and proliferation. Method: The present study investigated the expression of the tetraspanins CD9, CD37, CD63, CD81 and CD82 in OCSS using immunohistochemistry. Tissue Microarray (TMA) of 127 cases of OSCC and 10 normal tissue of oral cavity were evaluated immunomorphologically and semi-quantitatively, and results were compared to the clinical-pathological features. Multivariate Cox regression, Kaplan-Meier method and qui-square test were used for statistical analysis. Results: Expression of CD63, CD37 and CD81 was not detected in the cases studied (tumors and controls). Expression of CD9 and CD82 was observed on the membrane of basal layer cells in the controls of normal oral mucosa. CD9 expression was downregulated/ negative in 42/127 (33%) OSCC cases. Loss of CD9 expression in OSCC correlated with incidence of cervical lymph node metastasis (p=0.028). A bordeline significant relationship between CD9 downregulation and advanced disease (clinical stage T3/T4) (37.0%) (p=0.055) was verified. Significance between CD9 expression and other clinicopathological features was not statistically established. Disease-free survival rate of patients with CD9 down-regulated/negative (48.6%) was significantly lower than that in patients with CD9 positive expression (71.6%) (p=0.010). The 5-year overall survival rate of patients with CD9 downregulated/negative was 44.0% against 64.0% of CD9-positive cases (p=0.071). CD82 was downregulated/negative in 100/127 (79.0%) specimens, but no correlation was observed between CD82 expression, clinicopathological parameters, disease-free survival and 5-year overall survival (p= 0.705, p= 0.549 respectively). Conclusion: Our results suggest that downregulation of CD9 might be an indicator of poor prognosis in OSCC patients.

PP4-163
MALIGNANT PERIPHERAL NERVE SHEATH TUMOUR (MPNST) OF THE NASAL CAVITY. REPORT OF A CASE AND REVIEW OF THE LITERATURE

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BACKGROUND: Peripheral nerve sheath tumours (PNST) are soft tissue neoplasms, arising from the neural sheath of autonomic, cranial, or peripheral nerves, which are rarely encountered in the nasal cavity and paranasal sinuses with 20 well-documented cases published in the English-language literature. We report a rare case of a high-grade MPNST of the nasal cavity in a woman. METHOD: A previous healthy 39-year-old woman was admitted to our hospital with left nasal obstruction without bleeding. Magnetic resonance imaging studies showed a obstructive mass of the left nasal cavity extending to the left paranasal sinuses, approaching the ipsilateral orbit with destructive malignant features. The patient underwent a total surgical removal of the mass and multiple tissue specimens were examined with hematoxylin/eosin as well as immunohistochemically for: Vimentin, Bcl-2, a-SMA, GFAP, S-100, α1-AT, C-Kit, CD34, CD31, CD57, HHV-8, Ki67, P53. RESULTS: Nasal mucosa covered by respiratory epithelium and infiltrated by a spindle cell, vascular neoplasm growing in a fascicular pattern, mimicking kaposi's sarcoma. The tumour was unencapsulated with indistinct borders and without necrosis. The cells had oval or elongated nuclei, with rare mitotic activity and the cytoplasm was clear or fibrillary eosinophilic. There were areas of mild cellularity and a chronic inflammatory infiltrate intra and extratumoral. Immunohistochemical staining of tumour cells showed diffuse intense positivity for Vimentin, Bcl-2, a-SMA, focal reaction for GFAP, S-100, α1-AT, C-Kit and all the other markers were negative. The Ki67 index was 5% while p53 protein was overexpressed (40%). Based on the immunophenotype a diagnosis of a high grade malignant peripheral nerve sheath tumour of the nasal cavity was made. The patient received adjuvant radiation with further surgical therapy. CONCLUSION: Approximately 45% of benign PNST occur in the head and neck region. These tumours undergo malignant transformation but MPNST can form de novo. High-grade MPNST require complete surgical resection, with wide tumour free margins, combined with adjuvant irradiation and chemotherapy. However, MPNSTs are thought to be incurable with current treatment modalities because of their metastatic potential. Our case required a differential diagnosis from a wide range of spindle cell neoplasms and particularly from leiomyosarcoma.

PP4-164
MALIGNANT TRANSFORMATION OF ORAL LICHEN PLANUS

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INTRODUCTION: Oral lichen planus (OLP) is a chronic inflammatory disease with low, but proved risk of transformation to oral squamous cell carcinoma (OSCC). The reported rate varies from 0,4 up to 5% of cases. Oral cancer in patients with OLP has been identified as arising on the erythematous, atrophic

and erosive lichen-planus-affected oral mucosa. Immunohistochemical and more recently genetic studies have provided evidence of cellular and molecular events in OLP lesions that could preceede malignant transformation, suggessting that molecular assessment may become a valuable clinical tool for patient's follow-up. MATERIAL AND METHODS: We present five patients with squamous cell carcinoma of the buccal mucosa arised on the pre-existing oral lichen planus. Immunohistochemical analysis of the precursor lesions was performed by using p53 and Ki-67 antigens, and compared with the unaffected mucosa of the same patient, as well as with the matched control patient's mucosa. RESULTS AND DISCUSSION: Significantly stronger expression of Ki-67 and moderatly stronger expression of p53 protein staining was seen in the overlying epithelium in the OLP lesions preceded to SCC, in comparison to the unaffected and control mucosa. Presented cases document and alert on the propensity of oral lichen planus to undergo malignant transformation and stress the importance of regular follow-up of these patients. CONCLUSION: Clinical diagnosis of OLP has to be confirmed by histopathological analysis. As a premalignant lesion, OLP need to be assessed by use of immunohistochemical and genetic markers as well. Further research is essential for better understanding of the mechanisms and propensity of some OLP lesions to undergo malignant transformation.

PP4-165
CALCIFYING EPITHELIAL ODONTOGENIC TUMOR OF THE MAXILLA - CASE REPORT

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INTRODUCTION: Calcifying epithelial odontogenic tumor also known as Pindborg tumor is very rare, locally invasive neoplasm, characterized by amyloid-like material that may become calcified. It accounts for less than 1% of all odontogenic tumors, without gender predilection and usually clinically presents as slow-growing, painless mass, most often envolving premolar and molar region. The mandible is affected twice as often as maxilla. Patients between 2nd and 6th decade, with a mean age of 40 years are usually affected. The vast majority of cases are intraosseous, with approximately 6% arising in extraosseous locations. CLINICAL DATA: A 36-year-old Caucasian male was admitted to our Hospital because of painless swelling, which lasted over 3 months in the region of hard palate and buccal aspect of maxilla on the right side. All radiographic findings showed a tumorous mass of the right maxillary sinus with disrupted structure of the ipsilateral upper jaw and intact orbital space. The last two loose molar teeth were extracted and the biopsy was performed which confirmed the diagnosis of calcifying epithelial odontogenic tumor. PATHOHISTOLOGY: The tumor volume was about 15 cm3 and histologically it was composed of small islands and sheets of polygonal cells with abundant eosinophilic cytoplasm, clear cell borders and noticeable intercellular bridges. Nuclei were pleomorphic, without mitotic figures. Eosinophilic, homogeneous hyaline material, within and around tumor cells, was proven to be amyloid-like, because when stained with Congo red, demonstrated the classic bright green birefringence viewed with polarized light under microscope. This amyloid-like material was often centrally calcified in the form of Liesegang concentric rings. DISCUSSION: There are around 200 cases described in the literature to the present. Long-term follow up is essential because there is a recurrence risk if the tumor was incompletely resected and in particular with the clear cell variant.

PP4-166
DEVELOPMENT OF A NEW FAST CHEMOSENSITIVITY TEST IN LARYNX CARCINOMA

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BACKGROUND: The aim of our preliminary study was to develop an accurate chemosensitivity test for larinx carcinomas (LKs). The final goal of our study was a test which could predict the effects of cisplatin on tumour cells prior the onset of treatment in order to individualize the pharmacological strategy. METHODS: We directly exposed for a short period (30 minutes) fresh samples from 3 LKs using cisplatin (Teva L01XA01) assessing 3 different cisplatin concentrations: low (6,25 µg/ml), medium (25 µg/ml) and high (75 µg/ml). We also utilized 2 control specimens, one to be immediately formalin-fixed and one to be incubated in the medium, but lacking cisplatin. Cases interpretation was performed by histological examination of H-E stained sections. The main objectives of this study were to characterize the morphological changes induced in tumour cells and to determine effects on molecular markers involved in proliferation and/or apoptosis. RESULTS: The preliminary observation of these 3 LKs allowed us to detect the elementary lesions that could have a role in the identification of the reactivity of tumour cells toward cisplatin: extracellular and/or intracellular oedema, apoptosis and at intratumoural necrosis. Although all 3 tumours showed an identical histotype and the same site of origin, case 1 and case 3 showed a striking morphological reactivity to cisplatin, on the contrary, case 2 did not show any significant morphological features due to cisplatin exposure. What's more cases 1 and 3 were characterized by decreasing nuclear immunoreactivity for cycline D1, p53 and PCNA. Therefore, at the molecular level, case 2 did not show any significant change in immunoreactivity for cycline D1, p53 and PCNA. CONCLUSION: Our data evidence that samples from 3 LKs may evidence different patterns to cisplatin exposure. Extracellular and/or intracellular oedema, apoptosis and necrosis were the most frequent findings observed in cisplatin-treated specimens. These observations induced us to focus our attention toward the follow up of these patients in order to compare our in "in vitro" data with the clinical response of patients, although, our findings need further validation on a larger number of neoplasias.

PP4-167
NGF-BETA/PRONGF AND THEIR RECEPTORS IN NORMAL HUMAN ORAL MUCOSA

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BACKGROUND: Nerve growth factor β (NGF-β) and its precursor proNGF are important for differentiation and survival of neurons and dermal keratinocytes. NGF-β is secreted in the saliva of mouse and man. The aim of this study was to determine whether NGF can play a role in the differentiation and wound healing of oral mucosa by investigating its effects on oral keratinocytes. METHODS: mRNA production and protein expression of NGF/proNGF and their receptors TrkA and p75 NTR were analyzed in cultured human oral mucosal keratinocytes (OMK) by Western blot analysis and reverse transcribed PCR respectively. Proliferation of OMK upon NGF-β

stimulation was measured by BrdU incorporation. The effect of NGF-β on the migration of OMK was studied using a scratch assay. Expression of NGF-β and its receptors in biopsies from normal oral mucosa (NOM) and oral mucosal equivalents were examined using immunological staining. RESULTS: OMK expressed mRNA for NGF-β /proNGF and their receptors TrkA and p75NTR. Lysates from OMK did not contain mature NGF-β but several NGF-proforms with molecular weights between 32 and 114 kDa. Culture medium from OMK contained 75 kDa proNGF. Addition of NGF-β significantly enhanced proliferation of OMK cultures and in vitro scratch closure. Immunostaining of biopsies from normal oral mucosa showed the presence of proNGF in all epithelial layers. NGF staining was observed in the granular and upper spinous cell layers. TrkA immunoreactivity was detected in basal and parabasal cells, with weak to moderate staining in spinous and granular cell layers. p75NTR staining was seen in basal cell layers. CONCLUSION: NGF-β /proNGF (from saliva or from the keratinocytes themselves) has mitogenic and motogenic effects on OMK and may therefore aid in healing of oral wounds. Differential expression of NGF and NGF-receptors throughout the epithelium suggests a role in epithelial differentiation.

PP4-168
KARYOMETRIC ANALYSIS OF POSTIRRADIATION SIALOADENITIS

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Aim: The aim of this study was to estimate karyometric variables in postirradiation sialoadenitis. Material and Methods. At Institute of Pathology, University of Nis, four patients with postirradiation sialoadenitis, and twelve patients with normal parotid gland (control group) were analyzed. Nuclear area, mean and mode optical density (OD), standard deviation of OD, minimal and maximal OD, intergrated OD (IOD), nuclear perimeter and circulariry were analyzed by ImageJ software at objective x63 on routinely stained histopathological sections. Results: Histologically, atrophy of parenchyma and fibrosis were found in patients with postirradiation sialoadenitis. In postirradiation sialoadenitis, the nuclei were more irregular in contour (circularity=0.84±0.03), compared to control group (0.88±0.02)($p<0.01$). Mean and maximal ODs were significantly lower in patients with postirradiation sialoadenitis (0.39±0.04, and 0.51±0.03 a.u.), compared to control group (0.44±0.04, and 0.56±0.06 a.u., respectively)($p<0.05$). Conclusion: Our results suggest that radiation therapy causes irregularities of nuclear shape and loss of chromatin density.

PP4-169
CRANIAL MEASUREMENTS USED IN SEX AND AGE ESTABLISHMENT

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Aim: The purpose of the study was to interpret and apply the most efficient parameters in establishing the sex and age of cranial fragments. Materials and Methods: Two skulls had been found in two different places, isolated, without other bones and unburied. After removing the leaves and the earth, the skulls were cleaned and measured. Results: The first skull was missing a mandible and had teeth 18, 23 ,24 and 27 with 11 and 12 lost post mortem and the other teeth lost ante mortem judging by the aspect of the alveolar margin. The second skull had the mandible, but with a fractured gonion and a coronoid process. The edentation was complete, with a partial fracture of the maximale alveolar process. The left zigomatic region presented a cominnutive fracture with the absence of some fragments of the anterior wall of the maxilar sinus. Cranial measurements highlighted some elements specifying the sex (the size of the foramen magna, of the left orbit), but also some elements unable to surely specify the sex (cranial parameter, cranial diameters, the mastoid and the aspect of the external face of the gonion). The teeth present in the first case presented decayed processes which lowered the possibility of appreciating the abrasion and the age. In the second case, for age establishment the aspects of the cranial sutures (the obliteration degree) were used. Conclusion: The sizes of certain cranial elements can be used for establishing the sex. In the absence of the teeth, age can be presumed by the degree of obliteration of the cranial sutures and the goniac angle.

PP4-170
CLEAR CELLS IN ODONTOGENIC TUMORS

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Background: Analysis of the English medical literature reveals a different comprehension of the clear cell (CC) component in odontogenic tumors, especially when tumors bearing both ameloblastomatous features and CC component are discussed. Four types of these tumors are described in the literature: three malignant (1. tumors entirely composed of CCs, 2. admixture of islands of CCs and cords of smaller cells, 3. admixture of islands of CCs and ameloblastomatous areas) and one benign (follicular ameloblastoma with extensive replacement of the stellate reticulum by CCs). Method: We performed an analysis of the microscopic characteristics of the CC component in 14 cases of odontogenic carcinomas (6 clear cell odontogenic carcinomas (CCOCs), 6 ameloblastic carcinomas (ACs) and 2 primary intraosseous squamous cell carcinomas (PISCCs)) versus the CC component in 30 otherwise ordinary ameloblastomas. We noticed the similar appearance of the CC in the different types of odontogenic carcinomas and we compared them with that of the CC in ameloblastomas. All statistical tests were performed with SPSS version 12.0.1 for Windows (Fisher's exact test and non-parametric Mann-Whitney-U test) with a level of statistical significance $P<0.05$. Results: There is definite similarity of the CCs in malignant odontogenic tumors without ectomesenchymal contribution despite the heterogeneity of the tumoral types. The cellular characteristics are statistically different in malignant versus benign odontogenic tumors. CCs with a nuclear/cellular ratio <0.5, elongated spindle-shaped nuclei, fine granular chromatin and small nucleoli are seen in benign lesions; CCs in malignant tumors are smaller with a higher nuclear/ cellular ratio, coarse granular chromatin and prominent nucleoli. ($P < 0.001$, except P nuclear shape = 0.03). These findings suggest that CCs represent a phenotypic cellular variant and not the hallmark of the lesion. We propose a reassignation of different types of CCOC: - CCOC, ameloblastomatous pattern (+/- squamous differentiation) - AC, CC variant; - CCOC, biphasic/monophasic pattern + squamous differentiation - PISCC, CC variant; - CCOC, biphasic/monophasic pattern, no squamous differentiation - odontogenic carcinoma not otherwise specified. Conclusion: CCOC in its current acceptation is an exceedingly rare tumor that might be reclassified as variants of different other types of odontogenic carcinoma. However, no matter if the CCOC name is retained or discarded, one should remember that benign odontogenic tumors may display CC component as well as the malignant ones.

PP4-171
EXPRESSION OF PROGNOSTIC AND PROLIFERATION MARKERS IN SALIVARY GLAND TUMORS
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Aims: The aim of the present study was to investigate the relationship between different prognostic and proliferation markers in salivary gland tumors. Methods: This study was performed to examine the expression of the antibodies, cathepsin D, methalloproteinases 1 and 2 (MMP-1, MMP-2), p53, MIB-1, PCNA, and Replication protein A (RP-A) in 100 salivary gland tumors. The expression of these antibodies was examined by immunohistochemistry in 9 normal parotid tissue, and various tumors of the salivary glands (14 pleomorphic adenomas (AP), 11 polymorphous low-grade adenocarcinoma, 16 mucoepidermoid carcinomas, 14 acinic cell carcinomas, 12 adenoid cystic carcinomas, and 8 not-otherwise-specified adenocarcinomas). Immunohistochemical studies were done on paraffin embedded tissue sections. Results: Cathepsina D antibody gave strong reactions in all neoplasms, P53 and Mib-1 were negative in normal parotid glands and AP but were highly expressed in all malignant tumors. MMP-1 was negative in normal parotid glands, and we observed low expression in AP, and high expression in carcinomas. MMP-2 was negative in normal salivary glands, whereas the expression was higher in both pleomorphic adenomas and carcinomas. Between pleomorphic adenomas, the myxoid type expressed MMP-1 and MMP-2 at higher levels than the cellular ones, although this latter type were showed significant expression of RP-A. The expression of MMP-2 in myxoid pleomorphic adenomas was similar to that of carcinomas. The expression of MMP-2, P53, MIB-1 and RP-A in high grade mucoepidermoid carcinomas was higher than in the low grade ones, although in this latter type, the protein expression of MMP-1 was high. RP-A expression was low in carcinomas. This protein plays an important role in DNA replication, recombination and repair. Conclusions: 1. - Pleomorphic adenomas of the myxoid type have an expression of MMPs similar to that of carcinomas. 2. - MMP-1 expression was low or negative in normal salivary glands and in pleomorphic adenomas, but was high in carcinomas. 3. - MMP-2 expression was negative in normal salivary glands, but was higher in pleomorphic adenomas than in carcinomas. 4. - Low grade mucoepidermoid carcinomas exhibited high MMP-1 expression whereas MMP-2 expression was high in high grade mucoepidermoid carcinomas. 5,-RP- A expression was low in carcinomas.

PP4-172
PROGNOSTIC SIGNIFICANCE OF FAS AND p53 IN THE SQUAMOUS CELL CARCINOMA OF THE LARYNX
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Background; Fas receptor expression makes tumor cells susceptible to the host immune system. In some cases of the squamous cell carcinoma of the head and neck, Fas has reported to be down-regulated, but about its relationship with clinicopathologic parameters and prognostic predictive value, controversy still remains. p53 is the most commonly mutated gene in human cancer, its prognostic and diagnostic value has been studied and contradictory results reported especially in the head and neck cancers. Method; Paraffin embedded tissue blocks of 53 primary squamous cell carcinoma of the larynx were investigated for expression of Fas and p53 by immunohistochemistry. Relationship of these two proteins with the clinicopathologic data has been studied. Results; The degree of Fas expression revealed a positive relationship with the histologic differentiation and there was a positive relationship with the expression of p53 and tumor size. We were unable to show statistically significant relationship with other prognostic parameters and these two proteins. Conclusion; Increased expression of Fas protein seems to have a positive effect on histologic differentiation and expression of p53 may have a role on the tumor progression with its possible effect on tumor size. But because of the lack of significant relationship with the majority of the prognostic parameters for both of these two markers, their prognostic value seems to be limited.

PP4-173
SYNCHRONIC TUMORS OF THE LARYNX: LEIOMYOSARCOMA AND SQUAMOUS CELL CARCINOMA. A CASE REPORT
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Background: The laryngeal smooth muscle tumors are rarely seen. In the literature one case of hypopharyngeal leiomyosarcoma, and two cases of laryngeal smooth muscle tumors including one angioleiomyoma, one leiomyosarcoma and one carcinoleiomyosarcoma were reported. On the other hand, squamous cell carcinoma is the most frequent tumor of the larynx. Coexistence of mesenchymal tumor and carcinoma is extremely rare in larynx. Case Report: A case who is 51 years old man was presented. He admitted to the hospital with disphagy complaint related with his smoking history. He had been smoked two pockets of cigarettes in a day for 20 years. In the laryngoscopy a mass on the left vocal cord was observed and performed biopsy procedure. In microscopic examination, leiomyosarcoma plus squamous cell carcinoma was detected. These two kinds of tumor were been seperately and side by side but they were not observed as intermingled areas of leiomyosarcoma and squamous cell carcinoma. Immunohistochemically smooth muscle actin was positive in leiomyosarcomatous area and pansitokeratin was positive in squamous cell carcinoma area and vice versa were negative. Discussion: These tumours may present diagnostic difficulties both histopathologically and clinically. The patients usually present no characteristic symptoms in early stage and the clinical symptoms are similar to squamous cell carcinoma however immunohistochemical investigation is helpful in distinguishing smooth muscle tumours from other connective tissue neoplasms and spindle cell squamous cell carcinoma. This report describes synchronic tumors of laryngeal leiomyosarcoma and squamous cell carcinoma along with its clinical and histopathologic features.

PP4-174
CORRELATION OF PCNA, Ki67 AND P53 EXPRESSIONS WITH HISTOPATHOLOGICAL GRADE IN MUCOEPIDERMOID CARCINOMA
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Background: Mucoepidermoid carcinoma constitutes almost 10% of all salivary gland tumours and the most common site is parotid gland. Even though the prognosis of mucoepidermoid carcinoma correlates with the histological grade, the occasional precence of metastasis in low grade tumours demonstrate that low grade histological features are not always indicative of low- grade biological behavior. In this study, PCNA, Ki67 and p53 expressions were corralated with histopathological grade of

mucoepidermoid carcinoma. Method: We reviwed a total of 31 mucoepidermoid carcinoma cases reported in our department. The distribution of the tumours according to the glands were as follows: 19 parotid, 3 submandibular, 9 minor salivary gland. In microscopic examination, AFIP grading system yield 11 (35.5%) low grade, 11 (35.5%) intermediate and 9 (29%) high grade tumours. For statistical analysis Kruscal Wallis and Mann-Whitney U tests were used. Results: Mean Ki67 indices of histopathological grades were calculated as 1.8% for low, 2.5% for intermediate and 7% for high grade tumours. PCNA indices, on the other hand, were 36%, 43%, 66% respectively. In statistical analysis, a significant difference was obtained between the Ki67 and PCNA indices with the increasing grade of the tumor ($p<0.05$). P53 expressions did not demostrate any meanignful correlation with the histopathological grade. Conclusion: In conclusion, Ki67 and PCNA demonstrates a positive correlation with increasing histopathological grade in mucoepidermoid carcinoma.

PP4-175

NUCLEAR PROFILES AND EXPRESSION PERCENTAGES OF P27 STAINED AND UNSTAINED TUMOR CELLS IN LARYNX CARCINOMAS: COULD IT BE USED AS A PROGNOSTIC TOOL?

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Background: This study was carried out on a set of twenty-eight laryngeal carcinomas to explore the P27 expression levels, as well as some nuclear planimetric variables of tumor cells. Our aim was to investigate the correlation of these data with conventional histopathological and clinical prognostic factors, if any. Method: Twenty-eight (28) laryngeal squamous cell carcinomas were studied retrospectively. We applied monoclonal antibody against P27 protein using streptovidin-biotin method. In addition to the expression level of P27 in each case, some nuclear planimetric variables of immunopositive and immunonegative tumor cells were measured using a computer assisted image analysis workstation. The expression level was considered as low when the tumor had $\leq$ 10% of P27 immunoreactivity. The potential relationships of these data to the tumor grade, stage, survival, and lymph node metastasis were assesed statistically. Results: Seventeen cases were Grade 1 (60%), remaining 11 (40%) were Grade 2 tumors. Among them, 14 cases had lymph node metastasis. In 19 cases (61%) P27 immunoexpressions were higher than 10%. In immunopositive cells, some nuclear variables suggested lower values than those in the immunonegative ones. These were: mean area (mean: 44.18), CV (mean: 43.39), perimeter (mean:25.8), f-max (mean:9.41) and f-min (mean:6) in immunopositive cells whereas mean area (mean:53.70), cv (39.36), perimeter (28.63), f-max (mean:1.39), f-min (mean:6.65) in immunonegative cells. All of these differences were statistically significant ($p<0.05$). Grade 1 tumor cells' nuclei which were labelled immunohistochemically showed significantly higher values of mean area, sd area and f-max than those in the Grade 2 ones. Differences between them are also significant ($p< 0.05$) Conclusion: Downregulation of P27 is known to be associated with poor prognosis in a number of malignancies. Although similar results were reported in larynx carcinomas, they are limited in number. Nuclear morphometric measurements have been reported as useful prognostic predictors in various cancers including larynx carcinomas. In this study, we concluded that P27 immunolabeling associated with the nuclear morphometry could be useful to predict the prognosis of larynx carcinoma. Further studies in larger series are needed to make more concrete deductions.

PP4-176

EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

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Objective: A case of an extranodal NK/T-cell lymphoma, nasal type is described. Methods & patients: A 75 years old female patient presented with malaise, weight loss and symptoms of nasal obstruction.. Clinical examination revealed an intranasal mass and an ulcerated lesion on hard palate with extensive mid-facial destructive lesions. Biopsy of the intranasal mass was undertaken. Results: The microscopic examination of the specimen using immunochemistry and the EBV + phenotype of the patient, suggested the presence of a T-cell lymphoproliferative lesion, characterized as NK/T-cell lymphoma, nasal type. Discussion-Conclusion: The nasal type NK/T-cell lymphoma is a rear type of predominantly extranodal non-Hodgkin lymphoma, previously known as lethal midline granuloma, which is associated with Epstein Barr virus. This lymphoma occurs most often in adults and is more common in males than females. It causes destruction of the mid-face, palatal and orbital walls. It can also involve skin, soft tissue, gastrointestinal and upper respiratory track. Histologic examination shows a diffuse lymphomatous infiltrate with angiocentric growth pattern and areas of necrosis. In most cases the lymphoma is composed of medium sized cells or a mixture of small and large cells. It must be mentioned that the cytological spectrum is very broad. Mitotic figures are easily found. Sometimes there might be a heavy admixture of inflammatory cells. The typical immunophenotype is: CD2+, CD56+ and cytoplasmic CD3+. Occasionally CD7 or CD30 are positive. CD43 and CD45RO are commonly expressed. CD4, CD5, CD57 are negative. It must be emphasized that the EBV positivity is essential for such a diagnosis. The prognosis is variable. Early stage disease may respond to radiotherapy alone, however late stage disease does not respond well to any available therapies.

PP4-177

A STUDY OF THE EFFECT OF PHARMACEUTICAL SUBSTANCES ON THE NASAL MUCOSA

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Background The study of the histological changes of the nasal mucosa, caused by local administration of vasoconstrictive agents (xylometazoline), with or without nasal rinsing with enriched sea-water solutions. Method Ten PMR-Landraze pigs (half of the total study population) were used. Therapeutic doses of the studied medication were administered over the period of one month and nasal mucosa biopsies were taken in pre-set times. Hematoxylin and eosin slices were reviewed under optic microscope. Each specimen was evaluated concerning the degree of: 1.Inflammation (quota of inflammatory cell infiltration)

2.Fibrosis (quota of slice surface covered by fibrous tissue) 3.Epithelial metaplasia (quota of squamous epithelial metaplasia) 4.Epithelial atypia (quota of atypic epithelial cells) 5. Necrosis Results Under the effect of the local acting vasocostrictive medication (xylometazoline) all of the studied parameters worsened. Similar results were observed when the administration of the agent was combined with nasal rinsing. However, inflammation worsened with a statistically significant slower rate, after ten days of combination of xylometazoline and nasal rinsing. Conclusion Preliminary results show the benefactory effect of nasal rinsing with enriched sea-water solutions, concerning the course of inflammation of nasal mucosa caused by the administration of local acting xylometazoline.

PP4-178
EVALUATION OF PROLIFERATION AND APOPTOSIS RATE OF OSTEOSARCOMA OF JAW

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Background: Osteosarcoma is the primary malignant bone tumour affecting more frequently long bones of young people. Approximately % 6 of all ostesorcoma occurs in the jaws. Jaw Osteosarcoma (JOS) is generally accepted to be a pathologically distinct entity due to older age at presentation, longer median survival, local reccurences and low incidence of metastasis.It has been suggested that better prognosis of JOS than long bones (LOS), may related to the type and the lower grade of the tumor. The aim of this study is to investigate the proliferation and apoptotoic rate of tumor cells in JOS by comparing LOS and discuss the possible corelation with grading, cellularity and histologic type. Methods: The study was conducted on the paraffin embedded samples of a total of 10 ostesarcoma (5 JOS and 5 LOS) cases. Immunohistochemistry was performed using antibody against K i67,while in situ hybridization (Tunel method) was done for detection of apoptotic cells. The number of positive cells was determined by software and histological grading was done . Results: The mean age of the patients of JOS and LOS were 26.5 and 19 years, respectively. Most of the JOS cases were from maxilla (80%), while femur was the most common site in LOS. 60% of JOS and 80% of LOS cases were grade 3. Proliferation rate is higher in LOS than JOS cases. Ki 67 positivity was seen 40% of JOS and 60% of LOS cases which were high grade tumors (grade 3). There was not any positivite staining for apoptosis in both tumor groups. Conclusion: Ki 67 Expression may related to the grade of JOS and have more value than apoptotic index. Studies with large series is needed to confirm these findings.

PP4-179
PREVALANCE OF EBV, HPV 16 AND HSV-1 IN ORAL LICHEN PLANUS

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Background: Oral lichen plnus (OLP) is a chronic inflammatory mucosal disease of unknown etiology. OLP occurs more frequently than the cutaneous form and tends to be more persistent and more resistant to treatment. Although the etiopathogenesis of lichen planus is unknown, it is generally considered to be an immunologically mediated process that histologically resembles hypersensitivity reaction. Lichen planus is probably of multifactorial origin, sometimes induced by drugs or dental materials, psyhological factors, infective agents, often idiopathic. Viral infections have recently been linked with OLP. HSV-1, CMV, HHV-6, EBV HPV, HCV are viruses that have been studied in the etiopathogenesis of OLP. The aim of the present study was to explore a possible association of HPV16, HSV-1 and EBV with OLP. Method: The study was conducted on formalin fixed paraffin embedded tissue specimens of 65 OLP cases. Five-micrometer thick sections of formalin fixed and paraffin embedded biopsy samples were processed by the avidin-biotin-peroxidase complex (ABC) method for Herpes Simplex type 1 , Epstein Barr virus and Human Papilloma virus type 16 primary antibodies. The red-brown nuclear staining was accepted as positive staining for each antibody. To confirm HPV 16 positivity, nested PCR technique was also performed. 15 normal oral mucosal tissue specimens were used as control group. Statistical analysis were performed by Fısher's exact test and Pearson Chi square methods. Results: The number of OLP cases that were positive for EBV was 23 (35%), while HPV 16 was positive in 14 (21%) cases and HSV-1 was positive in 6 (9%) cases. One case was positive for all 3 viruses. 3 cases were positve for both EBV and HSV-1, 5 cases were positive for both EBV and HPV 16, and 1 case was positive for both HSV-1 and HPV 16. The control group was totally negative for all 3 viruses. EBV and HPV 16 positivity in OLP were statistically significant. Conclusion: Although the high prevalance of EBV and HPV 16 does not imply a direct causative relation, it suggested that these viruses might be involved in the pathogenesis of OLP, while HSV positivity might be a secondary infection as a result of possible local immunosupression. EBV and HPV 16 positivity in OLP which are both oncogenic viruses should be considered seriously.

PP4-180
EVALUATION OF HOST IMMUNITY BY THE DETECTION OF NATURAL KILLER CELLS AND THE EXPRESSION OF TUMOR NECROSIS FACTOR ALPHA AND ITS CORRELATION WITH PROGNOSIS IN ORAL SQUAMOUS CELL CARCINOMAS

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Background: Natural Killer (NK)cells are able to show early and spontaneous cytotoxic reaction against tumor cells. NK cells have responsibility in cytokine secretion that have role in antitumor immunity. These cytokines are able to regulate NK cell activity by the otokrine effect. In addition to its well known biological effects, TNF- α, also produced by NK cells, shows cytotoxic effect on some tumor cell lines. This research aimed to give a new insight to the interaction between host local immune response and the biological behaviour of the tumor by evaluation of intratumoral NK cells and TNF-α expression in oral squamous cell carcinoma Method: In 46 cases of OSCC, CD57(NK) and TNF- α antibodies were immunohistochemically detected. Levels of CD57 and TNF- α expressions were microscopically evaluated in 2 histological groups that were categorized in accordance with their grading scores of invasive margins. Furthermore, the clinical stages of 15 cases were correlated with CD57 and TNF- α expression levels. Results: While the number of CD57(+) cells was statistically lower in the group with worse prognosis, the level of TNF alpha was found statistically higher. Conclusion: Increased level of TNF alpha might have direct role on the decrease in NK cells for the high invasive group. TNF alpha that causes connective tissue destruction might support the invasion of the tumor cells.

PP4-181
GIANT CELLS LESIONS - RETROSPECTIVE ANALYSIS

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Giant cell lesions mostly originate from the soft tissues, but rarely also from the bone. Giant cell lesions of gums are peripheral

giant cell granulomas (PGCG), but there are a number of lesions that occur in the jaws that contain giant cells within them: central giant cell granuloma (CGCG), giant cell tumor (GCT), brown tumor (BT) and cherubism (Ch). Histologically all of the giant cell lesions appear similar, if not identical, and they usually can be distinguished by clinical history, immunohistochemistry or genetic markers. During ten years period at the Clinic for Maxillofacial Surgery of the Military Medical Academy there were 29 patients with lesions containing giant cells: 22 PGCG, one CGCG, three GCT, two BT and one cherubism. All patients with PGCG were successfully treated by single wide surgical excision of exophitic gum tumor and ostectomy of underlying bone. CGCG appeared in a male mandible. It has been removed together with adjacent bone, with no relapse. Two of three cases of the GCT of the mandible appeared in women of the fifth decade and after one local relapse they died of lung metastases within three years time; the third case is a 14 years old boy with GCT of maxilla: during the last three years he went through the parcial, and ten moths later the subtotal maxillectomy and he is in a stable remission for almost two years. Both young men with terminal renal failure and BT of maxilla were successfully operated. The 16-year old boy with cherubism was only diagnosed by bone biopsy. Conclusion: Although these lesions are histologically similar, their precise distinction is important because of their different biologic behavior and treatment.

PP4-182
OUR EXPERIENCE WITH LOCALIZED CASTLEMAN'S DISEASE AT THE NECK
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Background: Castleman's disease (angiofollicular lymph node hyperplasia or giant lymph node hyperplasia) appears as localized or ? rare - multicentric benign lymph node mass of the mediastinum, but it has been reported in abdominal anad retroperitoneal cavities, lungs, axillary and cervical region. Case report: during last 16 years there were four cases of Castleman's disease localized at the neck: 54 years old lady and three males: 20, 32 and 37 years old. All of them had asymptomatic, slowly growing tumor mass; diameter 9, 7, 8 and 9 cm respectively; without data about previous inflammatory episodes; under the upper part of the sternocleidomastoid muscle, with clinical presentation resembling lateral branchial cyst and clear ultrasonographic description of a solitary, clearly limited lymph node with abundant vascularization. Intraoperatively they were soft, fatty, bloody and easy removable. After pathohistologic verification of hyaline-vascular type of Castleman's disease in all three cases, patients are followed up during a period of 16, 5, 2 and one year with repeated ultrasonographic examination of the neck, axillas and abdomen and CT examination of the chest, but none of them developed any sign of local or multicentric relapse. Conclusion: Castleman's disease is the rarest benign lymph node hyperplasia in our surgical praxis. These four cases were successfully operated and carefully followed up for years. As we expected, none of them turned into multicentric form.

PP4-183
ANALYSIS OF THE POSTOPERATIVE AND POSTIRRADIATION RELAPSE RATE OF INTRAORAL PLANOCELLULAR CARCINOMA
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Frequency and importance of etiologic factors in onset of oral squamocellular carcinoma (OPCC) at tongue and the floor of the mouth, together with their pathohistologic and clinical features, point to high incidence of its relapse. According to available prognostic parameters, including tumor parameters (pathohistologic and TNM classification), it is not possible for certain to explain the disease relapse in patients with the same histologic type, stage and size of tumors which were treated in the same way (surgery and postoperative irradiation) and which were all pathohistologicly proved to be completely excised. During three years long clinical follow up of 84% male and 16% female patients, 43-80 years old, with OPCC of tongue and the floor of the mouth, we analyzed 12% patients in the second and 88% patients in the third stage of the disease, with 10% of T-1, 76% of T-2 and 14% of T-3 tumor size. The lesion was mostly localized at the middle third of tongue margin. 54% of patients showed the infiltrative tumor growth. All of the patients underwent tumor excision as well as regional lymphadenectomy. In 88% patients the dissection of the neck was performed: in 80% of patients it was suprahyoid ? 63% unilateral and 17% bilateral ? dissection, while 14% of the patients underwent radical and 7% supraomohyoid dissection of the neck. Pathohistologic examination confirmed positive lymh nodes in 71% cases of suprahyoid and all cases of radical and supraomohyoid dissection. Clinical follow up demonstrated relapse of the disease in 46% of the patients: in 65% of them it appeared early (during the first twelwe months after treatment), while in 35% of them in the period 12-36 months after treatment. Locoregional relapse appeared in 13% of the patients, but only as a late lesion. Simultaneous appearance of both local and locoregional relapse appeared early in 21% and lately in 8% of the patients.

PP4-184
EXPERIENCE WITH LARGE DENTIGEROUS CYSTS OF THE MANDIBLE
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Mandibular odontogenic cysts are common, but could be challenging when exceed more than 5 cm of mandibular lenght. During last ten years there was 100 large mandibular cysts: 40 developmental (25 dentigerous, 12 keratocysts, 1 eruption, 2 calcifying odontogenic cyst) and 60 inflammatory (59 radicular and 1 residual cyst). There was 67 males and 33 females; 14-62 years old (mean age 36 years). Most of cysts involve angular region (37), mandibular body (31), ascendent ramus (3), symphysis (7) and two or more of these regions (22). Two patients had two different cysts, and two patients simultaneously had some other intraoral lesion: fibroma and hemangioma. More than half caused external visible assimetry, pain and drainage. All of them were enucleated and residual defect has been reconstructed with aloplastic material (Osteovit), rarely marsupialized (8) or mandibula enhanced with metallic plate over remaining buccolabial cortex. In three cases reoperation was needed after two-seven years. In two cases of keratocyst nevoid basal cell carcinoma syndrome (Gorlin syndrome) has been present: 59 years old woman (keratocyst found after several excisions of facial basalioma) and 35 years old man (keratocyst operated 12 years before occurence of multiple facial basaliomas); both with epidermal cysts, enlarged head circumference, rib anomalies and mild hypertelorism, as well as pectus excavatum at male.

PP4-185
PATHOHYSTOLOGIC ANALYSIS OF EFFECT OF CONCENTRATED PLATELETS ON OSTEOINTEGRATION OF DENTAL IMPLANTS
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INTRODUCTION: Biointegrity of an implant is based on expectation of their integration after placement within bony tissue by processes of remodelation and osteogenesis, so they could overtake functional load of the suprastructure. Aiming to create better conditions that influence to bone healing of dental implant, last few years growth factors are used in implantology. They are mediators of direct or indirect growth regulation of cells and tissues. Growth factors within CT-PRP important for bone regeneration are PDGF, TGF?, VEGF, EGF and other. AIM of this paper is to establish whether the application of CT-PRP method provides better osteointegration of dental implants. METHODS: The study was performed on six experimental dogs in which 24 BCT dental implants were placed: each got four implants ? two on the left side with CT-PRP and two on the right side without CT-PRP application. Osteotomized segments of the mandible were taken after 42, 70 and 98 days, and after preparation all were pathohistologically analyzed. Contact region of the implant with adjacent tissue has been analyzed pathohistologically by following of characteristic features of tissue reaction: connective tissue, osteoblasts and necrosis presence. RESULTS: Results obtained by pathohistological analysis demonstrated increase of connective tissue, outstanding osteoblastic activity and absence of necrosis in group where CT-PRP was applied, and increase of connective tissue, poor osteoblastic reaction and presence of necrosis during the first six weeks in group of implants placed in classic manner. CONCLUSION: According to results obtained from pathohistologic analysis the CT-PRP method has an advantage thanks to better osteointegration due to distinct osteoblastic reaction.

PP4-186
PRIMARY EXTRANODAL NON-HODGKIN MALIGNANT LYMPHOMAS OF THE HEAD AND NECK REGION ACCORDING TO THE WHO CLASSIFICATION
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BACKGROUND: The Working formulation commonly used to classify non-Hodgkin's lymphoma (NHL) in Bulgaria, has been recognized as imperfect for primary extranodal lymphoma in head and neck regions. PURPOSE: To study the clinicopathological and immunohistochemical features of extranodal NHL according WHO Classification. METHODS: Routine histology was performed and stained with H&E and immunohistochemistry, and clinical characteristics were recorded. RESULTS: Between 1997 and 2006, 22 patients with primary extranodal NHL of head and neck region were treated surgically.The patients included 12 male and 10 female; age 24-80 years; tumor localization: tonsil (n=10, 46%) sinunasal areas(n=4, 1.8%) nasopharynx (n=2, 0.9%), salivary glands 2, floor of mouth/gingiva 2, hypopharynx 2. Immunohistochemically, 20 tumors were of B- cell phenotype and 2 tumors were of T-cell phenotype. According to the WHO classification , 12 cases were diffuse large B-cell lymphomas (8 in tonsil,2 sinonasal), 3 were extramedulary plasmocytoma (paranasal sinuses-2, gigiva-1),1 was precursor B-lymphoblatic lymphoma (tonsila), and 1 was marginal zone B-cell lymphoma of mucosa associated lymphoid tissue (MALT), 1 was mantle cell lymphoma,1 was Burkitt lymphoma. Among the T-cell lymphomas, 1 was peripheral T-cell lymphoma (tongue) and 1 extranodal NK/T-cell lymphoma (epipharynx). CONCLUSION: Our date correspond with series from Western population, but there is a significant difference from Japan, Hong Kong and Korea

PP4-187
HER-2 OVEREXPRESSION AND GENE AMPLIFICATION IN SALIVARY DUCT CARCINOMA
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Background Her-2 receptor overexpression in salivary duct carcinomas (SDC) was described in 1994. In the management of the look-alike tumour in the breast, Her-2 targeted therapy has been introduced and the evaluation of Her-2 receptor and gene status has been standardized and correlated to drug effects. In SDC, reports have confirmed Her-2 overexpression and gene amplification has been described. However, a variety of procedures has been used in these studies. Method 31 SDC were identified from the institutional archive, 28 of these were available for further analysis, including 8 of the 9 cases in the report of Hellquist. One block was stained and scored according to the Herceptest protocol (Dako, DK). Fluorescence in situ hybridization (FISH) including a Her-2 probe and a centromere 17 probe was performed and evaluated in 20 cells (Vysis, USA). A ratio (Her-2:centromere) of ≥2.0 was considered amplified. Cases with a high, not countable, signal were considered as clusters. Results Herceptest was negative (score 0) in twelve cases, 2+ in three and 3+ in eleven. All negative tumours had a normal genotype with a ratio ≤1.4. Of the three cases with score 2, one showed a normal FISH pattern, one low level amplification with ratio 2.2, whilst the third showed Her-2 cluster. All eleven cases with Herceptest 3+ showed gene amplification as clusters. In two cases a more than two-fold increase in centrome signals, one as clusters, indicated a more complex genotype. Two cases with intratumour heterogeneity were found, score 0/2+ and 0/3+. FISH was negative in Herceptest negative areas. 2+ area showed increased number of Her-2 as well as centromer signals indicating aberrations at chromosomal level, 3+ area showed clusters. Conclusion We conclude that Her-2 overexpression occurs more frequently in SDC than in ductal carcinoma of the breast. In breast carcinomas Her-2 gene amplification is considered the dominating mechanism for Her-2 overexpression and a prerequisite for targeted therapy. In SDC, a high frequency of overexpression in spite of a normal gene copy has been reported. In our series, no case showed overexpression at the 3+ level with a normal pattern for Her-2 gene signals. Gene amplification is the dominating mechanism for Her-2 overexpression also in SDC whilst a few cases with more complex genomic aberrations occur. The data imply that SDC is a potential target for anti-Her2 therapy and that Her-2 status should be evaluated with the same algorithm as in breast carcinomas.

PP4-188
PRIMARY LEIOMYOSARCOMA OF THE THYROID GLAND
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Primary leiomyosarcoma of the thyroid gland is extremely rare and clinico-pathological information on this tumor is scant. We herein report a case of epithelioid leiomyosarcoma of the thyroid gland in which anaplastic carcinoma was suspected based on clinical and cytological features. The patient was a 79-year-old male who presented with a painful mass in the left neck region that had increased in size over a number of months. Ultrasonography revealed a mass lesion in the left lobe of the thyroid gland. Fine needle aspiration was performed. The cytological diagnosis was a malignant tumor compatible with carcinoma. In the surgical specimen of the thyroid left lobe and isthmus, there was a mass lesion measuring 6 cm in its greatest

dimension that was fleshy, necrotic and uncapsulated. Histologically, there was a solid, lobular pattern of growth comprised of epithelioid cells with increased cellularity, pleomorphism, a high mitotic rate, necrosis, and hemorrhage. Immunohistochemistry of the tumor cells clearly showed smooth muscle differentiation; the cells were positive for desmin, muscle-specific actin and vimentin and negative for cytokeratin, epithelial membrane antigen, carcinoembryonic antigen, thyroglobulin and calcitonin. The tumor cells also showed positive reactivity to c-kit proto-oncogene product. There was no evidence of metastasis in the radiological and clinical examination. No local recurrence and metastasis developed within 4 months after the initial operation. Leiomyosarcomas of the thyroid gland are distinctive tumors and can be distinguished from anaplastic carcinoma with the aid of immunohistochemistry.

PP4-189

HPV-RELATED SQUAMOUS PAPILLOMA OF THE OROLARYNGEAL TRACT. REPORT OF TWO CASES FROM THE TONSILS AND THE LARYNX

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Background: A variety of HPV-related lesions have been reported in the oral cavity and in the larynx. A wide spectrum of architectural patterns ranging from simple Epithelial Hyperplasia (EH), to Verruca Vulgaris (VV) and from Condylloma Acuminatum (CA) to Squamous Papilloma (SP), characterizes these lesions. Here we report two cases of SP, one from the larynx and one from the tonsil. Method: The first case was traced in a 45-year old sailor who referred to our hospital for a sore throat and endoscopic removal of a small laryngeal, warty lesion took place. We received 6 tissue specimens measuring 0.3-0.5cm. The clinical history of the patients wife, revealed an HPV infection of the cervix. The second case involved a 26-year old female with a small papilloma measuring 0.5cm on her right tonsil. Results: Histologically, both lesions were warty-papillary with branching projections consisted of squamous epithelium overlying thin fibrovascular cores. The basal, parabasal cell hyperplasia, the mild anisonucleosis and the non-uniform perinuclear halos of both lesions, motivated us to screen the positivity with the different subtypes of the papilloma viruses. Immunohistochemistry revealed strong staining for HPV types 6 and 11 in both cases. Conclusion: The SPs are the most common benign laryngeal tumors (84%) caused by HPV. The adult-onset oral and laryngeal SP is a frequently relapsing disease. HPV types 6 and 11 which do not carry a high risk of malignant transformation have been detected with a variable frequency (8-60%). Virus transmission appears to be mostly horizontal or by autoinoculation. These patients have a history of a genital wart in 16% and their female sex partners 12.5%. Primary HPV infection may remain latent, requiring cofactors to develop into the clinical disease. A lack of orogenital contact does not protect the patients from adult-onset SP since they seem to be prone to HPV infections. Differential diagnosis involves kerstinized SP, CA and verrucous carcinoma. The diagnosis plays an important role for the appropriate treatment since the relapse is very common. Therapy in laryngeal papillomatosis involves 6 sessions of laser ablation of the papillomas and intralesional injections with cidofovir.

PP4-190

ATYPICAL LYMPHOCYTIC INFILTRATION (LYMPHOCYTOMA) OF THE INFERIOR ALVEOLAR NERVE: A CASE REPORT

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Lymphocytomas are non-neoplastic processes occurring mainly in the skin that are characterised histopathologically by polyclonal lymphocytic infiltrate. They may be associated with Lyme's borreliosis but in many cases etiology remains unclear. Differential diagnosis includes also small cell malignant lymphomas. 33-year-old male was referred to the Department of Oral and Maxillofacial Surgery at Oulu University Hospital for gradually developed sensory loss of the left lower lip. Radiological examinations (panoramic x-ray, CT-scan and MRI) revealed enlarged left mandibular canal, with an expansive, but well-defined soft tissue lesion. In the bone scan a local accumulation was seen in the left body of the mandible. A biopsy was taken from the inferior alveolar nerve. Histopathological findings suggested a lymphocytic infiltration of the nerve tissue. Immunohistochemistry showed both B- and T-lymphocytes in the infiltrate. Some of the B-lymphocytes were Bcl-2 and CD23 positive, which was considered to be related to residual mantle zones. Later the whole enlarged section of the inferior alveolar nerve was resected, and showed similar histopathological findings. Further medical examinations have not revealed any systemic disease and the patient has been well in the 16-month follow-up period.

PP4-191

THE IMPORTANCE OF HYALURONIC ACID IN VOCAL FOLD LESIONS IN DIFFERENTIAL DIAGNOSIS

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Background: We examined the influence of hyaluronic acid and basement membrane thickness alterations on biochemical properties of vocal fold lesions including vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema. Method: Fifty-six laryngoscopic vocal fold biopsies from 49 patients, reported between 1.7.2005 and 27.2.2007 in Yeditepe University, School of Medicine, Department of Pathology, were evaluated retrospectively. Twenty-seven of these cases were vocal fold polyps, 7 were sulcus vocalis, 13 were vocal fold cysts, and 9 were Reinke's edema. Ten normal vocal fold mucosa samples from autopsy cases were included in the study as control group. In order to evaluate the hyaluronic acid in extracellular matrix and basement membrane alterations; hematoxylin-eosin, alcian blue pH 2.5, PAS, and colloidal iron stains were performed histochemically. We evaluated the staining pattern for each lesion and compared with that of control group. Data analysis was performed using SPSS Version 8.0 (Chicago, IL) statistical package. Frequency data were evaluated by the Kruskall Wallis test. A P value ≤ 0.01 was considered to be statistically significant. Results: We detected that sulphated hyaluronic acid in extracellular matrix was increased with neomatrix formation in vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema when compared with control group. The difference was statistically significant ($p<0.01$). This increase was more remarkable in vocal fold polyps than sulcus vocalis

lesions, vocal fold cysts, and Reinke's edema. It was detected that basement membrane thickness increased in 4 pathologic lesion groups compared with control group in statistically significant manner ($p<0.01$). This increase in basement membrane thickness was most remarkable in sulcus vocalis lesions. Conclusion: The results suggested that hyaluronic acid plays an important role in determining biochemical properties of vocal fold lesions including vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema. Furthermore we think that hyaluronic acid and basement membrane thickness alterations can be useful in differential diagnosis in vocal fold lesions.

PP4-192
THE RELATIONSHIP BETWEEN p63 EXPRESSIONS IN BASALOID AND CONVENTIONAL SQUAMOUS CARCINOMAS OF THE LARYNX

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INTRODUCTION: p63 is a p53 homologue that seems to play distinctive roles in the physiology of the squamous epithelia. p63 is similar to p53 but it has different activities. Our aim was to obtain new insights into the role of p63 in basaloid squamous cell carcinoma (BSCC) and conventional squamous cell carcinoma (CSCC). MATERIALS and METHODS: We examined the presence of p63 in 20 BSCC and in 20 CSCC cases retrospectively at immunohistochemical level. The expression of p63 was analyzed in paraffin-embedded specimens of 20 patients with BSCC and 20 patients with CSCC by using immunohistochemical staining methods. RESULTS: All cases with BSCC and CSCC stained positively with p63. Diffuse staining rates with p63 in the BSCC was significantly different from the ones in the CSCC group ($p<0.05$). 11 cases in the basaloid group and 4 cases in the conventional group showed diffuse staining with p63. Diffuse staining with p63 was evident on the sections from the specimens with poor prognostic factors in the basaloid group. CONCLUSION: In the present study, we determined p63 positivity in the all BSCC and CSCC cases of the larynx. In addition, diffuse staining pattern with p63 in the BSCC cases may have a value in distinguishing between the CSCC and BSCC. We suggest that p63 diffuse positivity has a relationship with the prognostic parameters and it seems to be a poor prognostic factor in BSCC of the larynx.

PP4-193
HR-CGH IN BASAL CELL ADENOMAS AND ADENOCARCINOMAS

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Background: Salivary gland tumors (SGT) are rare neoplasms comprising from 2 to 4% of all head and neck tumors. The overall low incidence and histological diversity are major limitations for all biological studies on the salivary gland tumors. The molecular events associated with their development and clinicopathological heterogeneity remain unknown. Previous cytogenetic and molecular genetic analysis of these tumors have been limited in scope and size and did not account for their inherent morphological and biological heterogeneity. Basal cell adenomas (BCAs) and basal cell adenocarcinomas (BCACs) represent, respectively, the benign and the malignant counterpart of salivary gland tumors characterized by the proliferation of basaloid cells. Histologically, they can be very similar, especially when a few amount of material is available for analysis, such as incisional and fine needle biopsies. Method: High resolution comparative genomic hybridization (HR-CGH) using metaphase chromosomes can detect changes in chromosome copy number with a resolution of 3-20Mb. We applied HR-CGH technology in 10 cases (from nine patients) of basal cell adenomas and 5 cases (from four patients) of basal cell adenocarcinomas in order to evaluate and to compare the gains and losses profile in both tumors. Results: Interestingly, it was detected a characteristic pattern of gains and losses in both group of tumors. Losses in 4p14-p15.1, 10q25-q26, 14q24-q31 and 19q13.2-q13 and gains in 22q13 were found in the BCACs. On the other hand, BCAs showed gains in 2q37, 3p24-p26, 4q33-q35, 5q23-q31, 7p22, 10q25-q26, 11p15, 11q25, 15q26, 18p11.3 and 20q13.2-q13.3. Two samples from the same patient (BCAC2 and BCAC5) showed different copy number alterations. In this case, one sample shared the same pattern of chromosomal alterations observed in the basal cell adenomas. Conclusion: In overall, the results support the hypotheses that a set of genes differentiate BCAs and BCACs. These results provide new information on potential genetic events of biological significance in future studies of these salivary gland tumors.

PP4-194
IDENTIFICATION OF METASTATIC ADENOCARCINOMA AND REACTIVE MESOTHELIAL CELLS IN PLEURAL EFFUSIONS. VALUE OF AN IMMUNOCYTOCHEMICAL PANEL OF CD44, CALRETININ, CK7, CK20 AND TTF-1

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Background: Pleural effusions are frequently first clinical manifestation of metastatic disease. Lung, breast, ovarian carcinomas and malignant mesothelioma (MM) are leading the causes of malign pleural effusions. Cytologic differentiation of metastatic carcinoma and reactive mesothelial cells in effusion is sometimes a problem that cytomorphologic criters are insufficient. Recently, extensive research has resulted in expansion of the antibody panel that is available for effusion diagnosis, thereby reducing the risk for error Methot: We have used a panel of five monoclonal antibodies; Calretinin, CD44, CK 7, CK 20 and TTF-1, so as to identify the primary tumor site of metastatic carcinoma cells and reactive mesothelial cells in pleural effusions. Results: Applying an algorithm of immunocytochemical marker constellations, we were able to correctly diagnose proliferating mesothelial cells (% 100) and primary tumor sites in 23 of 30 patients. The best result was achieved for the identification of metastatic carcinomas of the lungs (88.1%).We established an algorithm comprising five immunocytochemical markers that enabled a correct diagnosis of primary tumor sites in 75%. Conclusion: The panel studied could be useful in diagnostic routine for the identification of primary tumors of unknown origin, metastatic to the pleural membranes and reactive mesothelial cells.

PP4-195

p16 EXPRESSION IN HUMAN PAPILLOMAVIRUS RELATED PREMALIGNANT HEAD AND NECK LESIONS

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Background: Human papillomavirus (HPV) induced orophraynx carcinomas has been postulated as a distinct tumor entity in terms of biological behavior and treatment modality. p16 is a tumor supressor gene product ,which is a strong candidate of a valuable prognostic marker ,shown to be overexpressed in most oropharangeal carcinomas associated with high risk HPV. Material and Methods: The study was conducted on paraffin embedded specimens of 43 cases of tonsillar and laryngeal lesions which comprised 15 cases of dysplasia and 28 cases of papilloma. Eleven of 15 dysplasia cases were from tonsils and 4 of 15 cases were from larynx. Most of the tonsillar dysplasia specimens were from the dysplastic epithelium adjacent to tonsillar carcinoma (10 of 11 cases). Twenty four papilloma cases were from tonsils (14) and larynx (14). p16 expression was evaluated by ABC immunoperoxidase staining whereas the presence subtypes of HPV DNA was determined by polimerase chain reaction (PCR) . All dysplasia cases were graded and p 16 positivity was graded as high, medium, low degree (score1-2) on the basis of staining pattern and density. Results: Seventeen of 43 cases (39.5 %) showed the presence of HPV DNA. Of positive specimens, 7 of 17 (41.2 %) were papilloma and 10 of 17 (58.8 %) were dysplasia lesions. All HPV positive papilloma specimens were from larynx . Of positive dysplasia cases, 9 of 10 were tonsillar and 1of 10 was larynx lesions. Nine of 10 dysplasia cases adjacent to carcinoma showed HPV 16 positivity, where as 1 laryngeal dysplasia case had low risk viral load (HPV 6). All HPV positive laryngeal papilloma cases showed the presence of low risk HPV DNA load (HPV 6/11). High degree (score 3) p 16 overexpression was seen 12 of 15 (80 %) dysplasia and 1of 28 (3.6 %) papilloma cases. All HPV 16 positive dysplasia cases had high degree p 16 over expression,. Conclusion: The results showed HPV was not detected in tonsillar papillomas while HPV 11 /6 was found half of the larynx lesions. High risk HPV (HPV 16) was detected in tonsillar dysplasia lesions and showed positive correlation with p16 overexpression.

PP4-196

MIDDLE EAR CHOLESTEATOMA: CORRELATION BETWEEN IMMUNOHISTOCHEMICAL FINDINGS AND DISEASE EXTENSION AND BONE DESTRUCTION

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Background: Cholesteatomas are accumulation of exfoliated keratin inside the middle ear, originated from keratinized squamous epithelium. These epidermoid cyst show independent and progressive growth with destruction of adjacent tissue, especially the bone tissue, with tendency to recurrence. According to some investigations, a characteristic signal of cholesteatoma is the infiltration in the perimatrix of immune system cells, and the increment in proliferation of the cholesteatoma matrix would be the results of the inflammatory process, suggesting that the perimatrix would be the main factor of cholesteatoma development. Method: The purpose of the current study was to analyze the immunohistochemical features of cholesteatoma in correlation with extension and bone destruction grades. Specimens were obtained from 30 patients (9 female and 21 male; mean age, 46 years) requiring middle ear surgery. To define the cell density and distribution of T and B cells, CD4+ and CD8+ cells, antigen-presenting cells, macrophages, Mercels cells, Langerhans cells, mastocytes, as well as expression of E-cadherin, we used CD3, CD4, CD8, CD68, CD1a, CD117, HLA-DR, triptase, CD19, CD38 and E-cadherin antibodies and ABC immunohistochemical procedures. Results: Generally, immunostaining suggests that the T cells (CD4+>CD8+) and antigen-presenting cells (HLA-DR+) are predominant cell populations in the cholesteatoma, as well as that triptase+ mastocytes more number in comparison to CD117+mastocytes. Langerhans cells (CD1a+) are often seen intraepithelialy. There was increase density of each CD3+, CD4+, CD68+ cells in cases of cholesteatoma with extensive erosion and sequestration of the underlying bone. In addition, it is evidence of increased number of intraepithelial CD8+ cells in same cases. No statistical differences in density of each HLA-DR+, CD1a+, CD117+/triptase+, CD19+, CD38+ cells was found between cholesteatoma with and without extensive erosion of the underlying bone. Also, results show that the expression and distribution of E-cadherin in middle ear cholesteatoma is not deranged with extensive bone erosion. Conclusion: It is well known that the progression of the cholesteatoma might be induced by the release of factors from the cholesteatoma matrix or perimatrix. Findings of our study suggested that cholesteatoma CD3+, CD4+, CD8+ and CD68+cells plays and active role in the process of bone destruction.

PP4-197

THE EFFECT OF CAPECITABIN OR 5-FLUOROURACIL (5-FU) ON PREVENTION OF EPISCLERAL FIBROSIS AFTER TRABECULECTOMY IN THE RABBIT – TWO WEEKS FOLLOW-UP PATOHISTOLOGICAL FINDINGS

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Background: The fundamental role of the immune system in conjunctival wound healing after glaucoma surgery is well established. Glaucoma filtering surgery fails most frequently due to fibrosis at the episcleral-conjunctival/Tenon's capsule interface. The success rate of glaucoma filtration surgery may be optimized through the use of a combination of pharmacological agents that modulate the inflammatory and proliferative phases of the wound healing reaction. The most commonly clinically used drugs are corticosteroids and the antimetabolites, including 5-FU. Currently, many new pharmacological agents are used to modulate the wound healing. Method: In a rabbit model of glaucoma, we were examined the effects of capecitabin, a 5-FU Prodrug, or 5-FU on prevention of episcleral fibrosis after trabeculectomy. Twenty four animals were included in the study. After trabeculectomy we placed 5% solution of 5-FU in one eye of each animals for two weeks and the fellow eye was received capecitabin. Eye specimens were investigated by histological examination. Results: Microscopic examination revealed no statistical significant change in the number of fibroblasts and granulocytes, as well as collagen fibers density in the episclera between experimental groups. However, we observed the significant decrease of number of mononuclear cells and

plasmocytes, and reticulin fibers density in the eyes treated with capecitabin. Conclusion: Although both 5-FU and capecitabin were effective in the prevention of episcleral fibrosis after trabeculectomy in the rabbit, capecitabin has stronger effect in suppression of chronic inflammation.

PP4-198

EFFECT OF TREPONEMA DENTICOLA ON HUMAN DENDRITIC CELLS PHENOTYPE AND FUNCTION

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BACKGROUND: Periodontitis is a bacterial infection characterized by chronic gingival inflammation, which leads to the loss of the tooth-supporting tissue. Dendritic cells play an important role as regulatory and effector cells in the pathogenesis of periodontitis and Treponema denticola is an important periodontopathogen. This study compares the effect of Treponema denticola on the phenotype and function of human dendritic cells obtained from peripheral blood mononuclear cells of volunteers with periodontal healthy or chronic periodontitis. METHODS: Peripheral blood was obtained from systemically healthy volunteers with periodontal healthy or chronic periodontitis. Monnonuclear cells were isolated and maturated in dendritic cells. Dendritic cells were pulsed with extract of Treponema denticola at 5µg/ml, 10µg/ml or 15µg/ml, irradiated and were then cocultured with allogeneic T cells for 7 days. Dendritic cells were marked with monoclonais antibodies anti-CD1a, anti-CD14, anti-CD80, anti-CD86, anti-CD83, anti-CD11c, anti-CCR7, anti-HLADR. RESULTS: In periodontal healthy volunteers the bacteria increased the maturation of the dendritic cells, while in the periodontitis volunteers, the maturation was decreased when HLADR and CD-11c were analyzed. Proliferation of T cells was higher in the healthy than in the periodontitis volunteers. CONCLUSION: Phenotype and function of dendritic cells from individuals with periodontal healthy and chronic periodontitis differs when dendritic cells are pulsed with Treponema denticola. This may explain at least in part the differeces in susceptibility to periondontitis.

PP4-199

CYTOKERATIN EXPRESSION PATTERNS OF PRECANCEROUS LESIONS OF THE LARYNX CLASSIFIED ACCORDING TO THE LJUBLJANA AND WHO CLASSIFICATIONS

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INTRODUCTION: Ljubljana system for the precancerous lesions of the larynx is claimed to be more predictive for malignancy than WHO system. The main difference between these two classifications is the high percentage of involved epithelium in high grade displasia in WHO classification that corresponds to atypical hyperplasia in Ljubljana system. In normal laryngeal mucosa cytokeratin 14 (CK14) expression in basal, ciliated and non ciliated epithelium, CK8, CK18 and CK19 existence in non basal cells and CK13 expression in squamous and cuboidal cells were reported. In this study, we examined the cytokeratin expression patterns of precancerous lesions of the larynx classified according to Ljubljana and WHO classification in order to compare these two systems and altered cytokeratin expression in laryngeal carcinogenesis. MATERIAL METOD: We included 1 selected block from 42 laryngeal biopsies in our study (2 simple hyperplasia, 18 abnormal hyperplasia, 15 atypical hyperplasia, and 7 carcinoma insitu according to Ljubljana classification), (1 normal mucosa, 12 mild dysplasia, 9 moderate diysplasia, 12 severe dysplasia and 8 carcinoma insitu according to WHO classification). CK 7, 10, 13, 14, 16, 17, 19, 20 were detected by immunohistochemical metod. The relation between cytokeratin expression and subgroups were classified according to Ljubljana and WHO classification were detected by Chi square test. RESULTS In Ljubljana classification, CK13 expression was observed in 95% of simple hyperplasia, abnormal hyperplasia and 72.7% of, atypical hyperplasia and carcinoma insitu. CK16 expression is decreased with the intensity of the lesion ($p<0.053$). In WHO classification, CK10 expression was observed in 76.9% of normal mucosa and mild dysplasia, 48.3% of moderate, high dysplasia and carcinoma insitu ($p<0.083$). CK16 expression was detected in 76.9% of normal mucosa and mild dysplasia 41.1% of moderate, high dysplasia and carcinoma insitu ($p<0.033$). There was CK19 expression in 38.5% of the normal mucosa and mild dysplasia 75.9 % of moderate,high dysplasia and carcinoma insitu ($p<0.019$). CONCLUSION: WHO classification is more related with the cytokeratin expression than Ljubljana system. The reason may be the different levels of involvement of the squamous epithelium in two classification systems. There is increase in CK19 and decrease in CK10, 13 and 16 expressions in laryngeal carcinogenesis process.

PP4-200

MUC1, MUC2, MUC4, MUC5AC, MUC6 AND PS2 EXPRESSION IN SALIVARY GLAND NEOPLASIAS

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INTRODUCTION: Mucins are high density molecules synthesized by epithelial cells. Thirteen different type of mucin is defined. MUC 1, MUC 2, and MUC 4 were observed in the salivary gland ductal system. MUC 6 expression has been found in normal and neoplastic salivary gland tissue. TFF-1 is the molecule which makes co-expression with MUC 5 AC and was detected in the salivary glands. MUC expression in salivary gland neoplasias is variable and not studied adequate. Salivary gland tumors are a group of the neoplasias with problematic differential diagnosis especially in small biopsies. In this study, MUC and TFF1 expression of a group of salivary gland neoplasia were examined in order to find an additional criterion for their differential diagnosis. MATERIAL METOD For this purpose, 41 salivary gland tumors (10 pleomorphic adenoma, 10 Warthin tumor, 10 adenoid cystic carcinoma, 5 mucoepidermoid carcinoma, 4 myoepithelioma and 2 basal cell adenoma) have been included in the study. MUC 1, MUC 2, MUC 4, MUC 5 AC, MUC 6, and TFF-1 expressions were detected immunohistochemically. The presence and the intensity of the expression were scored as 0 to 4. Relation between existence of MUC 1, MUC 2, MUC 4, MUC 5 AC, MUC 6, TFF-1 expressions and MUC 5 AC and TFF-1 expression patterns (MUC 5 AC +/TFF-1 +, MUC 5 AC -/TFF-1 -, MUC 5 AC +/TFF-1 -, MUC 5 AC -/TFF-1 +) of different tumors, malignant and benign groups were detected by Chi-square test. RESULT: MUC 1 and MUC 4 expression was detected in all mucoepidermoid carcinomas, MUC 2 expression was detected in 4 cases (80%). MUC 6 existence in the mucoepidermoid carcinoma and adenoid cystic carcinoma was 20% and 40%. MUC 5 AC+/TFF-1 + staining pattern was observed in 62.9% of the benign neoplasias, and in 6.7% of the malignant salivary gland tumors ($p< 0.0001$). MUC 5 AC -/ TFF-1 - staining pattern was observed in 11.5% of benign and 53.5% of malignant salivary gland tumors ($p < 0.004$). CONCLUSION MUC 5 AC+/TFF-1 + staining pattern is more common in benign salivary gland neoplasias compared with the malignant group. Co expression of these two molecules in benign salivary gland neoplasias is similar to their normal expression in normal tissue. MUC-5 AC and TFF-1 expression may be an additional differential diagnostic parameter in salivary gland neoplasias.

PP4-201
CHRONIC HYPERPLASTIC CANDIDIASIS OF THE LARYNX

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Background: Candida species induce chronic inflammatory changes in the laryngeal epithelium comparable to chronic hyperplastic candidiasis (candidal leukoplakia) of the oral mucosa. However, hyperplastic candidiasis of the larynx is assumed to be rare. We here report a prospective series of 15 patients with chronic laryngeal candidiasis and discuss the diagnosis and management of these lesions. Methods : The histopathologic diagnosis of candidiasis was based on morphological features of causative fungi as well as associated epithelial changes. Patients were treated by type I, type II or type III resections. Excisions were followed by antireflux medications and systemic flucanozole treatment and patients were advised to stop smoking . Results : Of 15 patients with chronic laryngeal candidiasis, twelve were men (%75) and 3 (%25) were women. The ages of the patients ranged from 36 to 82 years. Hoarseness was the most common presenting symptom (13 cases). In 11 cases the history of smoking and in three cases associated alcohol consumption was recorded. Clinical impression was that of a hyperkeratotic lesion in 10 cases. Histopathological features associated with candidal organisms in biopsy specimens included; epithelial hyperplasia, keratosis and parakeratosis of varying severity, and polymorphonuclear leukocyte infiltration at the superficial layers of the laryngeal epithelium. Candidal hyphae were often scarce and located within the keratotic layer. Epithelial invasion by the hyphae was limited to uppermost epithelial layers. Periodic acid Schiff stain was very useful for the detection of fungal organisms. Mild epithelial dysplasia was noted in 1 case and moderate dysplasia was encountered in 2 additional cases. Histopathologically, in 8 cases fungal hyphae involved right vocal cords while in 5 cases they were noted in the left vocal cords. In one case bilateral vocal cords were affected. In the remaining case candidiasis was associated with squamous cell carcinoma. Conclusions: The possibility of hyperplastic candidiasis should be kept in mind when managing cases of keratosis and leukoplakia of the larynx. The pathologist must be aware of histopathological changes associated with hyperplastic laryngeal candidiasis and should apply special stains in order to detect fungi. This may influence the low incidence of laryngeal candidal leukoplakia, which in fact seems to be relatively higher in our experience. Laryngopharyngeal reflux may be an important predisposing factor in the development of laryngeal candidal leukoplakia.

PP4-202
DERMATOFIBROSARCOMA: CASE REPORT

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Dermatofibrosarcoma is rare tumor in head and neck region, it is asymptomatic and it rarely metastasizes. Here we present a case of on extremely aggressive, rapidly growing and mutilating DFS seemingly non-responsive to wide surgical excision. 42 years old men underwent surgical excision of discreet, painless left cheek soft tissue lesion. There were no palpable lymph nodes on the neck and chest radiography was normal. On histopathological examination, the lesion was thought to be dermatofibroma. Three years later, he presented with a recurrence at the site of excision. After excision the recurrent lesion was suggestive of dermatofibrosarcoma. During almost next three years the patient has been operated six times: each time the tumor rapidly and aggressively had grown outwards, destroying the whole middle third of the face, so at the moment of death only forehead, left eye, tongue and part of mandible had left. Comparing to dermatofibrosarcoma protuberans which appeared in our three patients, slowly relapsed and never tended to be aggressive, dermatofibrosarcoma itself, on the other hand, acted completely reverse, especially in this case where we have observed visible rapid growth of huge tumor masses destroying face, never giving regional or distant metastases.

PP4-203
EXTRAMEDULLARY PLASMACYTOMA OF NECK

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Plasma cell neoplasms and related entities are a group of lymphoid neoplasms of terminally diferentiated B cells that have in common the expression of a single clone of Ig secreting plasma cells. Multiple myeloma is the most common disorder. It is characterized by multiple tumorous mases of neoplastic plasma cells scattered throughout the sceletal system. About 3-5% of plasma cells neoplasms present as a solitary lesion of either bone or soft tissue. Aproxymately 80 % of all extramedullary plasmacytomas occur in the upper aerodigestive tract, especially the sinonasal tract, and 20 % in the other body sites. CASE: We present an anusual case of extramedullary plasmacytoma located in the neck of a 73 years old man. The patient initialy presented with tumorous mass in the right side of the neck. Ultrasonography showed tumorous mass. Ultrasound –guided fine needle aspiration of that lesion had been performed. Cytologic differential diagnosis was plasmacytoma or oncocytic tumor. In bone marrow aspirates we have found 8% plasma cells. Tumor was removed and hystological analysis revealed tumor composed of sheets mature plasma cells with round eccentric nuclei and abundant cytoplasm. Imunohystochemical analysis shoved positive reaction for CD 138 and IgA and negative for CD 20, CD 3 and IgM. Hystological analysis and immunohistochemistry confirmed diagnosis of plasmacytoma. CONCLUSION: Extramedullary plasmacytomas comprises less than 1% of all head and neck tumors. Extramedullary plasmacytoma of the neck region is extremely rare. Treatment consists of surgically or/and radiation therapy with tipically good prognosis.

Neuropathology

PP4-204
EXPRESSION OF THE PARATHYROID HORMONE-RELATED PROTEIN IN GLIAL TUMORS

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BACKGROUND: It has been found that Parathyroid hormone-related protein (PTHrP) is expressed in a variety of tumors including breast, prostate, colon, lung, renal and ovarian cancers. In some recent publications, it has been noted that there is a relation between PTHrP expression and prognosis in glial tumors. The aim of this study is to evaluate the expression of PTHrP in glial tumors and to find out whether there is a relationship between this expression and grade. METHODS: We have examined the expression of PTHrP in glial tumors of 16 glioblastoma multiforme , 2 anaplastic astrocytoma, diffuse astrocytoma grade II and pleomorphic xanthoastrocytoma grade II using immunohistochemical staining. RESULTS: It has been found that PTHrP staining has been seen in 6 glioblastoma multiforme, a pleomorphic xanthoastrocytoma. The strongest and the most diffuse staining has been seen in the case of pleomorphic xanthoastrocytoma. The patient was a child. It has also been found that staining is very weak in 4 out of the 6 fixed glioblastome. Four cases showed weak staining out of six positive gliobastome. CONCLUSİON: PTHrP is expressed very low in human glial tumors in our study. Further studies of large series having more low grade astrocytomas should be done.

PP4-205
RARE OCCURRENCE OF OLFACTORY COLLOID CYST

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Background. Colloid cysts are rare benign intracerebral lesions accounting for 0.2% to 2% of all intracranial neoplasms. They are predominately located in the anterior aspect of the third ventricle; however, there are occasional reports of colloid cysts found in the fourth ventricle, cerebellum, leptomeninges, parietal region and frontal lobe. We present herein a highly unusual case of a colloid cyst residing in the olfactory lobe. Materials and Method. A 74-year-old patient underwent a brain CT scan that incidentally revealed a space-occupying lesion measuring approximately 2.5X1.8X2.5 cm, involving the olfactory lobe. Magnetic resonance (MR) imaging showed the lesion as hyperintense on T1, T2 and FLAIR sequences, without surrounding brain edema. The differential diagnosis included a mucocele, dermoid cyst, meningioma and a glioma. Brain scintitomography showed a lesion of low metabolism, a finding consistent with benignity. The patient underwent surgery and intraoperative a cystic lesion was revealed. The cyst was eroding the dura and the frontal lobe and due to the danger of CFS leak, a dura repair was performed Results. Histological examination of the resected material showed that the cyst was lined by a single and focally by a pseudostratified layer of columnar ciliated epithelial cells resting on a basal lamina. Mucus-filled cells were not found. The content of the cyst was consisted of an amorphous, eosinophilic, colloid-like material. Immunohistochemical examination showed positive staining of the lining cells for cytokeratin, epithelial membrane antigen and focally for carcinoembryonic antigen. The histologic diagnosis was consistent of a colloid cyst. Conclusion. Colloid cysts should be included in the differential diagnosis of lesions in the anterior fossa and that although benign they may be aggressive by eroding the dura and producing a mass effect.

PP4-206
SEVERE PRIMARY INTRACEREBRAL HAEMATOMA – CORRELATIONS BETWEEN MAIN CLINICAL, IMAGISTIC AND MORPHOLOGICAL ASPECTS

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Background: The study is an integrated assessment of clinical, imagistic and morphological parameters in patients with severe primary intracerebral hemorrhage (PICH) who died during hospitalisation. Method. The selected group consisted of 183 cases and was divided into 2 groups: 102 cases who lived more than 48 h and were confirmend by computed tomography (CT) and 82 cases that died within 48 h and were confirmed by authopsy. The study was retrospective. The studied material consisted of patient's medical records (medical records, CT films, autopsy protocols and histopathology - HP records). Assessed parameters were: clinical (seasons relation, age, sex, arterial blood pressure - HT, motor deficit - MD, degree of coma - C, Glasgow score at admission) and morphological static (PICH sites, size, perilesional edema - pE, microhaemorrhages - mH,) and dynamic (mass effect - ME, ventricular effusion - VE and subarachnoid effusion - SE). Results: The severe PICH showed a predilection for winter and summer but men (M) are affected more frequently in transition seasons. Sex distribution showed a slight M predominence. The most affected life decades are the 5th and the 7th with a regressive trend for M after the 6th decade and an increasing trend for women (W) towards the 7th decade. Around 70% of the patients had IIIrd and IVth stage HT at admission, 85% had MD and almost 50% Glasgow scores lower than 6. The most common sites of PICH were cerebral hemispheres, slightly more frequent in the left one. They had huge dimensions as compared to hosting encephalic structures. In lobar sites, more than one lobe were involved, mostly parietal, temporal and frontal lobes. Both CT and HP examinations revealed in many cases multiple mH both near and distant to PICH. Other morphologic predictive factors as pE and ME were constantly present. The VE involved at least one of the lateral ventricles. C is more frequent in W, after 60 years of age, in PICH occuring in winter or summer, and non-lobar PICH. That is also the case of MD, which is more frequent in PICH occuring in autumn and winter and lobar PICH. VE is more frequent in M, after 60 years of age, in PICH occuring in autumn and lobar PICH. Although less frequent, SE is slightly more frequent in W, PICH occuring in winter and non-lobar PICH, regardless of age. Conclusions: Our data suggest that PICH associated with one or more clinical and morphological poor outcome predictors (HT, MD, C, ME, VE and SE) results in patient's death, despite any sustained therapeutical intervention.

PP4-207
ANAPLASTIC MENINGIOMA ASSOCIATED WITH MENINGIOANGIOMATOSIS: CASE REPORT OF A RARE ENTITY

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Background: Meningoangiomatosis (MA) is a rare malformative lesion that is associated with seizure and/or headache. It occurs both in patients with neurofibromatosis (NF), as well as in sporadic cases. Rarely, MA has been described coexisting with meningiomas. As far as we could reach, anaplastic meningioma together with MA has not been reported yet. Material and Methods: A 16-year-old boy had 2 year history of seizure. There was no history of NF. Magnetic Resonance Imaging (MRI) at the second year revealed a contrast-enhancing lesion, with dural tail and peritumoral edema near the left tentorium.The lesion was completely excised. Results: Microscopically, the lesion composed of mitotically active spindle cells with moderately large hyperchromatic nuclei, in a coarsely fasicular pattern resembling sarcoma. Limited areas showed elongated cells in interwoven fasicles and in whorls with psammoma bodies consistent with transitional meningioma. In the cortex, there were abundant small intracortical vessels ensheathed by meningothelial cells; typical of meningoangiomatosis. Conclusion: We present a unique case of anaplastic meningioma which have arisen from meningoangiomatosis without NF-2 history and discuss the pathogenesis, radiologic, clinic and pathologic features on the base of the literature.

PP4-208
ANGIOCENTRIC GLIOMA: A RARE EPILEPSY-ASSOCIATED NEOPLASM

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Background: Angiocentric glioma is a distinct epilepsy-related entity which was not included in the World Health Organization (WHO) classification of Central Nervous System (CNS) Tumors yet. There are only two reports with 18 cases in the literature. Material and Method: A 3-year-old girl was admitted to the hospital with a history of seizure. MRI showed lesion at the right hippocampus and excised. Result: Microscopically, the lesion was composed of cells that were strikingly angiocentric to both large and small vessels. Tumor cells were uniform and usually bipolar. Atypical mitosis, necrosis were absent. Proliferation index was also negative with Ki-67. Conclusion: We present a case of newly described entity which should take part in the WHO classification of CNS tumors. And discuss the clinic, radiologic, histologic and ultrastructural features of this tumor on the base of the literature.

PP4-209
HISTOLOGICAL DISCREPANCIES IN MALIGNANT GLIONEURONAL TUMORS: A REPORT OF 4 CASES

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Background: Although not included in WHO 2000 Classification of CNS tumors, malignant glioneuronal tumor (MGNT) is widely accepted as a new entity which resemble any type of high grade (grade III or IV) malignant glioma with tumor cells expressing both neuronal and glial markers without mature ganglion-like cells. In this case series, 4 immunohistochemically proven cases of MGNT are reported in order to emphasize the histological discrepancies among seperate cases of this poorly understood tumor group. Cases: Four cases of MGNT from three different institutions are studied. Two of the patients were female and the other two were male, age 13 (Case 1), 50 (Case 2), 70 (Case 3) and 18 (Case 4) years and localizations were right parietooccipital-intraventricular, left occipital, right temporal and right occipital, respectively. All cases showed peritumoral oedema and contrast enhancement. Except for Case 1, tumors were well circumscribed and gross-totally excised followed by radio-chemotherapy. Patients Case 1 and Case 3 died because of the disease 12 months and 21 months after the initial diagnosis with recurrences while others are still alive for 16 months (Case 2) and 5 months (Case 4). For all cases, glial fibrillary acidic protein, synaptophysine, neurofilament protein and Ki-67 were applied and it was shown that first three of these markers were much or less co-expressed by the tumor cells despite the histological discrepancies among cases. Case 1 and Case 3 was composed of PNET-like areas and glioblastoma areas. Case 1 additionally contained loose and spindle-cell areas while Case 3 showed perivascular arrangement of tumor cells and some pseudopapillary structures. The bulk of the tumor in Case 3 was composed of areas of typically oligodendroglioma or anaplastic oligodendroglioma, which are found in also Case 4. Case 4 also presented psedopapillary structures, spindle-cell areas and epitheliod gemistocyte-like cell clusters intermingled with indifferentiated small cells. Necrosis, pseudopalisading of tumor cells around necrosis and brisk mitotic activity were apparent in all cases. Ki-67 proliferation indices were 55%, 72%, 20% and 43%, respectively. Conclusion: As histology of MGNTs can diverge greatly, all CNS tumors resembling malignant gliomas (especially glioblastoma) or supratentorial PNET should undergo immunohistochemical analyses with at least two neuronal markers as well as with glial fibrillary asidic protein in order to make accurate diagnosis and prognostic presumptions.

PP4-210
SYMPTOMATIC SPINAL LUMBAR JUXTAFACET CYST: A CASE REPORT

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Background: Juxtafacet cysts (JFC) are rare intraspinal lesions associated with the facet joints. Although they can be seen along the spinal cord, the lumbar region is the most commonly affected area. The putative mechanisms of cysts formation include spinal instability, arthropathy and trauma. Histologically, two types of JFC are recognized: the synovial and ganglion cysts or

pseudocysts. The two lesions have different histological features, however, studies based on detailed histological analysis revealed dual findings in the same sample, suggesting that these cysts are either parts or progression of the same disease. We report herein a case of symptomatic juxtafacet cyst presenting as L4-L5 radiculopathy. Case report: A 53-year-old man presented with a history of progressively increasing severe pain in the right lower extremity. Physical examination revealed a L4-L5 sensitive and motor deficit. Magnetic resonance imaging (MRI) revealed an intraspinal cystic mass at the segment L4-L5, with hypointense T1-weighted and hyperintense T2-weighted signal and peripheral enhancement. Surgical treatment was planned due to severe pain and neurologic deficit. Histological examination of the resected material showed the presence of a cystic formation with no evidence of epithelial or synovial lining. The wall of the cyst was fibrovascular, with myxoid areas, focal hemorrhage, rare hemosiderin deposits, collections of multinuclear giant cells and small calcifications. The immunohistochemical staning for glial fibrillary acid protein (GFAP) and neurofilament (NF) were negative, showing that the lesion was not derived from neural tissue. The diagnosis was consistent with a cystic formation of spine or juxtafacet cyst with morphology of a ganglion cyst. The postoperative course was uneventful and the patient was discharged on the third following surgery day. On follow-up examinations at 3 months and 1 year after surgery, the patient remained free of pain and symptoms. Conclusion: Juxtafacet cysts are uncommon cause of radiculopathy. We emphasize the need to be thoroughly familiar with this pathologic entity that should be considered in the differential diagnosis of spinal lesions.

PP4-211

EMBRYONAL TUMOR WITH ABUNDANT NEUROPIL AND TRUE ROSETTES: A RARE PEDIATRIC EMBRYONAL NEOPLASM OF CNS. REPORT OF FOUR CASES

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BACKGROUND Embryonal neoplasms of CNS affect early years of life and share an aggressive behavior. WHO classification includes five different well-defined entities: medulloepithelioma, ependymoblastoma, medulloblastoma, supratentorial PNET and ATRT. Recently, a new tumor combining features of ependymoblastoma and neuroblastoma named "embryonal tumor with abundant neuropil and true rosettes" have been reported. This lesion is characterized by the presence of areas of well-differentiated neuropil containing ependimoblastic rosette and undifferentiated neuroepithelial cells resembling classic PNET. We report four cases of this rare entity. METHOD The tumoral specimens were routinely processed, paraffin embedded, and H&E stained. Slides were processed for immunohistochemistry using antibodies for Vimentin, S-100, Desmin, Synaptophysin, Neurofilaments, Smooth Muscle Actin, GFAP and BAF47/INI1. The proliferation index was evaluated using anti-MIB-1 antibody. RESULTS Patient #1 was a 1.5 years-old female with a right frontal lesion; patient #2 was a 1.5 years-old male with fronto-temporal lesion; patient #3 was a 3 years–old female with bi-frontal tumor; patient #4 was a 1.5 with pontine lesion. The tumors showed a variable cellular density with fields composed by fibrillar neuropil-like matrix and by hypercellular areas, formed by small hyperchromic cells. Rare Homer-Wright rosettes were focally observed. Mitoses and apoptosis were frequent. The distinctive feature was the presence of numerous ependimoblastic rosettes, found in the hypercellular areas and/or in the fibrillary neuropil-like matrix. The rosettes were composed by pseudo-stratified embryonal cells, arranged around a central lumen. In one case, a sarcomatous-like component of elongated pleomorphic cells was present. The undifferentiated cells were negative for GFAP and synaptophysin whereas the neuropil-like matrix stained intensely for sinaptophysin and neurofilaments. The sarcomatous areas present in one case showed diffuse immunoreactivity for smooth muscle actin and for desmin. The tumors showed a nuclear staining for BAF47/INI1. The MIB-1 ranged from 10 to 30 % in the higher cellular areas. Interestingly, the ependimoblastic rosettes in the neuropil-like areas contained the more proliferative cellular component. CONCLUSION Embryonal tumor with abundant neuropil and true rosettes although presents some features common to other embryonal tumors, seems to have specific histopathological, neuroradiological and clinical features, delineating it as a new entity among pediatric brain tumors.

PP4-212

EXPRESSION OF NUCLEAR FACTOR –κB IN HUMAN ASTROCYTOMAS: RELATIONSHIP WITH PIκBA AND PROGNOSTIC SIGNIFICANCE.

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Background: NF-κB is a family of dimeric transcription factors that play a critical role in host defence by regulating the expression of immune genes. Several investigators have reported its constitutive activation in various human tumour cell lines, whereas its immunoexpression has been regarded as a marker of poor prognosis in several tumours. NF-κB activation is tightly regulated by its interaction with IκB inhibitory proteins. However, little information is available about the role of IκB phosphorylation in relation to NFκB activation, and the clinical significance of this interaction in astrocytomas. This study aimed to elucidate the role of NFκB in astrocytomas, focusing on p50/NFκB1 subunit, to determine its association with phosphorylated IκB, and to define the clinical impact of this interaction. Method: The levels of p50/NFκB1 and pIκBa proteins expression were quantified immunohistochemically in paraffin-embedded tissue from 73 patients with astrocytomas, by using a rabbit polyclonal anti-NFκB p50 and a mouse monoclonal anti-pIκBa antibody. Nuclear and cytoplasmic immunoreactivity was assessed separately for both antibodies. Results: Nuclear NFκB1/p50 immunoreactivity was detected in 66/72 cases (91.6%), whereas cytoplasmic immunoreactivity in 17/72 (23%). Concurrent cytoplasmic and nuclear expression was recorded in 17 cases (22%). pIκBa expression was found in 72/73 (98.6%) cases and was predominantly cytoplasmic with scattered nuclear positivity. Concurrent pIκBa and nuclear NFκB1/p50 immunoexpression was detected in 66 cases. All cases with nuclear NFκB1/p50 expression manifested pIκBa immunoexpression. pIκBa expression was positively correlated with nuclear NFκB1/p50 expression ($p=0.0054$) and adversely correlated with cytoplasmic NFκB1/p50 expression ($p<0.0001$). Nuclear NFκB1/p50 and pIκBa expression increased in parallel with tumour grade ($p= 0.0031$ and $p<0.0001$). In univariate survival analysis nuclear NFκB1/p50 and pIκBa expression adversely affected survival ($p<0.0001$ and $p=0.002$). Multivariate survival analysis selected NFκB1/p50 nuclear expression ($p=0.001$) as a significant prognostic factor. Conclusion: Our study reinforces the concept that NFκB1/p50 activation is largely dictated by its interaction with IκBa in human astrocytomas.

Additionally, NFκB1/p50 nuclear expression parallels tumour grade, a result that suggests its contribution to tumour agressiveness. Finally, our results suggest that nuclear NFκB1/p50 expression may serve as a useful independent marker for stratifying patients with astrocytoma in terms of prognosis.

PP4-213
CEREBRAL NEUROBLASTOMA IN ADULTS: REPORT OF THREE CASES

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BACKGROUND: The term "cerebral neuroblastoma" identifies an embryonal neoplasm of CNS belonging to the group of the supratentorial PNET (sPNET) showing various degree of neuronal differentiation. These tumors affect preferentially infants or children and appear to be extremely rare in adult life. We present three cases of cerebral neuroblastoma in adult patients. METHOD: Tumoral specimens were routinely processed, paraffin embedded, and H&E stained. Slides were processed for immunohistochemistry with the ABC system, using antibodies for Vimentin, S-100, Desmin, Synaptophysin, Chromogranin, Neurofilaments, Smooth Muscle Actin, EMA, CK, MAP-2, GFAP, p53 and Neu-N. The proliferation index was evaluated with anti-MIB-1 antibody. RESULTS: In patient 1, a 63 years-old female, MRI revealed a 4 cm infiltrating lesion affecting the left occipital lobe, with non-homogeneous contrast enhancement and necrosis. In patient 2, a 61 years old female, MRI showed a 7 cm well-demarcated right frontal mass, with intense non-homogeneous contrast enhancement. In patient 3, a 59 years old female, CT scans revealed a left parieto-occipital lesion with edema, midline shift and homogeneous contrast enhancement. Histopathological examination showed lesions with similar architecture but with large spectrum in cell composition. Tumors were formed by lobules separated by thin fibro-vascular septa, filled by a mixture of small elements with neuroblastic features as well as larger cells with neuronal or ganglion-like morphology, merged in a variable amount of neuropil. However, cells showed also a variable range of "anaplasia" characterized by bizarre giant cells, apoptotic bodies, necrosis and atypical mitoses. Immunohistochemistry revealed a variable positivity for synaptophysin, neurofilaments, chromogranin and Neu-N in isolated cells. GFAP and mesenchymal or epithelial markers were negative. The proliferation index, assessed by Ki67/MIB1 antibody was variable but focally elevated. p53 was negative. CONCLUSION: Cerebral neuroblastomas in adults are histologically similar to those occurring in pediatric age, although they seem to show a higher degree of severe anaplasia. They lack specific clinico-radiological features. Moreover they have to be distinct from malignant neoplasms more frequent in the adult age group as mixed glio-neuronal tumors and pure gliomas such as glioblastoma and anaplastic oligodendrogliomas.

PP4-214
ANCIENT SCHWANNOMA OF THE SPINAL CORD. A RARE CASE

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Background: Schwannomas of the Spinal Cord (SSC) are rare benign tumors. Most are sporadic and some can be part of hereditary syndromes. Histological malignancy of this neoplasm is rare. Method: A 51 year old patient admitted to our hospital for neurologic evaluation with symptoms of spinal compression because of a CT scan lesion on the spinal cord. The lesion was surgically removed and we received a well-circumscribed, elongated, cylindrical specimen which was measuring 8.4x2.2cm. The tumor was encapsulated and on dissection it was partly solid with whitish tint and partly multicystic. The cysts were filled with a bloody-serous liquid. Results: Histologically, multiple cysts comprised most of the tumor while the rest consisted of an inconspicuous or focally cellular component (Antoni type A) with a hint of nuclear pallisading that rendered the diagnosis difficult. Hyalinization, hemosiderin deposition and some nuclear atypia were the minor characteristics of the tumor. In Antoni B areas edema separated tumor cells. No mitotic figures were found. Immunohistochemically the tumor showed reactivity for S-100 protein, vimentin and CD68. Keratin, desmoplakin, neurofilaments and desmin are not expressed. Conclusion: In this presentation we aim to emphasize the polycystic appearance of a so called ancient SSC in a rare location. SSCs generally agreed that are neoplasms originating from Schwann cells, hence their name. Malignant transformation is an exceptionally rare event. Ancient SSC are usually large tumors of long duration. Although histologically are benign, biologically mimick malignancy.

PP4-215
CONGENITAL SUPRATENTORIAL CYSTIC HEMANGIOBLASTOMA; A CASE REPORT AND REVIEW OF THE LITERATURE

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Congenital supratentorial hemangioblastomas are rarely encountered tumors even in pediatric population. Extensive review of the literature revealed that approximately 118 cases have been reported in the literature so far. However, only 6 of these occurred in infants, and 4 of them occurred during the first two months of life. A 5-week-old boy presented with emesis, irritability, bulging anterior fontanelle and a gradually expanding head circumference since birth. His previous medical and family history was uninformative in terms of cancer or inherited diseases. The MR scans demonstrated a large loculated cyst with a 3-cm heterogeneus mural nodule, first pushing the left frontal and parietal lobes, and then displacing in this region. The histopathologic diagnosis was reticular variant of hemangioblastoma. We reviewed the literature on congenital supratentorial hemangioblastoma and discussed the histopathologic characteristics and differential diagnosis associated with such lesions in this report

PP4-216
THE EXPRESSION OF OSTEOPONTIN IN HUMAN ASTROCYTOMAS: INTERSTITIAL EXPRESSION CORRELATES WITH MALIGNANCY GRADE AND ANGIOGENESIS

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BACKGROUND: Osteopontin (OPN) is a phosphorylated protein secreted into the extracellular matrix by a variety of cell types. Numerous functions have been ascribed to osteopontin, including roles in bone remodelling, cell-adhesion, cell-mediated immunity, the ability to act as a cytokine in cell signalling,

resulting in proliferation and/or cell survival, and angiogenesis. OPN has also been detected in a number of human tumour tissues and assessed as a potential marker of tumour progression. METHOD: This study was aimed to analyse by immunohistochemistry the expression of OPN in 76 human gliomas of various grades of malignancy: 8 pylocytic astrocytomas, 10 grade 2 astrocytomas, 8 anaplastic astrocytomas and 50 glioblastomas. The staining results were scored in a semi-quantitative manner by assessing the percentage of positive tumour cells and the intensity of staining. We also assessed the intercellular distribution of staining, which was categorized as focal or diffuse, with the staining intensity expressed either as low or high. Since OPN has been implicated in angiogenesis, we analysed the correlation between OPN expression and microvessel densitiy in glioblastomas. Microvessels were stained with α-endoglin antibody (CD105) and counted in predominantly vascular areas (hot spots) at x 400 magnification. RESULTS: In normal brain tissue the expression of OPN was present in some glial and neuronal cells in the form of cytoplasmyc granular staining of low intensity, while microglial cells were strongly positive. In glial tumors intracellular OPN expression ranged from absent or low (score 0-5) to strong (score 6-10). Strong OPN expression was observed in 3 grade I (37.5%), 4 grade II (40%), 2 grade III (25%) and 21 grade IV (42%) astrocytomas. In some tumors OPN was also present interstitially, between tumor cells. This staining pattern was accentuated around tumor necrosis and, in most of the cases, also around blood vessels. Tumours with either a diffuse or strong expression were considered positive in intercellular distribution of OPN staining. Interstitial OPN expression was observed in 1 pylocytic astrocytoma (12.5%), 2 grade II astrocytomas (20%), 2 anaplastic astrocytomas (25%) and 25 glioblastomas (50%). We found the association of interstitial OPN expression and angiogenesis i.e. microvessel density was higher in tumors with high interstitial OPN expression (Mann Whitney test, $p<0.03$). CONCLUSION: Our results show the overexpression of OPN in human astrocytic tumors and suggest the role of OPN in astrocytoma progression and angiogenesis.

PP4-217

CAFFEIC PHENETHYL ESTER (CAPE) SUCESSFULLY REDUCES BRAIN DYSFUNCTION IN SEPSIS

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Background and goal of study: Sepsis and ensuing multi organ failure continue to be the major causes of morbidity and mortality in the intensive care units. Nuclear factor-Kappa beta (NFKB) activation is supposed to be one of the targets in the treatment of sepsis and ensuing mortality. We studied the effectiveness of caffeic phenethyl ester (CAPE), a known NFKB inhibitor, in cecal ligation and puncture (CLP) induced sepsis model of brain dysfunction. Materials and Methods: 40 rats are randomized to 5 groups. All rats were operated to induce sepsis with cecal ligation and puncture (CLP) except control and CAPE groups that were operated just with laparotomy. CAPE (50 µmole/kg) was administered to rats intramuscularly at the time of operation in CAPE and CA+SEP (0) groups. CAPE was administered to rats in CA+SEP (12) group 12 hours after CLP. All rats from each group were sacrificed 24 hours after CLP; half of brain coronally was taken out for histopathological, and the rest brain was taken out for oxidative stress parameters. Apoptosis was examined with Tunnel staining. Induced nitric oxide synthase (iNOS) and heat schock protein(HSP70) were examined with immunohistochemistry. Malonlydialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were studied for oxidative stress evaluation. Results and Discussion: Immunohystochemical studies revealed that sepsis induced brain dysfunction in our model. Histopathologically apoptosis and iNOS and HSP70 expressions were studied in hippocampal CA1 region, prefrontal cortex and plexus choroideus. Although apoptotic measurement revealed no change between groups in hipocampal CA1 region, iNOS and HSP70 expressions were significantly increased in all other groups in comparison to control group. However, iNOS and HSP70 expressions were significantly reduced as CA+SEP (0) <

PP4-219

MEDICALLY INTRACTABLE EPILEPSY: REPORT OF THREE CASES

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Background: There is no precise definition of intractable epilepsy. Among the considerations are seizure frequency, seizure type, severity of attacks, and impact on quality of life. Twenty percent of epilepsy is intractable and surgery is an option in the attempt to cure or reduce the severity of medically resistant cases. We report herein 3 intractable epilepsy cases diagnosed as cortical dysplasia, Rasmussen encephalitis and Sturge-Weber syndrome for discussing their histopathological features. Case 1: A 2-year-old male has been suffering from generalized tonic and clonic seizures since 15 months. The histopathological examination revealed cortical dislamination, neuronal heterotopia in the white matter. Neuroimaging and histopathological findings were found compatible with cortical dysplasia. Case 2: A 14-year-old male has been suffering from progressive, focal and generalized seizures and also left hemiparesy on the forearm and leg since 3 years. Cranial MR showed right frontal and temporal atrophy. Functional right hemispherectomy was performed. The histopathological findings revealed neuronal loss, neuronophagia, microglial nodules and perivascular lymphocytic infiltration. The clinical, radiological and histological findings let us to diagnose the case as Rasmussen encephalitis. Case 3: A 2-year-old male has been suffering from progressive, generalized and focal seizures since 20 months. A pink macular lesion in the dermatome of N.trigeminus was remarked. MRI showed cortical atrophy and also increase in the thickness of leptomeninges. Being accepted medically intractable epilepsy, surgical procedure was performed. Leptomeningeal angiomatosis and cortical atrophy let us to diagnose Sturge-Weber syndrome with the presence of port-wine stains. Conclusion: Surgery in epilepsy is the resection of epileptogenic focus The pathological lesions are classified recently as: (a) hippocampal sclerosis (b) malformative lesions, (c) vascular lesions (d) tumors, (e) chronic inflammatory and infectious lesions, (f) ulegyric lesions (g) gliosis-astrocytic proliferation, (h) concomitant calcified lesions and (i) unspecified lesions. It is impotant to remember that Rasmussen encephalitis is a rare, chronic inflammatory disease that usually affects only one hemisphere. Furthermore, special attention has been given to the coexistence of focal cortical dysplasia and tumors, which include mixed neuroglial tumors. Finally, our cases and literature data stress that clinical, neuroradiological and histopathological correlation is important for the diagnosis of these lesions.

PP4-220

PROGNOSTIC FACTORS IN MENINGIOMAS

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ABSTRACT: Meningiomas are mostly benign tumors cured by surgical resection. But the behaviour of meningiomas are sometimes diffucult to predict. Some of them tend to recur, therefore longterm management in subtotally resected tumors remain controversial. The microscopic brain invasion, mitotic rate higher than 4/ 10 HPF, the presence sf sheeting, hipercellularity, prominent nucleoli, nuclear pleomorfism, small cells, decreased recurrence free survival (RFS). In this study MIB-1 LI , PR, ER, P 53 and EGFR expressions are compared with histopathological changes. METHOD: 148 meningioma cases operated in our Neurosurgical Department of Okmeydanı Hospital 20 cases were chosen for study.(4 angiomatous meningioma, 2 of which recurred, 5 meningioma cases, 5 atypical grade II meningioma cases, 6 malignant meningioma cases) are examined immunohistochemically using MIB-1 monoclonal antibody estrogen-progesteron receptor P53 and EGFR. RESULTS: There were a close association between MIB-1 LI indices and mitotic index and tumor grade. According to Spearman's rho (Spearman's Rho= 0,493) (p=0,027) P53 and EGFR immunohistochemistry revealed no useful prognostic information. ER, PR immunohistochemistry were variable, but usually ER were not expressed.

PP4-221

EXPRESSION OF HORMONE RECEPTORS PR & ER, ANGIOGENIC FACTORS VEGF – A & VEGF-R3, P53, MDM2, CERBB2 AND CD117 IN MENINGIOMAS

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Background: Meningiomas are mostly frequent and benign central nervous system tumors. Independently from the extent of tumor resection, reccurence and tumor progression often determine clinical outcome. In an attempt to understand meningioma tumor biology, we evaluated biomarkers possibly involved in tumor formation, reccurence and malignant transformation, such as progesterone and estrogen receptors, angiogenic factors VEGF – A and VEGF-R3, p53, MDM2, cerbB2 (Her-2/neu) and CD117 (c-kit) and their possible implication in therapeutic approaches. Methods: We examined 34 meningiomas operated in our hospital the last 4 years, 30 of which benign, 2 reccurent (WHO grade I) and 2 atypical (WHO grade II) immunohistochemically for Progesterone Receptors (PR), Estrogen Receptors (ER), Vascular Endothelial Growth Factor (VEGF-A) and its Receptor (VEGF-R3), p53, MDM2, cerbB2 and CD117. Results: PR were expressed in 27/32 (84%) WHO grade I meningiomas in a mean of 44,15%. ER were demonstrated in 12/32 (37,5%) in a mean of 5,5%. Neither PR nor ER were expressed in atypical meningiomas. VEGF-A was intensly demonstrated in most WHO grade I and II meningiomas mainly with cytoplasmic granular or dot-like distribution. In 2 reccurent cases it was faintly or negatively expressed. VEGF-R3 was variably detected with cytoplasmic and nuclear distribution in all meningiomas. p53 nuclear staining was shown in 18/32 (56,25%) benign meningiomas in a mean of 13,05% with negative staining in 2 atypical meningiomas. Nuclear MDM-2 overexpression was variably demonstrated in all benign, recurrent and atypical meningiomas in a mean of 60,90%. cerbB2 was scored 3+ positive staining in 1 benign meningioma (2%), 2+ in 6 meningiomas (17,6%), 1 of which atypical and 1+ and less in the rest (79,4%). CD117 was expressed focally and faintly in 4/34 (11,7%) benign mengiomas. Conclusion: Loss of progesterone receptors' expression is shown to be associated with increased reccurence, implying a better prognosis for positive expression, as were most of our benign and 2 atypical cases. Instead, presence of ERs in meningiomas may correlate with karyotype abnormalities. Hormone receptor status, especially in females, seems to be a possible anti-hormonal target of therapy. Angiogenic factors like VEGF-A and its Receptor VEGF-R3 may be involved in meningioma vasculature and likelihood of reccurence, so antiangiogenic agents should be encountered in therapy. Inactivation of p53 tumor suppressor and overexpression of MDM2 protein may promote meningioma progression and future reccurence, revealing a possible candidate to an aggressive treatment. In our cases the amount of expression of MDM-2 seems not to be correlated to histological grade and reccurence. cerbB2 (Her-2/Neu) and CD117 (c-kit), both members of the tyrosine kinase receptors' family, have not yet been detected in meningiomas but their possible overexpression, especially cerbB2, as in our limited number of cases, must be studied further.

PP4-222

TYPICAL AND UNUSUAL PATHOLOGICAL CHARACTERISTICS AND RADIOLOGICAL FINDINGS IN PRIMARY NEOPLASMS OF THE SPINAL CORD: UCSF EXPERIENCE

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Background: Limited biopsy samples and overlapping clinical/radiological features can complicate the diagnosis of primary neoplasms of the spinal cord. Typical features of each entity are well known, but deviations from these features are not well recognized. Our study is aimed to determine typical as well as unusual radiological and pathological characteristics of the primary spinal cord tumors. Material and Methods: We reviewed the clinical, radiological and pathological features of all primary neoplasms of the spinal cord diagnosed and treated at our institution between 1996 and 2005. Radiological and pathological materials were analyzed using standard evaluation forms, and the results were analyzed using a statistical software package (SPSS 11.1 for Windows). Appropriate permissions were obtained from the institutional Committee on Human Research (H41995-25437). Results: The entire cohort of 136 patients with primary spinal cord neoplasms consisted of 79 males and 57 females with a mean age of 43 years (range 1-89 years). Only 13 (10%) of these neoplasms occurred in the pediatric age group (<15years). There were 57 ependymomas, 44 schwannomas, 15 neurofibromas, 10 astrocytomas of various grades, 8 hemangioblastomas, 2 gangliocytomas, and 1 subependymoma. Forty four percent of schwannomas demonstrated cystic change radiologically as well as pathologically, 32% had marked inflammatory infiltrates, and 48% had significant degenerative atypia. Radiological evidence of hemosiderin was most prevalent in ependymomas. A significant number of non-pilocytic tumors demonstrated Rosenthal fibers in the neuropil surrounding the tumor. Radiologically some ependymomas could not be distinguished from schwannomas or pilocytic astrocytomas. Clinical features, radiological presence of cyst and T1-T2 characteristics were not helpful discriminators. Pathological material was insufficient in three cases, in which radiological features were helpful. Conclusion: In our series, a significant number of primary spinal cord tumors did not show typical features radiologically, and to a lesser extent histologically. Radiologically, presence of hemosiderin correlated best with an ependymoma, but otherwise pilocytic astrocytomas could not be distinguished from ependymal tumors. The main challenge in the pathological diagnosis was the sample size. Recognition of prevalence of atypical features is critical in correct diagnosis primary spinal cord tumors.

PP4-223

CEREBRAL MEDULLOEPITHELIOMA

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Introduction: Intracranial medulloepithelioma (ME) is an uncommon, highly malignant primitive neuroectodermal tumour of the central nervous system (CNS) which pathologically recapitulates the neural tube. It usually develops in early childhood and may occur anywhere within the CNS, the most common site being periventricular. Aim of study: The aim of our study was to describe clinicopathological features and immunohistochemical profile of ME and to discuss differential diagnosis. Patients & Methods: Between January 1991 and March 2007, 4 cases of cerebral medulloepithelioma were diagnosed at the pathology department of La Rabta Hospital. Medical record and microscopic slides were available in all cases and were retrospectively reviewed. Results: Our study included four male children aged between 4 and 13 years (mean age = 10,75 years). All patients presented with symptoms of increased intracranial pressure including headache vomiting and lethargy. CT scan showed an isodense or hypodense non-enhancing well-circumscribed tumour involving the temporoparietal lobe in two cases, the frontoparietal lobe in one case and the temporoparietooccipital lobe in one case. Gross-total resection of the tumour was performed in 3 cases and partial resection in one case. Pathological examination of the surgical specimen showed a tumoral proliferation made neural tube-like structures composed of primitive appearing cells that formed a pseudostratified epithelium arranged in tubular, papillary or trabecular configuration. The neural tube-like structures were outlined by a continuous, PAS-positive basement membrane. Mitotic figures were abundant and tended to be located near the luminal surface. Immunohistochemically, tumour cells were in all cases strongly positive for vimentin but were negative for PS100, NSE and GFAP. Postoperatively, 3 patients underwent adjuvant radiotherapy in the tumour bed. During the follow-up period which ranged between 1 and 3 years, one patient died.

PP4-224

RT-PCR: DIAGNOSIS VALUE IN DOGS WITH SPONTANEOUS ACUTE-, SUBACUTE-, AND CHRONIC-DEMYELINATING DISTEMPER ENCEPHALITIS

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Background: Distemper is an endemic disease, that is not restricted to carnivorous or to a single specie. Encephalitis due to canine distemper virus (CDV) has been associated with multiple sclerosis (MS) because of extensive demyelization in both and the postulated viral origin in MS. A domestic dog barely spends its life without contact with distemper virus (DV), mainly in developing countries; also, contact may occur through vaccination programs. Methods: Within three years, 45 dogs with signs of canine distemper (CD) were attended in Zoonosis Control Center of Taubaté, São Paulo, Brazil. Clinical data were recorded according to a protocol. The animals were euthanized, necropsied, photos were taken, and a brain sample was randomly selected for RT-PCR to CDV. In 7 healthy dogs (control group), submitted to euthanasia for different reasons, the same procedure was adopted. This study followed guidelines prescribed by the Brazilian Medical Research Centre and received approval from the Research Ethics Committee of the State University of Campinas. Results: in 31 out 45 dogs, a positive RT-PCR reaction was found in brain samples (group A); in the 14 remained dogs, negative results were obtained (group B); in all the 7 healthy dogs samples, positive PCR results were found (group C). On histological view, chronic-demyelinating encephalitis, typical of CD, was identified in group A animals and in 11/14 dogs in group B; 3/14 dogs in group B displayed no histological abnormalities. Focal lymphoid infiltrate was found in 5/7 animals and no abnormalities in 2/7 dogs in group C. Conclusion: For the endemic nature of CD and vaccination programs, positive results of RT-PCR do not allow the distinction of CDV from other diseases that share with it the same clinical manifestation. By the other side, false-negative RT-PCR could be the result of inadequate brain sample selection or be due to technical reasons. In this way, our findings stress the importance of clinical evaluation for proper CD diagnosis. The results may be useful for clinical research on CDV and sanitary programs of control of the disease.

PP4-225

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA IN IMMUNOCOMPETENT PATIENTS: RETROSPECTIVE ANALYSIS OF 15 CASES

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Background: Primary central nervous system lymphoma (PCNSL) is a rare disease. It represents approximately %3 of the central nervous tumors and is most often seen in inmmunodeficient patients. The incidence has increased in last two decades. In this study we aimed to review retrospectively PCNSL cases which were diagnosed in Ankara University, Faculty of Medicine, Department of Pathology with their clinical, responses to therapy regiments and survival. Material-method: Total 15 cases diagnosed in 2002-2006 and confirmed immunohistochemically were included into the study. The patients were analysed in relation to sex, age, time of the symptoms, procedures, treatments, survival and pathological features. Results: All of the patients (8 male, 7 female) were immunocompetant and the time of the initial symptoms to pathological diagnosis was in range between 1 month to 4 months. The mean age of the patients was 47.7 (range, 27-66 years). The most common symptom was hemiparesy (%80), headache (%46.6) and seizures (%26.6). The lesions were mostly located in cerebral lobes. Except 1 case which had operated, all of the cases were dignosed by steriotactic biopsy. All of the cases were diffuse large B-cell lymphoma, which were confirmed by immunohistochemistry. In one case clonality was showed by PCR. Two cases were not recieved any treatment because they rapidly proggressed to death after the diagnosis. Ten cases received combined treatment of chemotherapy and radiotherapy. The others were treated with radiotherapy or chemotherapy alone. Two cases were excluded from the survival analysis as the absence of the follow-up data. Six cases were died of the disease during the therapy in a period of ranging from 1 month to 24 months after the diagnosis. Five cases were still alive with a follow-up period ranging from 6 months to 48 months. Coclusion: PCNSL is very agressive tumor despite the combined therapy regiments. Steriotactic biopsy is very valuable diagnostic procedure as it is less invasive procedure, especially for deeply located lesions and it provides rapid diagnosis for this type of tumor which is treated non-surgical therapies. Besides molecular methods may be used for conforming the clonality of the tumor, especially for differential diagnosis.

Molecular Pathology

PP4-226
AN ALL-IRELAND CANCER BIOBANK AND INFORMATICS NETWORK FOR COLLABORATIVE TRANSLATIONAL RESEARCH

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INTRODUCTION: Translational research in cancer requires a large tissue sample volume and linked patient data. In Ireland, as in other European countries, cancer samples for research have not been collected with due quality control or stored consistently in accordance with standardised procedures. Insufficient sample aliquots have mitigated against the possibility of collaborative research. We therefore decided to develop an all-Ireland cancer biobank network with common informatics, to provide an infrastructure for collaborative translational research in the main hospitals of Ireland, north and south. METHODS: The Spanish Tumor Bank Network www.cnio.es was considered the most appropriate model for the Irish network, which includes two jurisdictions. The network concept was promoted among numerous stakeholder groups, including professional bodies, hospital management, medical staff, researchers, patient advocate groups, industry, and the public. Biobank Ireland Trust www.biobankireland.com was formed for public awareness and education, for fundraising, and to enable development of a restricted access online sample and informatics resource for potential research projects. No grants were sought, and no full-time personnel were assigned to the project. Disseminations included formal proposals and (international) presentations (ISBER 2005), and hosting and attending biobanking conferences. RESULTS: Progress has been slow and fitful! Informal networks of international biobanking experts were invaluable. Presentations, fundraising events, discussions with members of the public, and media coverage were all helpful. Also of significance was the inclusion in Ireland's national cancer strategy (2006) of an aspiration to develop a national cancer biobank infrastructure. Biobank Ireland Trust's business proposal forms the basis of the Irish network's proof of concept phase: the network will commence in two hospitals, following which it will be extended to 10 others. The estimated cost of the network, excluding capital costs, is €8-10m. over 6 years. COMMENT: A biobank network is more about people than science. Patient advocate groups and the public must be consulted at the outset. When biobanking becomes standard of care in pathology, a biobank network will improve cancer care directly and indirectly. The Irish network will be a template for research infrastructure in other human diseases, will facilitate collaborations with other countries and will enable provision of biobanking assistance to developing nations.

PP4-227
CYCLOOXIGENASE IN THE NORMAL HUMAN TISSUES – IS COX-1 REALLY A CONSTITUTIVE ISOFORM, AND COX-2 AN INDUCIBLE ISOFORM?

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BACKGROUND: Cyclooxygenase is a key enzyme in prostanoid synthesis. It exists in at least two isoforms: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Despite the fact that COX isoforms have been widely studied in various pathological conditions, the data on their distribution and role in the normal tissues is still controversial. It is generally accepted that COX-1 is constitutively expressed in normal tissues as a housekeeper enzyme maintaining tissue homeostasis, whereas COX-2 is normally not present in most tissues, but can be induced by various stimuli. Some studies, however, suggest that distribution and function of COX isoforms is more complex. We therefore analysed the distribution of COX isoforms in the normal human tissues. METHOD: Our study included autopsy samples of various organs and tissues from 10 healthy trauma victims. Immuno-histochemistry was performed by a peroxidase-streptavidin method on formalin fixed, paraffin-embedded tissue, using monoclonal antibodies against COX-1 and COX-2. In addition, western blot analysis of COX-2 protein expression was performed on frozen tissue samples of the brain, heart, kidney, liver, lung, thyroid, adrenal gland, testis, aorta and coronary artery. RESULTS: Using immunohistochemistry, we found COX-1 expression in blood vessels (in endothelial and smooth muscle cells) in all tested tissues, and in occasional inflammatory cells and macrophages, particularly in mucosal membranes. COX-2 was widely expressed in the parenchymal cells of endocrine glands (adrenal cortex, pituitary gland, thyroid, pancreas), testis, liver, central nervous system, kidney and in the glands of the gastric and colonic mucosa. COX-2 was focally expressed in the lung, and almost absent in the spleen and prostate. In the heart, occasional positive cardiomyocytes occurred with increasing age. In all organs and tissues, we observed COX-2 positivity in ganglion cells, occasional inflammatory cells and macrophages and focally in endothelial cells of blood vessels. With western blot analysis, we found detectable levels of COX-2 protein in all the tissues tested, presenting with bands of different intensities. CONCLUSION: Our results indicate that COX-2 is widely expressed in the parenchyma of many human organs and tissues, whereas COX-1 is predominantly found in blood vessels. Therefore, tissue distribution and role of COX isoforms under physiological conditions is clearly more complex than generally believed. We will probably have to revise the concept of "constitutive" and "inducible" COX isoforms.

PP4-228
ERK AND PERK EXPRESSION, B-RAF MUTATIONS AND MICROSATELLITE INSTABILITY IN COLORECTAL CANCER

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Background: The RAS/RAF/MEK/ERK signaling pathway plays a pivotal role in controlling cellular growth, differentiation and survival mediating cellular responses to growth signals. Activating B-Raf mutations which stimulate RAF/MEK/ERK kinase cascade resulting in phosphorylation and subsequent activation of ERK, have been associated with colon cancer exhibiting microsatellite instability (MSI) -an alternative pathway of carcinogenesis caused by defective mismatch repair (MMR) system. The aim of this study was to determine the B-Raf mutational status and to asses the immunohistochemical pattern of ERK and activated ERK (pERK) in relation to MSI and hMLH1 promoter hypermethylation, of patients with colon cancer so as to elicit any potential associations with prognostic value. Methods: B-Raf exon 11 and 15 mutations were investigated by single stranded conformation polymorphism (SSCP) and DNA sequencing in 94 patients diagnosed with colon cancer. Additionally, immunohistochemical expression of both total ERK and pERK was examined. The presence of MSI was determined by analysis of sensitive mononucleotide markers (BAT-25, BAT-26) and the methylation status of hMLHI promoter was assessed by methylation specific PCR. Results: Total ERK cytoplasmic or nuclear immuoreactivity was detected in 92.7% (51/55) and 85.4% (47/55) of the cases respectively. Nuclear pERK immunoreactivity was found in 71.7% (32/45) while 11 (24.4%) cases displayed cytoplasmic positivity. There

was no correlation between nuclear or cytoplasmic ERK LI and the presence of nuclear and/or cytoplasmic pERK immunoexpression. pERK nuclear immunoreactivity was positively correlated with tumor grade (p=0.0133) and Duke's stage (p=0.09), whereas the latter was of marginal significance. B-Raf mutations in exon 15 were found in 12 cases. The presence or the score of ERK and pERK immunoexpression, either nuclear or cytoplasmic was not correlated with B-Raf mutations, MSI status or hMLH1 promoter methylation. Conclusion: Our findings indicate that ERK and activated ERK (pERK) are frequently expressed in colorectal cancer, independently of B-Raf status. Moreover nuclear pERK expression correlated with tumor grade and Duke's stage, suggesting a possible further implication in tumor aggressiveness. The project is co-funded by the European Social Fund and National Resources-(EPEAEK II)-PYTHAGORAS II.

PP4-229

DOUBLE IMMUNOSTAINING METHOD. WHAT IS THE SIGNIFICANCE?

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Background.Immunostaining is a technique for identifying cellular or tissue antigen. Immunohistochemistry has been part of the routine technique of the pathology laboratory for over 20 years.These technique have significantly expanded the capabilities of the pathologist in diagnostic procedures.Immunohistochemical techniques were very sensitive and specific methods which utilize antigen-antibody complexes.Among the immunostaining methods the enzyme labelled streptavidin-biotin method is now widely used in routine testing. In study, immunohistochemical localization of α-Smooth Muscle Actin (α-SMA),Proliferative Cell Nuclear Antigen (PCNA) was performed using the streptavidin-biotin peroxidase (Str.ABC/HRP) and streptavidin-biotin alkaline phosphatase (StrABC/AP) immunostaining methods.The double immunostaining method described may have wide application since it can be used both for paraffin sections and cytology. It does not require specialized technique or apparatus. The aim of our study to compare the immunoexpression of PCNA and α-SMA in anti-Thy1.1 diffuse experimental glomerulonephritis rat model by Str.ABC/HRP,StrABC/AP and double (Str.ABC/HRP and Str.ABC/AP) immunohistochemistry methods (DIHC). Methods.Ten male Wistar Albino rats were divided into two groups of fifth: Group I Control(C): 5 rats treated with 0.1ml /100g normal saline intravenously (i.v) for four weeks; groupII glomerulonephritis (GN): 5 treated with anti-Thy1.1(0.25µg/100g) i.v at zero day. Kidneys from rats were collected as Instituional Animal Care and use Committee approved procedures.Representative tissue samples were stained with hematoxylin eosin (HE) and Gomori One Step Trichrome.In each case PCNA and α-SMA expression were semiquantatitively scored on glomeruli and tubulointerstitium by Str.ABC/HRP,Str.ABC/AP and double immunohistochemical methods staining slides. Results:At the end of the study period morphological changes including tubulointerstitial injury and glomerular cell proliferations were significantly increased in glomeulonephritis group compared to control group.With in immunohistochemical methods,glomerular immunoexpression of α-SMA was similar in two groups.Tubulointerstitial immunoexpression of α-SMA was similar in Str.ABC/AP and Str.ABC/HRP in GN compared to two groups,but different in DIHC.Immunoexpression of PCNA in all immunohistochemical methods on tubulointerstitial and glomeruli was significant increased in GN group compared to C. Conclusions.Double immunostaining was the method to detect two antigen in same site.That method was simple and reliable.

PP4-230

THE EFFECTS IN SKIN WOUND HEALING AND CELL PROLIFERATION OF THE PLASMID DNA ENCODING GM-CSF AND CHITOSAN

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Background: Granulocyte macrophage colony-stimulating factor (GM-CSF) regulates proliferation, differentiation and function of hematopoietic progenitor cells. Whereas a very important problem with the use of GM-CSF is that it has short biological t50 and it is necessary to administer frequent injections of high dose of GM-CSF, however this approach has been limited by the toxicity of this protein. Chitosan (CS), a natural cationic polysaccharide, has a high potential as a non-viral vector for gene delivery. Topical delivery of therapeutically relevant genes to wounded skin may help accelerate wound healing. The objective of this study was to investigate the encapsulation of hGM-CSF encoding gene into chitosan complex and in vitro transfection efficiency and therefore in vivo effects of GM-CSF complex application were identified in normally healing wounds. Method: pORF-hGM-CSF plasmid DNA was extracted by the alkaline lysis method.CS-pDNA complexes were prepared.In vitro transfection studies, the effect of the proliferative of the GM-CSF was determined by MTT test. We have used the assay to measure proliferative GM-CSF at the 24,48 and 72 hours. For skin topical application, Wistar rats were first anesthetized by ketamine, their backs shaved and 6 mm long punch biopsy was made at two individual sites on the dorsum of the back of each animal.Thereafter, 100 µg GM-CSF/CS complex (1:1,N/P) and chitosan per wound sites were topically applied.Skin samples were harvested 24,48,72 hours (acute term) and 1,2 weeks (chronic term) after application.Specimens were paraffin embedded, sectioned, stained using H&E and Gomori One Step Trichrome. Results: The better in vitro GM-CSF gene expression was found with 1:1 (N/P ratio). The dose of plasmid in complex is also important in protein expression. In vivo study, the neovascularization, epithelization, cellular content and vascularity increased in GM-CSF/CS complex group compared to chitosan group in acute term. In chronic term, the chronic inflammation, neovascularization, epithelization, cellular content, granulation tissue formation and vascularity increased in GM-CSF/CS complex group compared to chitosan group. In acute and chronic terms, the collagen deposition increased in chitosan group compared to GM-CSF group. Conclusion: According to our results, chitosan/DNA complex is thought to have ability to be a good potential in the treatment with GM-CSF gene so that it is to be a suitable delivery system for gene of chitosan, be applied from skin with chitosan of the gene and able to potential therapeutic effect to wound.

PP4-231
FASCIN AND MIB-1 EXPRESSION IN SEROUS OVARIAN TUMORS
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Background: Fascin-1 is an actin-bundling protein that contributes to the formation of actin-based structures, including cellular surface protrusions that mediate cell movement. Studies in carcinomas suggest the emergence of fascin as a new prognostic indicator. Rare reports concerning fascin expression in ovarian carcinoma have not confirmed a clear-cut relation between immunostaining and established markers considered to reflect biological aggression. The aim of our study was to examine a group of serous ovarian tumors for possible relationships of fascin expression. The proliferative activity was also examined, since some studies suggested an inverse relationship between Ki-67 and fascin. Method: Fascin immunostaining was assessed in 70 specimens of serous ovarian tumors, with complete surgical staging when indicated, (45 carcinomas, 9 borderline tumors and 16 cystadenomas), retrieved from the files of the Pathology Department, University Hospital of Thessaly. Immunostaining was performed using two antibodies, IM20 (Novocastra, U.K.) and 55k-2 (Cellmarque, U.S.A.). Extent and intensity of immunoreactivity were semiquantitavely evaluated. Double staining for fascin and Ki67 was performed in carcinomas using the double Envision kit (Dako, Denmark) and MIB-1 antibody (DakoCytomation, Denmark). Western Blotting was performed on proteins isolated from tumor tissue kept at -80oC. Results: Fascin score was highest in carcinomas. Cystadenomas showed a score lower from carcinomas and borderline tumors ($p<0.0001$). Increased values (mean 4.70 vs 2) were observed in carcinomas of advanced stage (III/IV vs I) ($p=0.04$).Immunoreactivity was increased in metastases in comparison to primary tumors. Positivity was also found in the stroma of carcinomas, often intensified in the immediate vicinity. An interesting association between fascin and MIB-1 immunoreactivity was observed, the latter being lower in tumors showing increased fascin score. Conclusion: Our study showed that increased fascin immunoreactivity in serous ovarian carcinomas is associated with certain features of increased tumor aggressiveness. Increased score in specimens from metastatic sites suggests that fascin may be an important factor in ovarian carcinoma metastasis, as expected from its role in cell motility. We have also showed that cycling cells (MIB-1+) show lower fascin scores. Therefore, the actually invading cells, i.e. the "invasion fraction", may differ from the "growth fraction" of a given tumor. Future studies could determine if fascin may become a helpful marker in gynecological pathology.

PP4-232
SYNDECAN-1 AND VLA-4 INTEGRIN MOLECULES MAY CO-OPERATE IN MYELOMA CELLS UPON STIMULATION
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Background CD138 (syndecan-1) expression is a distinctive feature of plasma/myeloma cells in the bone marrow (BM). The integrin VLA-4 (α4β1, CD49d/CD29)is the most common integrin on multiple myeloma (MM) cells. Both molecules are involved in adhesion of MM cells to matrix proteins and possibly in selective localization of multiple myeloma to the BM. Adhesion induces pro-survival signaling cascades, increases proliferation, promotes drug resistance and thus may contribute to tumor progression, although extramedullary spread of MM is believed to be associated with loss of cell adhesive properties. Adhesion of MM cells is substantially increased in vitro following stimulation by cytokines, mainly HGF,IGF-1 and SDF-1α. Existing data on CD138 and VLA-4 on MM cells with regard to clinical stage/progression are controversial, but soluble syndecan has been proved to be a strong negative prognostic factor. Possible interaction/cross-talk of integrins and syndecans is widely discussed; intriguing data have been demonstrated for carcinoma cells, but little evidence exists in MM so far. The aim was to study the expression and localization of syndecan-1 and VLA-4 on MM cells. Methods and Results 62 BM trephine samples from MM patients (9 pts in Durie stage I, 24 – st II and 29 – st III) were screened for CD138 and CD29 immunoreactivity. There was no significant difference between the groups. Precise localization of syndecan-1 and α4-integrins in the membrane of MM cells was studied by fluorescence laser scanning confocal microscopy (LSM-510, Zeiss, Germany) in INA-6 cells (MM cell line) following adhesion to fibronectin with or without stimulation with HGF, IGF-1 and SDF-1α. Scans were taken at the interface. In unstimulated cells α4 signal appeared as a central spot at the adhesion surface, surrounded by a ring-like CD138 signal, with clear separation of both signals in the majority of cells. Upon stimulation the signals from α4 and CD138 got co-localized in the majority of the cells in a ring-formed pattern, α4 moving laterally and merging with CD138-positive area. The same tendency was found in a primary MM cell sample. Conclusion This study is, to our knowledge, the first to indicate a possible co-operation between VLA-4 and syndecan-1 in MM cells. This involves lateral movement of VLA-4 molecules leading to co-localization of syndecan-1 and VLA-4 signals. Total syndecan-1 and VLA-4 integrin expression on MM cells has not been related to clinical stage in our setting. Further investigation is needed to elucidate possible role of the phenomenon in tumor progression.

PP4-233
ACTIVATED ERK EXPRESSION IN RELATION WITH B-RAF GENE MUTATIONS IN UROTHELIAL BLADDER CARCINOMA
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Background: Ras/RAF/MEK/ERK signaling pathway is commonly activated in human cancers. However the effect of ERK signaling on the prognosis of primary urinary bladder cancer (UC) is not clearly understood. Activating mutations in the serine/threonine kinase B-Raf are observed in several tumor types such as melanomas and thyroid, colorectal and ovarian carcinomas. The aim of this study was to examine the prognostic significance of phosphorylated ERK1/2 expression as a hallmark of ERK activation in relation with the presence of B-Raf somatic point mutations in Greek patients with primary UC. Methods: Tumor samples from 132 patients with primary urinary bladder cancer were examined for pERK1/2 immunohistochemical

expression. PCR-SSCP and sequencing analysis were performed in 90 cases in order to detect activating mutations in exon 15 of the B-Raf gene. Statistical package STATA 9.0 for Windows was used for all statistical calculations. All results with a two-sided p ≤ 0.05 were considered statistically significant. Results: Nuclear immunoreactivity for pERK was detected in 99.2% (131/132) of cases, whereas cytoplasmic immunoreactivity in 97.7% of cases (129/132). The pERK nuclear expression was marginally related to tumor's histological grade and stage (1/2 vs 3, Fisher's exact test, p=0.082 and Ta vs T1/T2/T3 Fisher's exact test, p=0.084). In univariate analysis for invasive carcinomas higher histological grade (p=0.0003), advanced tumor stage (p<0.0001) and high nuclear pERK expression (p=0.039) were associated with worse prognosis. However, when analysis was restricted in muscle-invasive carcinomas, advanced stage (p=0.001) emerged as the only factor adversely associated with survival. Multivariate analysis in invasive as well as in muscle-invasive carcinomas selected only tumor stage as significant prognostic factor (p=0.024 and p=0.039 respectively). Mutations in exon 15 of B-Raf gene were not detected in the examined cases. Conclusion: ERK activation as well as B-Raf mutations are frequently detected in certain tumor types implying their importance as potential targets for anticancer treatment. Our findings suggest that pERK expression is common and occurs in a B-Raf-independent manner in primary urinary bladder cancer. The correlation of pERK expression with high histological grade and advanced tumor stage suggests a potential implication in tumor aggressiveness while elevated nuclear pERK expression seems to be an adverse prognostic factor, although not independent of classical clinicopathological prognosticators.

PP4-234
OVEREXPRESSION OF GLUTATHIONE SYNTHESIZING ENZYMES IN A MOUSE MODEL FOR DRUG-INDUCED STEATOHEPATITIS

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Background: Mallory bodies (MBs) are a characterictic, cytoplasmic inclusion found in hepatocytes in alcoholic steatohepatitis and other chronic non-alcoholic liver diseases. Oxidative stress participates in the pathogenesis of such disorders as an imbalance in cellular oxidant/antioxidant homoeostasis can influence inflammation, fibrosis and apoptosis. Glutathione is the major antioxidant in hepatocytes that serves several biological functions and alterations in the glutathione level induced by toxic factors may promote the development of chronic liver disease. Our aim was to study the glutathione metabolizing pathway in an animal model that shows features of drug-induced steatohepatitis. Methods: Livers of 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC)-intoxicated swiss albino mice were harvested after 1 week DDC-treatment, 2.5 months DDC-treatment, 1 month DDC-free diet (=recovery) and 3 days DDC-refeeding. Total RNA was extracted and enzymes involved in glutathione metabolism were analyzed by Real-time quantitative RT-PCR. Results: Real-time PCR data revealed a deregulation of glutamate-cysteine ligase (GCL) and GSH synthetase (GSHS) in DDC-exposed murine livers. Compared to normal mouse liver, the enzymes GCL and GSHS were both overexpressed after 1 week (1.7 and 2.6 fold) and 2.5 months DDC intoxication (1.9 and 3.1 fold), respectively, but returned to a normal level after administration of a DDC-free diet for 1 month after 2.5 months of DDC intoxication (recovery). Refeeding recovered mice a DDC-containing diet for 3 days after one month of DDC-free diet induced GCL (1.6 fold) and GSHS (3.4 fold). Conclusion: Our data suggest an activation of the glutathione synthesis pathway in liver exposed to DDC intoxication. The increased expression of GCL and GSHS may be in part a compensatory effect for a depletion of homocysteine/cysteine in the affected liver, as these metabolites are precursors in the glutathione synthesis and alterations of these metabolites are found in human ASH. Otherwise since oxidative stress is an important factor in the development of steatohepatitis, overexpression may be an indicator for increased oxidative stress defence in drug-affected hepatocytes.

PP4-235
MOLECULAR AND IMMUNOHISTOCHEMICAL ANALYSIS OF P53 MUTATIONS IN BREAST CARCINOMAS

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Background.: Alterations of the p53 tumor suppressor gene are probably the most common genetic abnormalities in human malignancies. The majority of p53 mutations are missense and occur in the conserved DNA-binding domain (exons 5-8). In all, about 1150 different point mutations have been described, with more than 95% affecting exons 5-8. These mutations may lead to loss of DNA binding that is believed to be critical for the biological activity of p53. In breast cancer, frequency of p53 mutation is reported about 20%, lower than in other solid tumors. The implications for breast carcinogenesis however are unclear; in several studies it has been associated with more aggressive disease and worse overall survival. As direct sequencing of the gene is a time-consuming and laborious procedure, several prescreening methods have been developed. A modification of Denaturing Gradient Gel Electrophoresis (DGGE) known as GC-clamped DGGE appears to be more efficient and sensitive. Methods: DNA was extracted from 60 breast carcinomas of various histopathology and corresponding adjacent normal tissues. We used a PCR and GC-clamped DGGE assay, followed by sequencing, to investigate the presence of mutations within exons 5-8 of p53 gene. Additionally, we looked for p53 protein accumulation by immunostaining. Results: Out of 60 cases analyzed, 12 (20%) seemed to be carriers of a p53 aberration (7 in exon6, 2 in exon7, 2 in exon8 and 1 in exon5). The vast majority of mutations were documented in invasive ductal type-high grade tumors. In all (but one) cases that produced a variant DGGE-pattern, gene alterations were identified by sequencing and validated in TP53 Mutation Databases. Of these, two mutations: a missense at g.13418T>A (p.Y220N) and a deletion at g.13270delG have not been previously reported in breast cancer. The presence of mutations revealed strong correlation with high-grade breast carcinomas (p=0.002). Positive immunostaining of p53 protein was observed more frequently in invasive breast carcinomas (51%) than in in situ component (27%), with statistical significance (p=0.043). P53 overexression was associated with the presence of mutations in p53 gene (p=0.004). Conclusion: Our data indicate that PCR-DGGE is an efficient and highly sensitive prescreening approach for p53 mutation detection in DNA samples of breast cancer patients. The presence of mutations and p53 protein accumulation in invasive carcinomas, as well as the significant correlation between mutations and high tumor grade, reveal their possible implication in advanced stages of breast carcinogenesis.

PP4-236
Withdrawn

PP4-237
HETEROTOPIC MESENTERIC OSSIFICATION: ULTRASTRUCTURAL FEATURES OF THREE CASES AND REVIEW OF THE LITERATURE

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Background: Heterotopic ossification is a reactive metaplastic bone-producing process, which occurs in soft tissues mainly as a consequence of trauma. This reactive metaplastic process is well known in somatic tissues and named "myositis ossificans". The histological finding of mature bone-tissue within the mesentery of the gastrointestinal tract is very uncommon and to our knowledge, less than 25 cases have been described in the literature. This kind of lesion may represent the soft-tissue counterpart of myositis ossificans and therefore, it has been designated under the term "heterotopic mesenteric ossification" (HMO). Purpose of the present paper is to describe the ultrastructural features of three cases. Method: Three patients were studied; they were male, aged 25, 48 and 58 years, respectively. All of them had a previous history of abdominal trauma and presented with intestinal obstruction. Results: Macroscopically, multiple, firm, greyish-white lesions were observed in the mesentery of small bowel in all cases and in one also in the omentum. Histologically the lesions were composed of steatonecrosis and haemorrhage surrounded by myo-fibroblastic proliferation, with spindle shaped cells, intermixed with an acute and chronic inflammatory infiltrate. At the periphery of these areas, eosinophilic material resembling osteoid was present. The "zone phenomenon" described in the myositis ossificans was observed. The myo-fibroblastic cells and osteoblasts were mitotically active, while they lacked atypia and atypical mitotic figures were absent. At ultrastructure, elongated cells immersed in the osteoid material were rich in rough endoplasmatic reticulum, contained vesicles and filaments and showed a well developed Golgi apparatus. The eccentric nuclei showed finely distributed chromatin with occasional prominent nucleoli. All these features are consistent with a secretory-like cell pattern and an active protein synthesis. Conclusion: The precise pathogenesis of HMO has still not yet been determined. Most probably it is related to metaplasia of mesenchymal stem cells, as a consequence of an exuberant reaction to trauma or injury, in predisposed individuals. The ultrastructural features observed here further support the hypothesis of stem cells actively producing osteoid material.

PP4-238
EWING SARCOMAS AND PRIMITIVE NEUROECTODERMAL TUMORS SEEN THROUGH THE PRISM OF EQUITY

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Background: It is now well known that these two entities are in fact two faces of the same malignant tumor designated ES/PNET. Not so long ago there were slightly different treatment protocols for the two entities, giving rise to debates among clinicians and pathologists about "give me the precise diagnosis". Methods: We analyzed 33 biopsy/operative archive specimens admitted at our institution from the Clinic for Orthopaedic Surgery, Skopje. All tissue samples had been fixed in 10% neutral formaline and paraffin embedded (FFPE), prior to sectioning and staining with H.E. and monoclonal antibodies. Twenty three cases were diagnosed as ES and 10 as PNET, according to the microscopic morphology and immunohistochemical staining pattern for the following markers: Chromogranin, NSE, Synaptophysin, S-100 and CD99. We performed additional immunohistochemical stainings for p53, Bcl-2 and Ki-67. All of the immunohistochemical stainings were performed using the DAKO Cytomation EnVision+HRP system. We also isolated DNA from FFPE for subsequent identification of EWS/FLI1 fusion [t(11;22)(q24;12)]. For EWS/FLI1 gene fusion identification we used Hot start–Touch down-direct PCR on Auto Q server-thermal cycler. Primer sequences were constructed using Gene Runner v.3.05 on reference sequences obtained from Ensemble Database ver. 41.36c with start:pos.11/ex7EWS; end: pos.478/ ex6 FLI1. Results: 61% of the patiens with ES were males, versus 60% with PNET ($p>0,05$). The mean age of the patients with ES was 19,5 years (min. 7, max. 55, SD=12,33), versus 20,3 (min. 4, max. 60, SD= 17,51) in PNET ($p>0,05$). As for the immunohistochemistry, we encountered slightly more frequent (but not significantly) positivity of PNET cases for p53 (weak 1+ staining signal) – 30%, versus 13% of ES cases ($p>0,05$). In PNET there were only 1+ intensity signals detected for Bcl-2 (33% cases), versus 38% of ES. More intensive expression (2+, 3+) was found exclusively in ES cases (9,5% and 4,8% respectively), however, with statisticaly non significant difference ($p>0,05$). Ki-67 showed similar expression frequency in both ES (13%) and PNET (10%) with 1+ intensity ($p>0,05$). EWS/FLI1 gene fusion was detected in 43,4% ES, compared 30% of PNET cases ($p>0,05$). Conclusion: Except for the potentially differential staining patterns of ES and PNET for NSE, Synaptophysin and Chromogranin, we found no other significant differences between ES and PNET neither for immunohistochemical expression of p53, Bcl-2 and Ki-67, nor for the presence of the EWS/FLI1 gene fusion.

PP4-239
A BRIEF REPORT OF SYNOVIAL SARCOMA CASES IN HACETTEPE UNIVERSITY: A MORPHOLOGICAL ANALYSIS

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Background: Synovial sarcoma (SS) is a morphologically, clinically and genetically distinct entity. Unlike its name, it does not arise from or differentiate toward synovium and is issued under the name Tumours of Uncertain Differentiation in the recent WHO classification. SS is a mesenchymal spindle cell tumour with variable epithelial differentiation including glandular formation and has a specific chromosomal translocation t(X;18)(p11;q11). Method: This study aimed at re-evaluation of the SS cases in our institution's pathology archive between 1990-2007 to create a brief idea about histopathologic features of SS and its relationship to prognosis. Results: From the 18 cases reported as malign mesenchymal tumor, some of which only favoring SS diagnosis, 15 were confirmed. More than half of the cases were male (8); the mean age at diagnosis was 32 years and there was a male predominance in the younger age group. Eight cases were consultation so macroscopic examination was available in 7 cases. Among those cases, the mean diameter of tumor varied between 2-10 cm. The most common locations were foot (3) and inguinal region (3), followed by upper extremity and head-neck region by 2 cases each, one case was located at the knee, the last 3 cases abdominal and lumbal. Excisional biopsy could be performed in 5 of the 6 cases operated in our institution and in none of the 5 cases in which lymph node dissection was done, metastasis was detected. Microscopically, 7 of 15 cases were biphasic, one consultation case was a monophasic ossifying SS located in heel. Tumor necrosis was present in 3 cases. An

immunohistochemical panel composed of at least EMA and pancytokeratin was performed in all cases. At least focal positivity with one of these markers were used in addition to morphologic features. Mitotic count in 10 high power field ranged between 2-43; 3 cases with counts more than 10 / 10 HPF. Five patients received their adjuvant therapy in our institution, 2 patients received chemotherapy. Three patients received chemotherapy and radiotherapy, one due to lung metastasis 2 years after the first diagnosis and all died due to disease. Seven cases are alive without disease and the mean follow up period is 11 months. Conclusion: In the literature up to 50% of SS recur, usually within 2 years, nearly 40% metastasize, commonly to lungs and bone. Tumor free local excision followed by postoperative radiotherapy improves the survival. Young age, tumors <5 cm in diameter, with <10 mitoses / 10 HPF, SS18/SSX2 variant gene and no necrosis are good prognostic factors.

PP4-240

OSTEOSARCOMA: REVIEW OF 107 CASES

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BACKGROUND: Osteosarcoma (OS) is the most common bone tumor of childhood and young adulthood. Despite new therapy modalities and therapy agents, prognosis is poor. Epidemiologic and genetic researches are the most promising studies to understand the nature of this tumor. METHOD: We reviewed 107 cases diagnosed in our department between 2000- 2007 according to the latest WHO classification; and analyzed them considering age, sex, etiology, localization, histological parameters (necrosis rates), recurrence, metastasis, and response to therapy. RESULTS: The patients were between 7-73 years; and the median age was 28. There was male predominance (59%). Knee localization was seen in 57% of the cases, and most of them (49%) were in the 3rd decade. After 3rd decade knee localization decreased gradually and from the 5th decade of life OSs were seen at the trunk and extremity parts except knee. Conventional and blastic OSs were the most common types (77%), and 23% of the cases were malignant fibrous histiocytoma like OSs. Metastasis and recurrence were mostly seen in males. Ninty-one percent of the patients had Huvos grade III-IV tumor necrosis. Metastasis occured in 15% of the patients and 10% of them recurred. Sixty-six percent of the recurred patients and 87% of the metastatic cases had Huvos grade I-II tumor necrosis. Epiphysis invasion was seen in 94% of the cases; but in 91% of them articular space was intact. There were 3 postradiotherapy OSs and an OS associated with retinoblastoma in our material. CONCLUSION: Age, sex and localization distribution of the cases are the same as the English literature. But the percentage of knee localization is fewer and upper extremity localization is slightly more in our review. Response to chemoradiotherapy is the most important factor that effects the metastasis and recurrence rates. Neoadjuvant chemoradiotherapy and surgery are the gold standarts of dealing with OS.

PP4-241

DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF AGNOR IN OSTEOSARCOMA, CHONDROSARCOMA, EWING SARCOMA AND GIANT CELL TUMOUR OF THE BONE

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Background: AgNOR method which is based on determination of nucleoli regulator regions (NORs) by silver staining is one of the cell proliferation markers and is useful for identification of prognosis as well as differentiation of benign and malign tumours. There are great advances on bone tumour differential diagnosis and establishment of prognostic parameters. Yet, the reason of different responses to chemotherapy at osteosarcoma (OS) and Ewing Sarcoma (ES) cases, sometimes non-existence of objective criteria to determine histological malignity degree at chondorsarcoma (CS), the reason of the aggressive behaviour of some bone giant cell tumours (BGCT) are certain questions waiting to be replied. Methods: In this study, 33 OS, 12 CS, 10 ES and 15 BGCT cases diagnosed at Ege University Medical Faculty Pathology Department are evaluated. Besides, 5 non-Hodgkin lymphoma cases are included for comparison with ES. Cytological preparations prepared by imprint method from tru-cut biopsies are fixed in ethyl alcohol. Silver staining is performed and slides are examined by light microscopy. AgNOR spots at 100 cell nuclei are counted and mean AgNOR value is calculated. Results: While OS cases have the highest AgNOR values, CS cases are on the second and ES are on the third rank. The lowest values belong to BGCT and AgNOR counts differ significantly among tumour groups. In addition, this method is helpful for differentiating ES and non-Hodgkin lymphoma or BGCT and OS. The histological grade of CS and AgNOR values have correlation since low grade tumours show less while high grade tumours show more AgNOR values. At OS cases where response to chemotherapy is higher than 90%, AgNOR values are also higher. Conclusion: AgNOR method has a limited value on differentiation of malign bone tumours as OS, CS, and ES. Although it is helpful for identification of response to chemotherapy at OS and prediction of metastasis at CS, such findings are not observed at ES. AgNOR seems to be useful for differentiating ES and lymphoma or BGCT and telangiectatic OS. At CS cases, it may be helpful for differentiation of low and high grade tumours.

PP4-242

EXPRESION OF p53 AND Ki 67 IN ATYPICAL MALIGNANT CHONDROBLASTOMA

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Chondroblastoma is a rare primary, usually benign bone tumor, but aggressive behavior is described. During the 20 years we report from Register of bone tumors of the Institute of pathology Belgrade 5 cases with unexpected clinical and histological characteristics which pointed at atypical chondroblastoma. Four patients were female and one was male, the youngest was 17 , the oldest was 50. The middle ages were older compared to the patients with typical chondroblastoma. Clinical features were: pain, swelling and restriction of movement. The history of the disease varied from 3 months to 4 years. Location were :2 humerus , femur, tibia and coxa. Radiological studies showed lytic, destructive and expansive lesion. In 3 patients clinically was presented recurrent tumor, after surgical therapy. Biopsy

tissue showed: fields with polyhedral tumor and giant cells, cartilaginous interstitial matrix, “chicken wire” calcification and cystic spaces with blood. But also increased cell and nuclear atypia, hyperchromasia, pleomorphism and abnormal mitotic figures pointed to aggressive chondroblastoma. Immunohistochemical studies revealed p 53 mutation (in 3 from 5 cases) and extensive proliferate activity KI67 positive in more than 75% of cells (in 4 of 5 cases). Surgical therapy was applied. Two years later one patient with p53 positive, and KI67 positive tumor have radiological confirmed lesion to the lungs suspected to metastatic deposits. Other patients are with no evidence of recurrent or metastatic lesions. These findings suggest that expression of p53 and KI67 are not in the correlation with biological behavior of aggressive chondroblastoma.

PP4-243
EXPRESION OF KI 67 IN MALIGNANT TRANSFORMATION OF CHRONIC PROLIFERATE FIBROBLASTIC PERIOSTAL LESIONS

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On the Register of bone biopsy, Institute of Pathology, Belgrade was registered 2 cases of malignant transformation of chronic proliferate fibroblastic periostal lesions. In our opinion this lesions should be analyzed as separate entities in bone tumors pathology. Both patients were males. First patient, 24 years old BM had a slow growing tumor in proximal part of tibia with 5 years long anamnesis. After the first biopsy lesion was described as a proliferate, posttraumatic fibroblastic lesion of periost. Patient had 6 relapses durind the 13 years until the final diagnosis- osteosarcoma. Second patient, ST was 34 years old when was performed the first biopsy of femoral lesion after trauma. In the period of 9 years he had 3 relapses and repeted byopsis. The final diagnosis was again osteosarcoma (low grade superficial). This cases have in common a few elements: sex of patients, long evolution of lesions (13, and 9 years), trauma in anamnesis, many relapses (6 and 3), primary diagnosis of proliferate periostal lesions and finally, lesions were firstly clinically manifested when patients were young adults (period of life when osteosarcoma is the most frequent). Radiography, CT scan, MR, scintigraphy and histopathology characteristics of lesions firstly pointed to fibroblastic periostal proliferate lesions with expressive osteoid production up to mature bone. After many relapses and repeated biopsies with identical morphological findings- no malignancy but intensive proliferation of periostal fiber tissue and production of mature bone arise transformation and lesions become low grade malignant tumors (low grade malignant osteosarcoma). Proliferate potential of this lesions was investigated by Ki67. Expression of Ki67 were les intensive in first biopsy and it become intensive in relapses, finale it was the most intensive in the last biopsy when was made diagnosis osteosarcoma. We conclude that increase of expression's intensity of Ki 67 prove to malignant transformations of posttraumatic proliferate fibroblastic periostal lesions.

PP4-244
MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 23 CASES OF DERMATOFIBROSARCOMA PROTUBERANS

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Aims: The dermatofibrosarcoma protuberans (DFSP) is a malignant soft tissue tumour, characterized by frequent recurrences and rarely metastases. The tumour occurs most commonly on the trunk, including chest, back, and abdominal wall. Less commonly, the neoplasm is located on the proximal extremities; it rarely involves the distal extremities. The head and neck, especially the scalp, are also commonly involved. The vulva and parotid gland are unusual sites of involvement. The tumour appears between 25 and 75 years old. DFSP presents under several morphological variants: fibrosarcomatous, myxoid, with giant cells, pigmented, myofibroblastic. This study aims, through the analysis of 23 cases of DFSP observed during 5 years, to specify the prognostic factors of these tumours. Methods: 23 cases of DFSP are viewed retrospectively. We made sections of paraffin blocks with hematoxylin-eosin and special histochemical stainings: P.A.S., Blue Alcian, Fontana, Trichrome of Masson. We also performed an immunohistochemical study using: CD 34, CD117, C Kit, S100 Protein, Factor VIII, CD 68. Results and conclusions: DFSP has a significant risk of local recurrence that depends on the local excision. A much higher recurrence rate is reported in tumours treated by superficial or incomplete excisions only. Local reccurence usually develops within three years after initial surgery. The global analysis reveals that the type of the variant, the cellular atypia, the mitotic index and necrosis are microscopic predictive factors for the evolution of the DFSP.

PP4-245
RHABDOMYOSARCOMA: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ASPECTS

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Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of the childhood and adolescent periods and can seldomly be seen in adults too. Despite all research, a single prognostic classification for modelling the treatment is still needed. The clinicopathological features of our recorded rhabdomyosarcoma cases are reviewed and the major prognostic factors are discussed. Materials and method: We present 12 RMS cases located in chest wall, back, flank, abdominal wall, upper and lower extremities. Our patients were between 27 and 45 years old. We made sections on paraffin blocks with hematoxylin-eosin and special histochemical stainings: P.A.S., Van Gieson, Gomori. We also performed immunohistochemical reactions. Results and conclusions: Microscopically, we identified these RMS variants: embryonal, alveolar and pleomorphic. Desmin, Actin and Myogenin positivity of different intensities were observed in all cases. All the cases were treated by chemotherapy and radiotherapy whenever was needed. According to the clinical follow-up, the evolution was unfavorable when the tumour had more than 5 cm, when was located in upper and lower extremities, when was an alveolar or pleomorphic type and when had local recurrences.

PP4-246
MULTIPLE GLOMUS TUMORS OF THE ANKLE WITH PROMINENT INTRANUCLEAR PSEUDOINCLUSIONS

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Glomus tumor is a neoplasm, composed of the modified smooth muscle cells of the glomus body. We report a case of multiple glomus tumors of the ankle which show varying histologic types: solid type (glomus tumor proper) and angiomatous type (glomangioma). Interestingly, the tumor cells of the present case show prominent intranuclear inclusions, a finding hitherto unreported in glomus tumors. Ultrastructural examination demonstrated that the nuclear inclusions are not true inclusion bodies but intranuclear cytoplasmic pseudoinclusions made by the cytoplasmic invaginations due to the deep and complex nuclear contours.

PP4-247
COMBINED STUDIES CONCERNING IDENTIFICATION OF HIGH STRESS ZONES AT THE ACETABULAR BONE-BIPOLAR PROSTHESIS INTERFACE

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Background The study is intending to determine the stress and strain state in the acetabular bone (AB) at the bone-bipolar prosthesis cup interface using combined numerical and experimental methods. Method AB stress state was analysed with the finite element method (FEM) and the photoelasticity on the plane models. For FEM, two calculus models were developed considering a frontal section (FP) and a sagital one (SP) through the upper part of a bipolar prosthesis and the surrounding zone of the AB. Numerical simulations were performed for both one and two legs standing positions (OLSP and TLSP) for both models. Main stress and equivalent stress fields distribution in the AB were obtained after processing of calculus results and the corresponding curves were plotted using a special post-processing program. With photoelasticity technique the isochromatic fringes field in the AB for the OLSP was recorded in a circular polariscope. Photoelastic data were processed and the distribution curve of the main stresses difference (б1–б2) on the AB surface was plotted. Results In OLSP, in FP, pmax was 25MPa and pressure area (PA) was situated on the upper acetabular wall (UAW), near the acetabular rim (AR) with values decreasing towards the medial plane. In SP, maximal pressure (pmax) is much smaller (8MPa) and PA was situated towards the anterior aspect of the UAW with a more uniform distribution of the values. The resulting PA is ovoid, with the greater diameter in the sagital plane and shifted towards the anterior aspect of the UAW. The pressure values have a decreasing trend from lateral to medial. In TLSP, overall pressure values were much smaller that those of previous condition. In FP, pmax was 4MPa and the PA was restrained to a small area in the close proximity of AR. In SP, PA is larger than in FP, placed on both sides of median line and with values decreasing from posterior (3MPa) to anterior (1,5MPa). The resulting PA is like an antero-posterior narrow strip, near the AR with decreasing values from posterior to anterior. The results of photoelastic investigations indicated the same area with high level on the contact surface as numerical simulations. Conclusion The experimental study revealed that PA is placed on UAW near the AR and is varying both in shape and dimentions (decreasing in TLSP) and loading level (decreasing in TLSP compared with OLSP, from lateral to medial and from posterior to anterior).

PP4-248
MICROARRAY-COMPARATIVE GENOMIC HYBRIDIZATION OF ALVEOLAR SOFT PART SARCOMA. -GENE COPY NUMBER PROFILING AND IDENTIFICATION OF CANDIDATE GENES-

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Background: Alveolar soft part sarcoma (ASPS) is a rare, histologically distinctive soft tissue sarcoma of unknown origin. Although ASPS is characterized by a specific alteration, der(17)t(X;17)(p11;q25), the entire spectrum of genetic events underlying the pathogenesis of ASPS is unclear. Method: Using microarray-based comparative genomic hybridization (aCGH), we examined DNA copy number changes of ASPS. aCGH composed of 4030 clones was performed with two cases of fresh frozen tumor tissue from 29 year-old male and 16 year-old female. Results: We identified 30 chromosome regions that were imbalanced in both tumors, and 11 of those regions were located on chromosome Xp (Xp11.3-p11.4, Xp21.1, Xp21.3, Xp22.11-p22.13, and Xp22.31-p22.33) Additional regions with increased copy number were observed at 1q25.1, 12p12.1 and 17p11.2. Loss was found on only one region, chromose 22q11.23. Several genes located within the amplified region of Xp included UTX, RPS6KA3, ZNF645, PHEX, USP11, PCTK1, UBE1, KAL1, ARSE, ARAF, SYN1, TIMP1, GYG2, BMX, ACE2, CXorf20, TMEM27, CA5BL, PDK3, PCYT1B, TMSB4X and XK genes. Conclusions: Our study showed genomic regions and new candidate genes that were associated with tumor pathogenesis of ASPS.

PP4-249
AN UNINVESTIGATED CASE OF A MEDIASTINAL RHABDOMYOSARCOMA WITH MASSIVE PERICARDIAL EXTENSION

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Background: Primary mediastinal rhabdomyosarcomas are extremely rare. The tumors can occur in adults and children, and follow a very aggressive clinical course. Method: A 27-year-old female, originated from an isolated ethnic region, was admitted in ER presenting cough, chest pain, and respiratory distress, superior and inferior vena cava syndromes that aggravated in the past 3 weeks. A CT scan of chest revealed a giant anterosuperior mediastinal mass with extension in the middle compartment, somewhat not well-defined borders, and inhomogeneous tissue structure. The tumor had a mass effect on the vessels at the basis of the heart with tracheal compression, left superior lobar bronchi obstruction, and pleural effusion in the left costodiafragmatic sinus. The clinical examination revealed a blood pressure of 80/ 40 mmHg, AV=126/min, turgescent jugular veins, bilateral lower limb edema, a dull sound at the percussion of the left hemithorax. Given the resectable appearance on CT surgical resection was undertaken, but the patient suddenly died of cardiac failure. Results: The autopsy of the corpse was made. The examination showed a giant anterosuperior mediastinal tumor with extension in the middle compartment, the compression of the large vessels at the heart basis, trachea and left lobar superior bronchi. The tumoral mass encircled the entire heart, which circumferential infiltrated the pericardium as numerous white nodules. Another findings: left superior lobe pneumonia, parapneumonial sero-fibrinous pleurisy, liver and renal stasis. Classical histological method and immunohistochemistry were used for pathological

evaluation. Embryonal rhabdomyosarcoma composed in a great percentage of primitive cells devoid of rhabdomyoblastic differentiation, disposed in a very loose connecting stroma and rare cells with rhabdomyoblastic differentiation . IHC showed intensely positive reaction for vimentin, actin and weak positiving for desmin. Cytokeratin markers, CD20, CD34, CD68, CD117 and S100 are negative. Conclusion: Primary mediastinal rhabdomyosarcomas are extremely rare. In our 3 years old experience in collaborating with the Cardiothoracic Surgery Department this was the only soft tissue tumor from 16 mediastinal tumors. Since these tumors can remain clinically silent, they may have already reached large dimensions by the time of diagnosis. This is especially true for those masses originating from the chest wall.

PP4-250
DESMOPLASTIC SMALL ROUND CELL TUMOR-PROBLEMS OF DIFFERENTIAL DIAGNOSIS IN UNUSUAL LOCATIONS

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Background: Desmoplastic small round cell tumor (DSRCT) is well defined neoplasm with distinct clinical, pathologic, immunohistochemical and molecular features. This tumor shows epithelial, mesenchymal and neural differentiation. DSRCT occurs mainly in peritoneal cavity of young males but can occur in unusual locations and then diagnosis could be difficult. The aim of the study is analysis histopathological and immunohistochemical features of primary, recurrent and metastatic DSRCT in soft tissue, cardiac muscle and cervical lymphnode locations and to make a detailed differential diagnosis. Method: three cases of DSRCT in unusual locations were found in our consultant files. We studied routine H&E-stained sections and immunohistochemically-stained sections (AE1/AE3, EMA, CK19, CK20, vimentin, desmin, WT1, CD56, CD99, synaptophisin, NSE, CD56, actin, SMA, Myf 4, Myo-D1, S-100, Melan A, HMB-45, TTF-1 and LCA). Results: All of the studied cases pertained to young adult men of ages: 25 (case 1), 27 (case 2) and 23 (case 3). The 25 years-old patient presented with mass in the left popliteal fossa and recurrent tumor after 9 months. The 27 years-old man presented with a neoplastic infiltration in the cardiac muscle. The third 23 years-old patient showed metastatic bilateral cervical lymphnodes. Tumors were made up of small nests and well-defined islands of cells separated by thin strands (case 1, case 3) or by broad desmoplastic strands (case 2). The neoplastic cells were small- to medium-size with scanty or focally clear, vacuolated cytoplasm. There was evidence of rosette formation in the case 1 recurrent tumor as well as glandular arrangement in the case 2 tumor. Necrosis was noted. The tumor cells of case 1 and case 3 showed typical immunophenotype of DSRCT- AE1/AE3, desmin, NSE possitivity accompanied by EMA, vimentin, WT1, CD56. The cells displayed diffuse cytoplasmic and membranous reaction for CD99. The tumors cells of case 2 were positive for keratin, EMA, vimentin, WT1, CD56, NSE, but negative for desmin. Considering their unusual location, the tumors presented herein yielded broad differential diagnoses including Ewing's sarcoma/PNET, alveolar and embryonal rhabdomyosarcoma, poorly differentiated synovial sarcoma, lymphoma, metastatic melanoma, metastatic small cell carcinoma, metastatic neuroendocrine carcinoma and Merkel cell carcinoma. Conclusions: DSRCT must be kept in mind when diagnosing primary tumors of soft tissue and metastatic lymphonodes in young men.

PP4-251
PRIMARY MYXOID TYPE LOW GRADE CHONDROSARCOMA OF THE XYPHOID PROCESS IN A YOUNG WOMAN

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Primary chondrosarcomas (CHS) of xyphoid process are rare and commonly occurs in elderly individuals. Herein we present a case of primary myxoid grade I CHS in a young adult. A 28 years-old woman presented clinically with a slow growing painless swelling on anterior chest wall. X-rays and CT examinations revealed an area of radiolucency with variable mineralization, indistinct margins, approximately 5 cm, centered in the xyphoid process. Macroscopic evaluation of surgically resected specimen showed a lobulated mass with a lucent glistering appearance with gritty areas. Histologically, the tumor formed nodules that invade the surrounding fibroadipose tissue. The extent of cellular atypia was mild and mitoses infrequently detected. Focally, increased cellularity was observed. The stroma was predominantly myxoid. A small areas of tumor necrosis was also found. It is important for the correct diagnosis of primary CHS to stress the attention for clinicopathological correlation of all the available data before arriving at a diagnosis.

PP4-252
CASE REPORT : ATYPICAL GRANULAR CELL TUMOR

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BACKGROUND: Granular cell tumor (GCT) is an uncommon soft-tissue tumor that is usually benign and readily treated with complete resection. It is found more commonly in the dermal-hypodermal tissue of the head, neck, chest wall and upper extremities. Apart from GCT s also have been observed in sites of previous trauma, such as surgical scars, and are associated with inflamation. We desciribe herein a GCT observed at the site of operation scar on the chest wall. CASE: The lesion was detected in a 69-year-old woman, located at the region of coronary artery bypass surgery scars on the chest wall. This operation was about 1 years ago. The lesion was painless subcutaneous nodule, which gradually enlarged. Excisional biopsy was performed. Histologically, there was a tumor, in the dermis and subcutis . It consisted of agregates of polygonal and spindle cells, sperated by thin bundles of mature collagen. The cytoplasm was eosinophilic and granular. Tumor cell nuclei was large and vesicular. There was no necrosis. mitotic activity was: 4-5/10hpf. Immunohistochemically, tumor was positive for S-100 protein and CD-68; negative for desmin, actin, HMB-45, CD-34, pancytokeratin. CONCLUSION : Malignant GCTs account for %1-2 of all GCTs. Because the malignant variant shares many striking pathologic similarities with reliable histologic criteria have been debated. Recently, Fanburg-Smith et al. classified atypical, malignant, and benign GCTs on the basis of six histologic criteria: necrosis, spindling, vesicular nuclei with large nucleoli, increase mitotic activity (>2 x10hpf), high nuclear to cytoplasmic ratio, and pleomorphism. Neoplasm that met three or more of these criteria were classified as atypical, and those that met one or two criteria were classified as atypical. The present tumor showed spindling and increasing mitotic activity. It can be classified as atypical GCT, suggesting malignant potential. Herein, we report this rare case of atypical GCT and present a brief review of the relevant literature.

PP4-253
HISTOLOGICAL, GENETIC AND MOLECULAR DATA IN 96 CASES OF SOFT TISSUE TUMOURS, DIAGNOSED ON IPO – PORTO (PORTUGAL CANCER CENTRE – OPORTO)
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INTRODUCTION: Diagnosis of soft tissue tumours is complex. Electron microscopy 20 years before and immunocytochemestry (ICC)nowadays, introduced important and decisive data to evaluate the histogenesis and differentiation of many sarcomas. In recent years, molecular pathology, essentially chromosomic alterations and genetic mutations studied by PCR techniques and/or FISH, are being added to the routine methods of diagnosis. The aim of this work is to evaluate the contribution of molecular pathology to the diagnosis of soft tissue sarcomas (visceral sarcomas were excluded) in Oporto Cancer Centre during the last 5 years (from 2001 until now). MATERIAL AND METHODS - A total of 96 soft tissue tumours diagnosed in the last 5 years was included in this study, with exclusion of visceral cases such as GIST. The material used was obtained from surgical specimens in most of cases (82 cases), and also from tru-cut biopsies (12 cases) and, in a small number of children tumours, also from fine needle aspiration material (2 cases). In these tumours, the following immunocytochemical antibodies were used in routine diagnosis: cytokeratins, EMA, vimentin, SMA, desmin, Myo D1, S-100 protein, CD31, CD34, CD99, CD10, HMB45. Genetic and molecular techniques included in most of cases: FISH and identification of fusion proteins using RT-PCR from paraffin sections. In a small number of cases a fresh tissue specimen was available, and conventional kariotype was performed. RESULTS In 17 cases diagnosed as synovial sarcoma by histology, the characteristic molecular alteration was present in 12. In 1 a diagnosis of Ewing sarcoma could be done and in 4 cases no other diagnosis was suggested.In Ewing sarcoma, concordance between histologic and molecular diagnosis was found in 12 from 14 cases. Regarding liposarcoma, 3 cases classified as myxoid by conventional histology were reclassified as well differentiated, and 1 as synovial sarcoma, after genetic study. On other hand, 2 cases classified as lipoma in conventional histology were reclassified as well differentiated liposarcoma after genetic study. COMMENTS: Molecular pathology was important to clarify the diagnosis of some cases of synovial sarcoma, Ewing sarcoma, liposarcoma and alveolar rhabdomyosarcoma, whose histology and ICC were not typical. In other cases, such as embryonal rhabdomyosarcoma, myxoid chondrosarcoma and malignant peripheral nerve sheath tumour molecular pathology was not so helpful but was informative to exclude other diagnosis and to give additional information whose real significance is not yet understood.

PP4-254
CLINICAL AND PATHOLOGICAL ASPECTS IN PROXIMAL AND DISTAL FEMORAL PATHOLOGICAL FRACTURES DUE TO THE METASTASIS
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This paper has investigated a group of 55 patients with surgically removed metastasis in proximal and distal femur, by clinical, pathological and therapeutic means. The patient mean age was of 53,5 years. The fracture site was described on the femoral neck (5 cases), on the trochanteric region (14 cases), on the subtrochanteric region (20 cases) and on the distal femur (16 cases). Part of the cases (42 patients) showed history of different primary tumor localization (lung carcinoma – 19 cases, breast carcinoma – 12 cases, prostatic carcinomas – 8 cases, renal carcinomas – 2 cases, larynx carcinoma – 1 case). All patients were operated by excising the tumor followed by cavity filling with acrylic cement and internal fixation, with immediate mobilization. In 52% cases the histopathological aspect of the metastasis was identical to the initial malignancy. In 13 cases where the primary malignancy was unknown, we have used histochemical methods to determine the tumor origin. Thus, we have determined more 4 primary tumors corresponding to the histopathological aspect of the metastasis (lung – 2 cases, breast – 1 case, ovary – 1 case). Despite the fact that rigid osteosynthesis provides the patient a psychological support by the early functional rehabilitation, the survival rate is influenced by the primary malignancy.

PP4-255
CATEGORIZATION OF MUSCULAR DYSTROPHIES : AN IMMUNOHISTOCHEMICAL STUDY OF 44 CASES
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BACKGROUND-The term " Muscular Dystrophy" which was first used by Erb in 1891 referred to a group of muscle disorders manifest by progressive muscle weakness of the voluntary muscles as a result of primary muscle degeneration.The disorders are frequently hereditary. By now many subsets of the disorders were described, all with nearly same clinical and histopathological findings; so further examinations such as Immunohistochemistry on muscle specimens & using blotting methods are essencial for differentiating the disorders . In Iran the IHC method first established in 2004 and a Neuromuscular core fascility formed in Genetics Research Center since then for the referred patients. METHOD-Patients:Muscle biopsies were obtained from Deltoid muscle of the patients who were clinically diagnosed as muscular dystrophy .High serum CK & myopathic pattern in EMG have been reported for all.Normal muscle tissue was used as normal control.Antibodies:Three settings of Immunostainings were applied for each patient ;1-Dystrophin antibodies,2-Alpha,Beta&Gamma sarcoglycan and Dysferlin,3-Merosin. RESULTS-From all 44 patients;5 cases revealed complete absence of Dystrophins(DMD);10 with partially stained with Dystrophins(suggested for BMD);8 cases of Sarcoglycans deficiency;4 patients with Dysferlinopathy;2 cases with Merosin deficiency (CMD);14 cases revealed positive staining with all of the antibodies and should be followed by the others (Calpain-3,Caveolin...);finally one of the patients showed only severe necrosis & degeneration . CONCLUSION-According to our study IHC is an essencial technique for the diagnosis & differentiation of muscular dystrophies.It should be followed by the blotting methods in most of the cases(BMD & Dysferlinopathy);Molecular methods also should be performed for definite diagnosis in some other cases(subtyping of Sarcoglycanopathies). In our pilot study , the most frequent muscular dystrophies was Dystrophinopathies followed by Sacoglycanopathies .A study on a larger group of Iranian patients with more antibodies should be followed.

PP4-256
INTRAABDOMINAL LOCALIZATION OF EXTRASKELETAL MYXOID CHONDROSARCOMA: CASE REPORT
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Extreskeletal myxoid chondrosarcomas with primary intraperitoneal localization are extremely rare with only several cases reported on Pubmed. We report a case of intraabdominal extraskeletal myxoid chondrosarcoma (EMC) in 57-year old

woman that was surgically treated because of the expansive tumor mass in her abdomen. The tumor that measured 11x11x7 cm, was located below diaphragm. On gross examination, it was a multinodular, chondroid tumor, well demarcated with central necrotic and gelatinous area. Microscopically, tumor was composed of chondroblasts arranged in cords, within myxoid stroma. Some of the cells were binuclear and bizarre. Peripheral area was less cellular compared with the central area. Mitotic activity of tumor cells was low classifying this tumor as a low-grade soft tissue tumor.

PP4-257

POLYVINYLPYRROLIDONE STORAGE DISEASE: A RARE CAUSE OF PATHOLOGIC FRACTURE

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[INTRODUCTION] Polyvinylpyrrolidone (PVP) had been widely used as a plasma substitute or a retardent in some drugs decades ago. In Taiwan, PVP has been prescribed inappropriately as blood tonics for nutrition support for a long time. However, local injection or systemic administration of PVP-containing drugs could cause PVP to be retained in the reticulo-endothelial system and lead to PVP storage disease. [CASE REPORT] A 65-year-old Taiwanese lady was suffered from right hip pain after a fall in Nov. 2005. Normocytic anemia (Hb: 9-10 g/dl) was noticed. Image studies showed right femoral subtrochanter fracture. During operation, the fracture site exhibited some soft, yellowish, and gelatinous material within the marrow space; therefore, a bony tumor or metastatic lesion was suspected. Histologically, it showed sheets of blue-grayish vacuolated cells in a myxoid and hemorrhagic background with minimal inflammatory reaction. These vacuolated cells were positive for mucicarmine and Congo red stains, but negative for Peroidic acid-Schiff (PAS) and alcian blue stains. Immunohistochemically, these cells were negative for cytokeratin and S-100 protein, excluding the possibility of metastatic carcinoma or myxoid liposarcoma. Tracing back this patient's medical history, she had been frequently given intravenous supplement of plasma substitutes as blood tonic at the local clinics for several years. The whole clinical and pathologic features suggested the diagnosis of PVP storage disease. [DISCUSSION] PVP storage disease, or PVP granuloma, occurred mainly in skin and soft tissue. Severe anemia and bony destruction were also noted in some cases, owing to massive infiltration of PVP storage cells in the marrow spaces. However, pathologic fracture due to PVP deposits in bones was rarely reported. For the pathologists, the differential diagnoses include metastatic epithelial tumors, such as signet-ring cell carcinoma and renal cell carcinoma. Chondromyxoid tumors of soft tissue, such as liposarcoma, chordoma, and chondrosarcoma, as well as other histiocytic storage disease, should also be considered. The characteristic histochemical staining results, combined with negative immunostains for cytokeratin and S-100, help reach the correct diagnosis. The clinicians and pathologists should be aware of this iatrogenic storage disease to prevent misdiagnosis.

PP4-258

MONOSTOTIC PAGET DISEASE OF THE PATELLA

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Background: To present an unusual case of Paget Disease located at the patella. Method: The patient was female 46 years old, presenting with pain and swelling of the left patella. The clinical, radiographic and CT findings were more suggestive of Paget disease and they did not show any other bone affected. The patient underwent a needle biopsy. Results: Microscopic examination revealed areas with prominent osteoclastic activity, while the osteoclasts were larger than usual, containing many nuclei per cell. There were also fewer osteoblastic areas with rimming of osteoblasts. No atypia was observed. The stroma was vascular and partially fibrotic. The bony trabeculae were disorganised, forming focally a "mosaic" pattern and few "cement" lines. The findings were suggestive of Paget disease and particularly of the osteolytic phase. Conclusion: Monostotic Paget disease of the patella is extremely rare. The etiologic factors of Paget disease are not clear yet. It seems that both genetic and non-genetic, perhaps viral, factors are assosiated with the disease. Molecular studies may contribute to the understanding of the pathophysiology of Paget disease and provide new therapeutical procedures in the future.

PP4-259

OSTEOBLASTOMA-LIKE OSTEOSARCOMA. REPORT OF TWO CASES

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Background: Osteoblastic-like osteosarcoma (OLO) is a rare (1% of osteosarcoma) variety of osteosarcoma of low-grade malignancy with potential for recurrence when adequate surgical margins are not achieved and metastases. Differential diagnosis between osteoblastoma and OLO can be very difficult, because osteosarcoma may resemble osteoblastoma (clinically, radiologically and histologically), certain variants of osteoblastoma (agressive osteoblastoma, osteoblastoma with pseudosarcomatous features, multifocal osteoblastoma) may mimic osteosarcoma and osteoblastoma may rarely undergo malignant transformation. Distinguishing these two lesions is of great importance because their prognosis and treatment are different. In most cases, combination of clinical, radiological and pathologic features affords a clear distinction. The strongest histologic criteria to differentiate between them is tumoral permeation of the surrounding host tissue. Design and results:We report two cases of OLO in 8 year-old children (one male and one female). The first presented with a 7 months history of increasing pain in the right shoulder. A lacunar lesion of the first rib was resected, suggesting an osteoblastoma histologically. The lesion reccured in the subclavian region 18 months later and histological examination displayed a typical OLO. This patient underwent chemotherapy and radiotherapy. Three years later, there was a tumoral progression towards vertebrae and spinal tube, leading to the death of this patient one year later. The second patient had an osteolytic lesion located in the right iliac crest. The tumour revealed morphologic features of a benign-appearing osteoblastoma. Local reccurence occured one year later, owing to a wide curretage. Seven months later, a further extensive local reccurence had developped. An OLO was then diagnosed and the patient was treated with chemotherapy followed by en bloc resection of the iliac bone. She is now free of symptoms (8 months). Conclusions: Pathological diagnosis of OLO based on purely morphological parameters is difficult, especially on small biopsies. These two observations show that it is necessary to define clear morphologic and immunophenotypic characteristics for this low grade osteosarcoma often misdiagnosed as osteoblastoma. Alteration in suppressor gene p 53 localized on chromosome 17p13 seems to be an early event in the tumorogenesis of malignant osteoblastic tumours. Its characterisation might be helpful and need further investigations in these circumstances.

PP4-260
MYOSITIS OSSIFICANS PROGRESSIVA: A CASE REPORT
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Introduction: Myositis ossificans progressiva is a rare autosomal dominant progressive disease of connective tissue. It consists on a heterotopic osteogenesis frequently associated to characteristic congenital malformations. Case report: We report a case of a 24 year-old girl which presented a heterotopic bone formation leading gradually to ankylosis. The diagnosis was clinically suggested and confirmed when we've discovered bone bridges. Discussion: Congenital malformations, most commonly of big toes and thumbs, are important criterias to draw on the diagnosis earlier and to avoid unnecessary biopsy or resection which may trigger off a flare of the disease. This lesion simulates microscopically an extraosseous osteosarcoma and juxtacortical osteosarcoma with a highly cellular stroma associated with new bone formation. The most important diagnostic feature is the zonal phenomenon of maturation. Therapeutic measures are essentially preventive and the prognosis is poor.

PP4-261
GIANT CELL GRANULOMA OF THE INFRA TEMPORAL FOSSA: A CASE REPORT
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Background: Giant cell granuloma (GCG) is an uncommon benign lesion that occurs almost exclusively whithin the jaw bone. Isolated cases of GCG occuring in the other cranial and facial bones have been documented. Location in temporal bone is extremely rare and less then 20 cases have been reported in the medical literature. Case report: A 46 year old man presented with a 3-year history of hearing loss on the left. Physical examination revealed a mass in the left preauricular area. Radiologic computed tomography showed a destructive multiloculated lesion of the left infratemporal fossa, the temporal and sphenoidal bones. This lesion eroded the left external auditory canal. Serum alcaline phosphatase, serum inorganic phosphate and calcium were all within the normal limits. A biopsy was performed. The microscopic examination of the specimen revealed a connective tissue stroma containing a large number of ovoid or spindle-shaped cells and multinucleated giant cells resembling osteoclasts. Foci of hemorrhage and deposit of hemosiderin were also present. There were some regular osteoid trabeculae. On immunohistochemical examination, both mononucleated and multinucleated cells expressed CD68 and focally S100 protein. Proliferate index assessed by KI-67 staining was estimated to 5-10%. The diagnosis of GCG was made. Conclusion: GCG is a rare benign lesion with distinct clinicopathological features. Modern neuro-imaging shows an expansile destructive multiloculated bone lesion mimicking a neoplastic mass. Histopathology is confirmatory of this benign lesion. Other giant cell containing lesions such as the giant cell tumour of the bone and a brown tumour of hyperparathyroidism need to be carefully excluded. Microsurgical excision is curative with a low recurrence rate.

PP4-262
A MODIFIED STAGING FOR SOFT TISSUE SARCOMA – PROGNOSTIC INDEX (STS-PI)
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This study was carried out on 122 patients suffering from different types of soft tissue sarcomas and ranging in their ages from 17 to 72 years. They were admitted to "Aswan Cancer Institute" in Egypt for surgical resection of their tumors. The resected specimens were proved to be soft tissue sarcomas of various microscopic types with a minimum follow up period of 60 months (5 years) . The aim of our study is to find out a reliable scoring system for grading and staging of such sarcomatous tumors that could reflect a convenient clinco-pathological prognostic index. Following proper gross morphological, histopathological and immunohistochemical diagnosis of the specimens under the study, they were given scores according to the following five parameters, as follows: (a) According to Tumor microscopic type: Score 1 indicates favorable prognosis (as in dermatofibrosarcoma protuberans) and Score 2: for other types of sarcomas.(b) According to Tumor longest dimension: Score 1: for tumors < 5 cm and Score 2: for tumors > 5 cm. (c) According to Tumor depth: Score 1: for Superficial tumors and Score 2: for Deep tumors. (d) As regards Tumor histological grade, according to the French Federation of Cancer Centers Sarcoma Groups (FFCCSG system) : tumors were given three scores; Score 1: for Grade I tumors (well differentiated), Score 2: for Grade II tumors(moderately differentiated) and Score 3: for Grade III tumors (well differentiated). Grading is best done in relation to histological morphological criteria including ;(degree of cellularity, pleomorphism, mitosis and necrosis), and finally the last parameter is (e) According to the presence or absence of Tumor metastases (LN or organ metastases); tumors were given either Score 0: for absent metastases and Score 6: when metastases are present. So our suggested STS-PI is as follows : The suggested soft tissue prognostic index (STS-PI) for the studied cases was calculated by summing up the previous scores relevant to each tumor and was found and described as 4 CLASSES as follows: -CLASS A : 4 (best score) -CLASS B : 5, 6, 7 (good score) -CLASS C : 8, 9 (medium score) -CLASS D : 10 – 15 (poor score) The 5 years survival rate for the studied tumors was recorded in 7/8 CLASS A tumors (87.5%), in 30/44 of CLASS B tumors (68.2 %) , and in 17/59 CLASS C tumors (28.8%) and was 0/11 in class D tumors (0 %), pointing to a reliable STS-PI.

PP4-263
EWING SARCOMA, A RETROSPECTIVE STUDY
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BACKGROUND. Recently, new concepts in Ewing sarcoma/PNET diagnosis and treatment were introduced. Since 1998, immunohistochemical verification of ES/PNET diagnosis, using CD99, is routinely performed at our Institute. Since end 2003, molecular methods (RT-PCR for EWS/FLI1) were introduced. In 2003, an algorithm was adopted including molecular analysis for residual disease and early recurrence. In order to establish a baseline patient population, we performed a partly retrospective and partly prospective analysis. PATIENTS AND METHODS. 78 Ewing sarcoma/PNET, diagnosed at the Institute of Pathology, Medical School University of Zagreb and treated at the Children's Hospital Zagreb, in the period from 1990 to 2006, were included in this study. Of all analyzed tumors, 8 were extraosseal. Retrospectively, we submitted all collected tumor samples for both immunohistochemical and molecular analysis. Immunohistochemical analysis was successful in all analyzed samples. RT-PCR could not be successfully performed on samples older than 2000. From 2003, together 96 samples – tumor tissue, peripheral blood and 28 bone marrows were analyzed for characteristic translocations. RESULTS. Immunohistochemically, all tumor samples showed moderate to strong positivity, while EWS/FLI1 could not be demonstrated in

6. From the samples submitted according to the new algorithm, 39 were positive by RT-PCR, among them 10 peripheral blood samples. In some cases multiple analyses of blood samples yielded only one positive. In two diagnostically problematic and immunohistochemicaly equivocal cases of small blue cell tumors molecular analysis fostered the final opinion. CONCLUSION. Introduction of molecular techniques enabled us to solve some diagnostic problems and to detect disseminated disease prior to clinical evidence producing a potent tool for improved patient care and disease management.

PP4-264
DESMOID TUMORS OF THE CHEST WALL

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Introduction: Desmoid tumors (DT) are rare benign soft tissue neoplasms that arise from fascial or musculo-aponeurotic structures. Despite their benign microscopic appearance, they are characterized by invasion of contiguous structures and recurrence. Purpose: We retrospectively reviewed our institutional experience of DT of chest wall with emphasis on the clinical and pathologic characteristics. Material & Methods: Between January 1993 to January 2007, we retrospectively collected 6 cases of DT of chest wall. Medical records and microscopic slides were reviewed. Anti-bodies selected for immunohistochemical staining included vimentin, desmin, smooth muscle actin, S100 and CD 34. Results: There were 4 males and 2 females with mean age of 40 years. Only pediatric case was noted. Three patients had a history of previous trauma or surgery (thoracotomy for lung adenocarcinoma and sternotomy for a aorto-coranory bypass). Complaints included pain (n=4), palpable mass (n=3), sensory symptoms and cough. In CT scan, lesions appeared as wall chest soft tissue tumor (n=4) with extension to the neck in one case. One tumor was located in posterior mediastinum mimicking neurogenic tumor and other was misdiagnosed as lung neoplasm. Surgical treatment was performed in 5 cases. The size of specimen varied from 3 to 14 cm and included ribs in 3 cases. Histology revealed a densely collagenated acellular lesion interspersed with areas of moderate cellularity consisting of fibroblastic and myofibroblastic cells. Mitotic figures were absent.There was no infiltration of surrounding structures. Chirurgical biopsy was performed in massive tumor with pleural, mediastinal and pulmonary involvement. Vimentin and smooth muscle actin were observed in all cases. S100 and desmine in one case. CD34 was not expressed by any of these tumors. One patient presented one recurrence and was treated by local resection and radiation therapy. Chemotherapy was beginned in a pediatric case. Discussion: Clinical presentation of chest wall DT in the literature is similar to our serie, but we not note a female predominance. The pathogenesis of DT is not clear. Trauma has been strongly implicated and recent work revealed hight incidence of B catenin gene mutations. Definitive diagnosis requires histopathology. Neurogenic tumors, fibrosarcomas, calcifying fibrous pseudotumors and localized fibrous tumors of the pleura should be considered in the differential diagnosis. CD34, smooth muscle actin and B catenin stains are useful in this setting.

PP4-265
CLINICO-PATHOLOGICAL AND OUTCOME FEATURES OF FIBROUS DYSPLASIA: RETROSPECTIVE STUDY OF A SERIES OF 26 CASES FROM TUNISIA

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Background: Fibrous dysplasia is a rare condition in which defective maturation of osteoblasts results in abnormal bone formation. Is typically occurs in patients before 30 years of age with an equal ratio between males and females. FD presents in two forms: monostotic and polyostotic. The purpose of this retrospective study was to analyze clinical, histological and radiological characteristics and the outcome after treatment of this lesion in Tunisian population. Methods: Twenty six cases of histologicaly proven fibrous dysplasia diagnosed during a 15-year period from 1990 through 2005 at the Department of Pathology of the CHU Farhat Hached of Sousse, Tunisia. The following medical records were subjected to a detailed scrutiny: In patient clinical records, imaging studies, histopathological observations and outpatient clinical follow-up records. Results: In the present series male/female ratio was 1:4.2. Their ages ranged from 6 to 71 with an average age of 28.6. Monostotic fibrous dysplasia was found in 24 patients (92.3%), and 2 patients had polyosotic fibrous dysplasia (7.7%). Mc Cune-Albright syndrome and Mazabraud syndrome witch associating fibrous dysplasia were not noted in any case. The majority of the lesions were located in the upper maxilla and the mandible (65.4%). Imaging studies often show lesion with a ground glass matrix. Histopathological examination in the majority of these cases showed dysplastic bone surrounded by fibroblastic, some osteoblastic giant cells and hemosiderin filled macrophages. In the monostotic form, the clinical outcome was considered good in majority of cases; however, one patient has developed an osteosarcoma, three years later. In the two polyostotic cases outcome was less satisfactory because of fractures and deformities. Conclusion: This clinicopathological study revealed a female predominance so this can support the hormonal theory of fibrous dysplasia suggested by some authors.

PP4-266
EGFR, P53 AND CAVEOLIN-1 EXPRESSION IN ADAMANTINOMA OF LONG BONES

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BACKGROUND: Adamantinoma of long bones comprises about 0.4% of all primary tumors. In 90% of cases, tibia is the main site of involvement. World Health Organization defined it as a low grade, malignant biphasic tumor characterized by a different morphological patterns, most commonly epithelial cells, which are surrounded by a bland spindle-cell osteo-fibrous component. Nowadays, the epithelial part of the tumor is accepted to be neoplastic. Aneuploidy and p53 overexpression have only been demonstrated recently in one study. Since many studies have indicated an important role for growth factors and associated receptors in tumor growth we studied the expression of epidermal growth factor receptor (EGFR) and also p53 immunohistochemistry. Caveolin-1 is a cell membrane protein and regulate intracellular signal transduction. It is usually found in mesenchymal tissue and is believed to act as a tumor suppressor or tumor promoter in many tumors. However, its expression has not been investigated in adamantinoma of long bones. For this reason we added caveolin-1 immunohistochemistry to our study. METHODS: A total of 8 resected adamantinoma of the tibia were included in this study.

All cases were classified according to the standard diagnostic criteria. Immunohistochemistry was performed by using anti-EGFR, p53 and anti-caveolin 1 antibodies. RESULTS: Nuclear p53, cytoplasmic and membranous Caveolin-1 and membranous EGFR reactivities were accepted as the positive immunostaining. Five cases (62.5%) showed focal and weak p53 expression only in epithelial areas. One case (12.5%) showed focal EGFR expression in the epithelial component. All cases (100%) showed strong caveolin-1 positivity in the epithelial part. Among them, 2 cases showed also focal caveolin-1 immunostaining in the fibrous component. Caveolin-1 expression was also found in endothelial cells. CONCLUSION: Although the percentage of p53 stained areas ranged from %10 to 30, our result supports that the epithelial part of the tumor is being neoplastic. As EGFR expression was present in one case it could not explain its relationship with tumor growth, but the down regulation of receptors may be one cause of this situation. Thus needs further investigations and correlations with epidermal growth factor expression. However, the high and strong caveolin-1 expression may be explained by its possible tumor promoter role in adamantinoma of long bones. Furthermore its predominant expression in the epithelial part of the tumor support the evidence that fibrous component of the tumor may be reactive.

PP4-267
CAVEOLIN-1 AND C-KIT EXPRESSION IN 12 MYXOID/ROUND CELL LIPOSARCOMAS

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BACKGROUND: Caveolin-1 is cell membrane protein and plays a major role in membrane traffic and signal transduction. Several studies have implied that caveolin-1 might have a tumor suppressor role; however, others have reported its tumor-promoting function. C-Kit expression has been utilized to identify patients with gastrointestinal stromal tumors (GISTs) who can be treated with imatinib. But its expression is not restricted to GISTs. Day by day, many tumors are adding to the c-kit positive tumors list and its expression in other soft tissue tumors is being an area of interest for therapeutic implications. Caveolin-1 and c-kit expression in sarcomas, especially in myxoid/round cell liposarcomas are not widely explored. For this reason, we decided to examine the expression of anti-caveolin-1 and c-kit antibodies in 12 cases of myxoid/round cell liposarcomas. METHODS: A total of 12 resected myxoid/round cell liposarcomas cases were included in this study. All cases were classified according to the standard diagnostic criteria. Three cases were composed of purely round cell liposarcoma and five cases were composed of purely myxoid liposarcoma. Four cases were composed of both myxoid and round cell liposarcoma. Immunohistochemistry was performed by using anti-caveolin-1 and anti-c-kit antibodies. RESULTS: The presence of membranous and/or cytoplasmic staining was accepted as a positive reaction for anti-caveolin-1 and c-Kit antibodies. Caveolin-1 expression was present in nine cases (75%) except three purely round cell liposarcomas and also in the round cell component of four cases containing both myxoid and round cell liposarcomas. Cytoplasmic c-Kit positivity was found in two cases. CONCLUSION: Round cell liposarcoma represents a histological continuum of myxoid liposarcoma. But the presence of round cell is a significance of unfavourable prognosis. Caveolin-1 expression has been investigated only in one recent study where focal and weak positivity was found in dedifferentiated liposarcomas. Our study results showed that caveolin-1 may have a potential role in the pathogenesis of myxoid and round cell liposarcomas. Furthermore, its absence in the non-lipogenic primitive round cell component let us to think that its down-regulation may be a hallmark of poorer outcome. As c-Kit has been found in only 2 of our cases, further investigations with larger series are needed to illuminate its usefulness for the therapeutic implications.

PP4-268
NEUROBLASTOMA IN ADULTS, PRESENTATION OF 2 CASES

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BACKGROUND: Neuroblastoma is one of the common malignant tumors of childhood but only about 100 cases have been reported in adults. The age range is 15-82 years with a median of 34 years. Male/ female distribution is equeal. Many of these tumors are reported in sites that are unusual for classical neuroblastoma (parotid, kidney, gluteal soft tissue, lower extremity). The survival rates are similar to those for INNS stage 3 and 4 childhood neuroblastomas. CASE 1: 50 year old male patient presented with paraoesophagial mass. Right thoracotomy was applied. 5x4x2 cm mass with a rib was excised and was sent to another pathology department, diagnosed as malignant tumor metastasis to paraoesophagial lymph node; and it was suggested that the tumor could be an osteosarcoma metastasis. The paraffin blocks were revised by Pathology Department of Cerrahpasa Medical Faculty. The diagnosis was neuroblastoma transformed from ganglioneuroma in paraoesophagial soft tissue confirmed by imunohistochemical staining with synaptophysin and chromogranin. CASE 2: 23 year old female patient presented with cervical mass. Two excision material, measuring 2x1.5x1 cm and 1.5x1x1 respectively were sent to another pathology center. According to the original pathology report, the materials were nodular and the cut surface was homogenous and yellow-white in colour. It was reported as neoplasm showing features of ganglioblastoma. The tumor cells were diffusely positive for NSE, focal positive with chromogranin negative for pancytokeratin and synaptophysin immunohistochemically. The paraffin blocks were revised by Pathology Department of Cerrahpasa Medical Faculty and diagnosed as neuroblastoma metastasis to lymph node. CONCLUSION: Neuroblastoma should be included in differential diagnosis of a small round cell tumor even in adult age especially in case of a background of schwannian stroma with scattered ganglion cells. This accompanying histology representing ganglioneuroma raises the possibility of dedifferentiation to neuroblastoma in the first case. The presence of an indolent form of a neuroblastoma is open to discussion for the second case.

PP4-269
THYROID METASTASIS FROM PELVIC MALIGNANT SOLITARY FIBROUS TUMOR AFTER 13 YEARS OF RESECTION AND COEXISTENT PARATHYROID ADENOMA

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Solitary fibrous tumor is a rare spindle cell neoplasm which can be malignant. Here we report a 70 year-old woman presented with elevated calcium level during routine blood test. Her blood parathormone level was also increased. Ultrasonography of the thyroid showed the presence of multiple nodules and a parathyroid mass. Total bilateral thyroidectomy was performed. On gross examination of the thyroid, there were multiple nodules varying from 0.3 to 1.6 cm in diameter. Microscopically the largest nodule was well-circumscribed and composed of spindle cells. Immunohistochemically, the lesion was negative for CD34, CD68, CD117, EMA, pancytokeratin, TTF-1, smooth muscle actin, S-100 protein and positive for CD99. It was found out that the patient underwent laparotomy and a large pelvic mass was removed in 1993. This mass was diagnosed histologically as low grade malignant fibrous histiocytoma. She received radiotherapy and chemotherapy. In 2005, during the folow-up, lung and liver metastasis were detected by computed tomography scan. At the same time an abdominal mass was seen and biopsy of this mass revealed a morphology consistent with the previous tumor. Microscopically all specimens were reevaluated. Hemangiopericytomatous and storiform patterns were observed in the tumors. When we take the changes in the classification of soft tissue tumors into consideration, we diagnosed the tumor as malignant solitary fibrous tumor. As it is known, cellular form of solitary fibrous tumor can show malignant behaviour, in the form of recurrence and/or metastasis. The patient also received a diagnosis of parathyroid adenoma by the microscopic examination of the contralateral lobe and this mass was the main purpose of thyroidectomy. We reported this case because yet to our knowledge this is the first case of metastatic solitary fibrous tumor of thyroid coexistent with parathyroid adenoma.

PP4-270
COX-1 AND COX-2 EXPRESSION IN OSTEOSARCOMA AND BENIGN BONE TUMORS

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Background: Cyclooxygenase (COX) enzymes catalyze the conversion of arachidonic acid to prostoglandins, which have a variety of physiologic and pathologic roles. Two COX isoenzymes have been described to date: COX-1, which is constitutively expressed by most tissues, and COX-2, which is inducible by cytokines, growth factors, and hormones. Overexpression of COX-2 has been discovered in a variety of solid tumors and pediatric sarcomas. In our study; we aimed to investigate the similarity and differences of COX-1 and COX-2 expression in benign bone tumors and osteosarcoma. Material and Methods: Paraffin-embedded tissue specimens from 35 bone tumors (20 osteosarcoma, 6 giant cell tumor, 6 fibrous dysplasia, 3 osteoblastoma cases) were retrieved from the files of the pathology department of Cukurova University, Medical Faculty. COX-1 and COX-2 were applied by immunohistochemical method. The results of staining for COX-1 and COX-2 were analyzed semiquantitatively by using an immunohistochemical scoring system (HSCORE) that combines the percentage of immunoreactive cells (quantity score) and an estimate of staining intensity (staining intensity score). Results: Strong (HSCORE:2-3)COX-1 expression was detected in five (33.4%) and negative (HSCORE:0) or weak positivity (HSCORE:1) in 10 benign cases (66.6%)and there was no strong positivity with COX-1 in osteosarcoma cases (P:0.005). Whereas; there was no significant differences with COX-2 between benign bone tumors and osteosarcoma cases (P:0.730). No correlation was found between COX-1 and COX-2 expression in osteosarcoma cases (r:0.15). Whereas, mild negative correlation was detected between benign bone tumors with COX-1 and COX-2(r:0.38, p:0.5). Conclusion: We conclude that most cases of the benign bone tumors express COX-1 and COX-2 to varying degrees. Whereas there was no moderate or strong positivity with COX-1 in osteosarcoma cases. These results may be explained that cyclooxygenase enzymes do not play an important role in osteosarcoma pathogenesis.

PP4-271
ACRAL MYXOINFLAMMATORY FIBROBLASTIC SARCOMA (REPORT OF 5 CASES)

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BACKROUND: Acral myxoinflammatory fibroblastic sarcoma (AMFS) was first described in 1998 as a rare tumor of distal extremities. AMFS is usually localized in the fingers within the subcutaneous tissues. It develops in patients of all ages with a median of late 40s. Grossly, it is an infiltrative multinodular mass measured 1-8 cm in diameter. AMFS consists of bizarre cells which have enlarged vesicular nuclei with inclusion-like nucleoli and large eosinophilic cytoplasms. The stromal component of the tumor contains various amounts of myxoid and hyalen matrix mixed with inflammatory cells. METHODS: We report 5 cases of AMFS, occuring in 3 males and 2 females with their clinical, histological and immunohistochemical features. The mean age was 45 (range 26-64). The tumor sizes were ranged from 1 cm to 6 cm (mean 2.8 cm). The localizations of the tumors were as follows: 2 ankles, 1 toe, 2 hands (D5 and extensor surface). Three patients presented with painless swelling, one with pain and mass and one with only pain. The symptom duration of cases was between 3,5 months and 4 years. Four cases, which were sent to us for consultation, were previously diagnosed as myxoid type malignant fibrous histiocytoma, chondrosarcoma, low grade fibrosarcoma and sarcoma (NOS). RESULTS: Immunohistochemical studies revealed positivity for vimentin (4/4), CD68 (5/5) and CD34 (2/3). Positive immune reaction was focally observed only in 2 of 5 cases. No positive immunostaining was detected for pancytokeratin (4/4) and smooth muscle actin (3/3). Ki-67 proliferation indices were between 2-5%. Four cases was treated with local excision, whereas 1 case with local amputation. Recurrence was seen only in one that was localized at extensor surface of the hand. None of the cases showed metastasis with a median follow-up of 17 months. CONCLUSIONS: AMFS is a very rare entity that must be kept in mind in the differential diagnoses of tumoral and non tumoral lesions, such as tenosynovitis, nodular fasciitis, giant cell tumor, inflammatory myofibroblastic tumor, liposarcoma, epithelioid sarcoma and malignant fibrous histiocytoma. Although we detected recurrence only in one case and no metastasis, AMFS is known to be a low-grade sarcoma that shows recurrence and has metastatic potential.

PP4-272
HMGA1 REARRANGMENTS OCCUR IN A SMALL SUBSET OF SPINDLE CELL LIPOMAS

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Background: Spindle cell and pleomorphic lipomas are benign subcutaneous adipose tissue neoplasms typically occurring in the neck and back of older men. Conventional cytogenetic analysis often shows monosomy or partial losses of chromosomes 13 and 16. Rearrangements of 6p21 have been reported in these tumors, and one study has identified HMGA1 overexpression by immunohistochemistry in a single example (Dumollard JM et al Ann Pathol 2001; 21:303-10). Because we have recently encountered a classic case of spindle cell lipoma in the face of a

66-year-old man with rearrangements of chromosome 6p21, we hypothesized that the HMGA1, a gene occasionally rearranged in ordinary lipomas and other benign neoplasms – was involved. The aim of this study was to investigate the frequency of HMGA1 rearrangements in a series of 17 of such tumors. Method: Seventeen cases of spindle cell lipomas with typical histopathologic features were studied for rearrangements of HMGA1 and HMGA2 by fluorescence in situ hybridization (FISH) on paraffin-embedded sections. Fresh and frozen tissues were available from the index case for standard cytogenetic analysis and reverse-transcriptase polymerase chain reaction (RT-PCR). Results: Cytogenetic analysis was performed in one case and demonstrated the following karyotype: 46,XY, t(1;6)(p32;p21.3), del(13)(q12q14)[19]/46,XY[1]. FISH analysis showed a balanced rearrangement of HMGA1, and semi-quantitative RT-PCR confirmed transcriptional upregulation of this gene. Molecular cytogenetic studies of 16 additional spindle cell lipomas revealed a balanced rearrangement of HMGA1 in a single case. HMGA2 rearrangements were not found in any of the cases studied. Conclusion: Rearrangements of chromosome 6p21 with involvement of HMGA1 seem to occur in approximately 10% of spindle cell lipomas (2/17 in the present series), a frequency similar to that observed in ordinary lipomas. This finding suggests that HMGA1 may play a role in the pathogenesis of a subset of these tumors.

PP4-273
HYDATID CYST OF BONE; REPORT OF FIVE CASES
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Background: Hydatid cyst is a parasitic infection caused by larval stage of Echinococus granulosus in man and domestic animals. The incidence of hydatid cyst in liver, lung, kidney and brain are 59-75%, 27%, 3% and 1-2% respectively. Hydatic cyst in bone is extremely rare and found in only 0.5-2% of cases. In endemic areas, this ratio can increase to 4%. Material and Methods: In a comprehensive study of 109 cyst hydatid cases from pathology files of Cukurova University Medical Faculty between 2002-2007 years; we identified five bone hydatid cysts (5.5%). Results: The age distribution was between 18 and 55 years. Three of them were male, two patients were female. Among the five cases; four cases were admitted to our hospital with pain and one patient with pathologic fracture. The localization of the lesions was femur (two cases), tibia (one case), iliac bone (one case), acetabulum (one case). Conclusion: In this study, we present five hydatid cyst in rare localization and review the literature.

PP4-274
SKULL BASE CHORDOMAS A RETROSPECTIVE STUDY OF 14 CASES
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Chordomas are uncommon malignant neoplasms arising from remnants of the notochord. They account for 1–4% of all primary malignant bone tumours and are mainly localized in the sacrococcygium (55%). Skull base chordomas are extremely rare representing less than 1% of all intracranial tumours. The aim of the present study is to analyze clinicopathological features of chordomas and to discuss differential diagnosis. Over the 12-year period from January 1997 to December 2006, 14 cases of skull base chordomas were diagnosed at the pathology department of La Rabta hospital. Relevant clinical information and hematoxylin & eosin stained tissue sections were available for review in all cases. Clinical features, symptoms at diagnosis, treatment and outcome were retrospectively analyzed. There were 11 male and 3 female patients (sex ratio M/F = 3,67) aged between 19 and 72 years with a mean age at presentation of 46,57 years. The most frequent complaints were headache, diplopia and decreased vision. Prior to surgery, each patient was investigated with contrast-enhanced magnetic resonance imaging (MRI) and contrast-enhanced computed tomography (CT). Seven tumours involved the clivus, 3 were intrasellar, 2 were located in the cavernous sinus and 2 involved the sphenoid sinus. Surgical resection of the tumour was achieved in all patients. Pathological examination of the surgical specimen was compatible with classic chordoma in 12 cases and chondroid chordoma in 2 cases. Immunohistochemical study was performed in only 2 cases and showed positive immunostaining of tumour cells with cytokeratin, EMA, vimentin and S-100 protein.

PP4-275
ANGIOMATOID FIBROUS HISTIOCYTOMA: REPORT OF THREE CASES WITH CYTOLOGICAL AND HISTOLOGICAL FEATURES
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Angiomatoid fibrous histiocytoma (AFH) is an intermediate grade histiocytic tumor which occurs in children and young adults. In this case report, we present the cytological and histological features of three AFH cases. The patients were all male, and 28, 22 and 36 years old. The localizations of the tumors were supraclavicular, forearm and inguinal region, and their diameters were 10 cm, 4 cm and 2.5 cm, respectively. In the fine needle aspiration material of the supraclavicular mass showed highly cellular smears displaying polygonal and spindle shaped cells, either dispersed or forming three dimentional clusters in a lymphocyte-rich background. The tumor cells had ovoid or spindle-shaped nuclei, fine chromatin, small inconspicious nucleoli. The excisional biopsy material of all three patients showed similar histopathological features. The tumors were composed of multiple cellular nodules surrounded by fibrous pseudocapsule and contained blood filled cavities in a lymphocytic and inflammatory backgound. Groups of hemosiderin-laden macrophages were also present in varying amounts. All three tumors were positive for CD68 and epithelial membrane antigen, negative for endothelial markers and one of them was positive for desmin. The tumor localized in cervical area had been resected with indeterminate surgical margins and recurred in 6 months time. The reexcision material showed similar histomorphological features. AFH should be included in the differential diagnosis in a child or young adult patient having a slow-growing subcutaneous mass with cytological and histological features of an intermediate grade histiocytic mesenchymal tumor. The morphological features as well as immunohistochemical findings make the accurate diagnosis possible.

PP4-276
CASE REPORT: MELANOTIC NEUROECTODERMAL TUMOR OF INFANCY
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Background: Melanotic neuroectodermal tumor of infancy (MNTI) is a rare benign neoplasm that may be locally aggressive. Since the first description by Krompecher in 1918, approximately

350 cases are reported in the medical literature. The tumor affects most commonly maxilla (%70) followed by the skull and mandible during first year of life. Early diagnosis and radical surgery are critical for long-term cure. Case presentation: We are presenting a 18 month old boy with a lump in the right temporal region since the age of 3 months. Because the tumor had a relatively fast grow, she was referred to our hospital. Radiological examination showed a tumor located in the right mastoid which was demarcated and radiolucent. It was not adhered to the dura mater. Tumor was completely excised by neurosurgeons. Microscopically, tumor have two components, one of them was composed of large polygonal epitelioid cells with deposits of melanin and the other component was smaller neuroblast-like cells characterized with round centrally located hyperchromatic nuclei and scant cytoplasm in a dense sclerotic fibrous stroma. Tumor cells were occasionally arranged as alveolar nests, small nests and solid sheets. Necrosis, hemorrhage, nuclear pleomorphism and mitoses were not evident. Immunohistochemically tumor was positive for HMB-45 and negative for synaptophysin, pancytokeratin, epithelial membrane antigen, protein S-100, glial fibrillary acidic protein, chromogranin A, ubiquitin. The postoperative course was uneventful. There was neither residual tumor nor recurrences at the control of 3., 6. and 15. months of post operation duration with radiologic evaluation (magnetic resonance images) .

PP4-277
PRIMARY SCHWANNOMA OF LYMPH NODE IN THE RETROPERITONEAL REGION

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Intranodal schwannoma is an uncommon benign tumor of lymph node. We report a case of an intranodal schwannoma in the retroperitoneum of a 61-year-old woman. The patient underwent left total nephrectomy for the retroperitoneal mass thought to involve the kidney. Macroscopic examination of the specimen revealed a well-circumscribed 4.5x4.0x4.0 cm nodule located in the perihilar fat. Microscopically, the nodule was composed of a proliferation of bland spindled cells, which were immunohistochemically strongly positive for S100 protein, and negative for Actin 1A4, HHF35, Desmin, Melan A, Cytokeratin AE1/3, CD 68, CD 34, and CD 45. The pathological findings led to a diagnosis of a very rare case of primary intranodal schwannoma presenting as a retroperitoneal mass.

Infectious Diseases

PP4-278
DIGESTIVE ACTINOMYCOSIS, REPORT OF 4 CASES.

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Background: Actinomycosis is a chronic granulomatous infection, that is relatively rare, caused by a Gram positive anaerobic bacteria. Digestive locations usually simulate a malignant process. Diagnoses difficulties usually lead to a surgical resection. Method: We report four cases of digestive actinomycosis, located to the appendix, the coecum and to the pancreas in respectively, 2 cases, 1 case and 1 case. The appendic ular location presented clinically as an appendicular syndrome. The two other localisations simulated a neoplastic process. Results: The 4 patients underwent a surgical resection. Histopathological examination has leaded to the correct diagnosis, showing a granulomatous infiltrate, surrounding colonies of actinomyces. Penicillin therapy was indicated in the 4 cases with a favourable clinical outcome. Conclusion: Abdominal location of actinomycosis is a rare with poorly specific clinical and radiologic features, the diagnosis is often made on surgical resection specimens. Treatment is based essentially on a well adapted antibiotherapy.

PP4-279
OCCURRENCE OF GASTRO-DUODENAL GIARDIASIS AND/OR HELICOBACTER PYLORI INFECTION AFTER GASTRIC ACID SUPPRESSION

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ABSTRACT Giardia lamblia (G. lamblia) is a common world-wide parasitic infestation. This study aims to detect gastric and/or duodenal G. lamblia colonization in patients receiving acid suppressing drug (Omeprazole) to assess its pathological repercussions on gastrointestinal mucosa and its link to Helicobacter pylori (H. pylori) micro-organisms.Forty patients (30-67 years of age) attending Endoscopy Unit of TBRI were enrolled. They were classified into two main groups; group (I): included 10 dyspeptic patients with no history of acid suppressing drug intake, group (II): included 30 patients complaining of dyspepsia or heart burn receiving proton pump inhibitor (Omeprazole) orally in a dose of 20 mg/day for 1-6 months [Benzimidazole = 5-Methoxy-2-(4-Methoxy-3,5-diMethyl-2-pyridyl-methylsulphinyl)] (C17H19 N3O3S= 345.4) as a treatment for gastro-esophageal reflux disease (GERD) or peptic ulcer.All cases were subjected to routine clinical examination, laboratory investigations and upper gastrointestinal endoscopy. Gastric and duodenal biopsies as well as duodenal aspirate were obtained from all cases. All biopsies were assessed using haematoxylin/eosin and Masson trichrome stains for histopathological evaluation, Geimsa stain for H. pylori detection and immunohistochemistry for proper identification of G. lamblia trophozoites.There was a clear male predominance in Giardia-positive patients. They tended to be older than control cases. Diarrhea, early satiety, abdominal pain or bloating were encountered more in positive cases. In acid suppressed patients (group II), giardiasis was detected in 40% of gastric biopsies, combined with H. pylori in 16.67%. Meanwhile; G. lamblia was evident in 76.67% of duodenal biopsies and 43.33% of duodenal aspirate. In gastric giardiasis, lymphocytic gastritis and intestinal

metaplasia were detected in 75% and 58.33% of cases respectively and were predominated in combined infection (100%). Duodenal giardiasis caused inflammatory infiltrate of the lamina propria in all cases (100%) with flattening and shortening of duodenal villi in 86.95% . Our results conclude that: Gastric acid suppression encourages G. lamblia colonization in the stomach manifested by lymphocytic gastritis or intestinal metaplasia. Gastric pathogenicity is more evident in combined G. lamblia and H. pylori infections. Duodenal biopsy is more accurate than duodenal aspirate in detecting G. lamblia infection. Caution in prolonged use of gastric acid suppressive drugs should be adopted in G. lamblia endemic areas.

PP4-280
RHEUMATOID ARTHRITIS ASSOCIATED WITH SPLENIC LEISHMANIASIS

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Rheumatoid arthritis (RA) is a chronic immune-complex disease which affects mostly women during the second and third decade of life. Due to prolong treatment with immunosuppressive drugs and the immunodeficiency which develops later in the course of the disease, it has rarely been described development of malignancies and opportunistic infections. Splenic leishmaniasis after treatment for RA has not been reported. We report the case of a 65-year-old woman who was admitted in our hospital with fever, night sweats, abdominal pain for the last 2 weeks, and weight loss (10 kgr) during the last 3 months. From her past medical history the patient was receiving treatment with methotrexate due to RA for the last 5 years. On physical examination it was found a palpable spleen, about 20 cm below the left costal margin. No peripheral lymphadenopathy, hepatomegaly or skin rushes was found. Hematological examination revealed pancytopenia. Because of the huge size of the spleen and the pancytopenia, a splenectomy was performed. Histological examination of the excised spleen revealed macrophages stuffed with ingested parasites, morphologically consistent with leishmania. The patient had no history of traveling to endemic areas with leishmaniasis. Test for leishmania using indirect immunofluorescence was strongly positive. A bone marrow biopsy revealed leishmania parasites in macrophages. The patient received treatment with amphotericine B and 3 years later she is in an excellent condition with the hematological abnormalities been resolved. In the present study we report the first case of splenic leishmaniasis after treatment for RA. Among other opportunistic infections, visceral leishmaniasis should be suspected after prolong treatment for RA.

PP4-281
SCHISTOSOMA HAEMATOBIUM (EGYPTIAN STRAIN): RATE OF DEVELOPMENT AND EFFECT OF PRAZIQUANTEL TREATMENT

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This study investigates the development of Egyptian strain of Schistosoma haematobium (S. haematobium) and the resultant immunohistopathological and biochemical changes of organs likely affected. Meanwhile the response of different developmental stages of S. haematobium worms to praziquantel (PZQ) was examined. S. haematobium-infected hamsters (400 cercariae/hamster) were classified into 4 main groups, they were treated 35, 55, 75 and 95 days post infection (PI). Each group was subdivided into three subgroups; two of them were treated orally with PZQ in a dose of 300 mg/kg or 500 mg/kg divided on two consecutive days, while the third one was left without treatment. Treated groups were sacrificed 20 days post treatment. Infection with S. haematobium became patent 75 days PI, tissue egg load and worm fecundity were higher 95 days and maximal 115 days PI with oogram pattern comparable to that in S. mansoni infection. In the liver, small cellular granulomas were observed 75 days PI with preponderance of CD4+ T-cell phenotypes. In the urinary bladder, only sub-mucosal focal Brunn's nests formation and angiogenesis without typical granulomas were observed. 95 and 115 days PI, confluent granulomata with multiple eggs in the center were observed in the liver and urinary bladder with preponderance of CD8+ positive T cells in the liver and hyperplasia of the urinary bladder lining epithelium with cystitis cystica and papillae formation. Higher worm eradication was recorded with the higher dose of PZQ tested in animals treated 75 and 95 days PI. In conclusion, in spite of the long prepatent period of the Egyptian strain of S. haematobium, sensitivity to PZQ was recorded early after infection. Granulomata were similar to that of S. mansoni in the livers and urinary bladders yet they were confluent with multiple eggs in the center, hyperplasia of the urinary bladder urothelium with cystitis cystica, papillae and Brunn's nests formation predictive of malignant changes with no hepatocyte dysplasia were detected.

PP4-282
A DIAGNOSTIC PITFALL IN SAMPLES FROM BRONCHIAL ASPIRATES: A FALSE HUMAN CONTAMINATION CAUSED BY AUREOBASIDIUM PULLULANS IN THREE PATIENTS

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Background: A. pullulans is a saprophyte dematiaceous fungi, which can exceptionnally caused infection in human. Peritonitis, cutaneous infection, meningitis, or keratitis, abcesses in the spleen and jaw due to A. pullulans have been described in adults. In the lower respiratory tract, Aureobasidium species have been very exceptionally isolated. Patients and results: Three cases were observed in a single laboratory, the same week. The patients were: a 60-year-old man presented with a left upper lobe tumor and carcinomatous cells in bronchial smears; a 39-year-old woman with pneumonia and numerous inflammatory cells in bronchial smears; and a 67-year-old man with chronic cough and normal epithelial ciliated cells in bronchial aspirates. In all smears, numerous yeasts measuring from 3 microns to 5 microns were observed. In 2 cases, ellipsoidal unicellular smooth conidia, variable in size, sometimes budding, and originating from rare septate hyphae were noted. These fungi were stained with Papanicolaou, May Grunwald Giemsa, Alcian blue, mucicarmin, and Gomori-Grocott methods. The same fungi were isolated from saponin solution used for red cells lysis before smear staining. Diagnosis was made by comparing the morphology of fungi, both in bronchial smears and in saponin. Finally, fungal culture from saponin isolated A. pullulans. It was well identified due to the presence of large thick-walled black cells that have small single-celled conidia budding. Comments and conclusion: exogenous contamination by microorganisms can frequently caused false diagnosis of infectious diseases in pathology. However, these contaminations should be suspected when the same features are observed in different samples from the same laboratory, when the morphology is very unusual, and/or when non inflammatory cells are detected. A number of fungi of low virulence, including A. pullulans are often considered contaminants when isolated from healthy hosts. However, a recent review of the English literature revealed 25 clinical cases of Aureobasidium spp. infection. Moreover, A. pullulans has affinity for synthetic materials and surgically implanted Silastic devices, causing infection in human and making the differential diagnosis with false contamination very difficult.

PP4-283
PATOGENETIC VARIANTS OF CASEOUS PNEUMONIA FROM THE POINTOF VIEW OF TEACHING ABOUT PATHOMORPHISM OF TUBERCULOSIS
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The aim of this research is to study the structure peculiarities of pathogenetic variants of caseous pneumonia (primary and secondary) in the 80th years of XX century and present days. Material and methods. The comparative patomorphologic analysis of the deaths cases of patients having tuberculosis of breathing organs based on the materials of specialized prosectorum of Chelyabinsk regional antituberculosis dispensary for 1986 (99 observations, 1st group) and 2006 years (105 dead patients, 2nd group) were held. Results. Was found the increasing of widespread destructive forms of tuberculosis infection such as fibrocavernous tuberculosis of the lungs (61%) and primary caseous pneumonia (PCP, independent clinic anatomic form of tuberculosis; 20%). The basic structural display of acute progressiveness of tuberculosis infection of the lungs is caseous pneumonia which can be as independent form of tuberculosis (PCP) and also it can be the stage of progress of another clinic morphologic forms of this infection (secondary caseous pneumonia: SCP). In 1986 PCP was registered in 3 cases (about 3%). The process was unilateral with the lesions of the higher lobe of the lungs. In 2006 PCP was founded in 20% of observations of tuberculosis of breathing organs. In the second group was a registered bilateral lesion of the lungs with the localization of the process more than in 3 lobes. Histological research showed that separating the zone of caseous necrosis polymorphocellular torus was introduced by lymphocytes, macrophages, neutrophils, few epithelioidcells. The density of demarcating cellular infiltrate in the observation of 2nd group was significantly lower than that of the 1st group. SCP developing in the terminal stage of another forms of tuberculosis: infiltrate, acute cavernous, fibrocavernous. The growth of the frequency of SCP with 23.2% in the 1st group, up to 68.6% in the 2nd group. In the most cases SCP was stipulated by progressiveness of fibrocavernous tuberculosis (60.8% in 1986 and 86% in 2006). Conclusion. For the last time the growth of observations with caseous pneumonia is registered. One of the structural peculiarities of modern pulmonary tuberculosis in the development of the (SCP) which in the most cases complicates the course of fibrocavernous tuberculosis. This situation from the point of the teaching about pathomorphism may be interpreted as a negative stage of pathomorphism of tuberculosis.

PP4-284
CYSTIC ECHINOCOCCOSIS OF THE THYROID GLAND: A CASE REPORT AND DIAGNOSTIC PROBLEMS
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In humans, cystic echinococcosis is one of the most serious helminthic diseases worldwide. The larval stages (cysts) of Echinococcus granulosis are mainly located in liver and lung. Other locations of infection are rare, which may occasionally hinder its diagnosis. A 65-year-old woman was admitted with a swelling in the right lobe of the thyroid gland of 2 years duration. Laboratory investigations and thyroid function were normal. Thyroid scanning showed a cold nodule in the lower pole of the right thyroid lobe. Thyroid ultrasonography showed multiple cystic nodules in the thyroid gland. To maka a differential diagnosis of the thyroid nodule, fine-needle aspiration biopsy (FNA) was performed and diagnosed as nonspesific inflammation. During aspiration biopsy, the patient did not present a clinical picture of anaphylactic reaction. Subtotal thyroidectomy was carried out and histopathologic examnation revealed a hydatid cyst. Subsequent to the histologic diagnosis (which was hydadit cyst), review of the FNA biopsy showed acellular laminated fragments; it had not been taken into consideration before. In endemic regions-like our country, patients presenting with cystic nodular lesions of thyroid-unusual location of hydatidosis- hydatic cyst should be keept in mind.

PP4-285
GASTRIC PRESENTATION OF STRONGYLOIDIASIS IN A DIABETIC PATIENT
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Strongyloides stercoralis is a common intestinal helminth. Strongyloidiasis is a worldwide parasitic infection affecting approximately 75 million people. Prevalence of up to 25% has been reported. In healthy hosts, the parasite usually does not cause any symptoms, or only mild symptoms those are limited to the small intestine. In immunocompromised host, massive infection may occur, that is causing hyperinfection syndrome or disseminated strongyloidiasis. There are few reports of gastric involvement, especially presenting as pylor stenosis. A 78 year-old male patient had admitted to local city hospital with complaints of epigastric pain, nausea and vomiting. He had those complaints since two months. An upper gastrointestinal endoscopy had revealed hyperemia and erosions at gastric antrum. The patient had been discharged after a twenty days treatment period with relieve of the symptoms. After a short period the same symptoms showed a recurrance and he was referred to our university hospital. Meanwhile he had a weight loss of 25 kg. At his admittance, physical examination was almost normal other than peripheral edema. Laboratory examination revealed a hyperglicemia of 161 mg/dl and hypokalemia with hypochloremia. An upper gastrointestinal endoscopic examination was performed again. In this procedure eosophagitis at the lower segment of eosophagus was observed. Cardia and fundus were normal, while edema was observed at corpus and antrum with pylor stenosis. In histopathological examination of gastric biopsy, adult forms of Strongyloides stercoralis were found in gland lumen and lamina propria accompained with mixed inflamatory cell infiltration and regenerative changes at gastric epithelium. As he refused the therapy, the patient did not receive a spesific treatment for strongyloidiasis. Here we report a case with diabetes mellitus and gastric strongyloidiasis leading to pylor stenosis.

PP4-286
UNCOMMON LOCATIONS OF HYDATID CYST
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Hydatid cyst is a parasitic disease, formed by Echinococcus granulosus, which is a social and economic problem in developing countries that effects public health directly. Liver and lungs are the most common locations of hydatid cyst but it may develop in any part of the body. In this study we analyzed 154 cyst hydatid and presented cases with uncommon locations as spleen, bone, intraarterial, ovary, adrenal, heart, mesenteric, retroperitoneal, subcutaneous tissue, breast, intramuscular tissue. We concluded that the diagosis of cyst hydatic should be considered in patient with cystic mass if the patient lives in geographic region that have a high risk for Echinococcus granulosus or migrated from or visited an endemic area.

PP4-287
THROMBOTIC THROMBOCYTOPENIC PURPURA (MOSCHOWITZ SYNDROME) AND DEATH FOLLOWING YELLOW FEVER VACCINATION

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A 40-year-old woman had, received yellow fever vaccination before her travel in French Guiana, presented after 10 days at emergency room of Regional Hospital of Cayenne (French Guiana) with complaints of fever and general malaise since 3 days. On examination, she has arthralgia, arm and leg ecchymosis and petechia, paleness mucosa, headache and discrete confusion. Laboratory test showed an haemolytic anaemia, an thrombopenia in favour a thrombotic thrombocytopenic purpura (TTP)(Moschowitz Syndrom). Evolution go to worse at death the 09 January 2006 and autopsy was performed revealed widespread microthrombosis of small vessels in all the examined organs in conformity with suspected TTP. The onset of symptoms soon after vaccination and failure to identify any other cause suggest that the TTP was an adverse reaction to the yellow fever vaccine.

PP4-305
SHOCK SYNDROME AND ADVERSE OUTCOME OF A CASE OF DENGUE FEVER CAUSED BY DEN-2 VIRUS INAUGURAL OF 2006 EPIDEMIC DISEASES IN FRENCH GUIANA

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If in neighbouring states, French Guiana is regularly affected all 2 at 6 years for a Dengue fever epidemiological crisis because local conditions are more favourable at Aedes aegypti development. The four serotypes of Dengue fever are present in French Guiana but DEN-2 was more predominant in 2006 strong epidemic. This affected more than 13 700 subjects and caused four death directly release with DEN-2. In this epidemic, our cases was the second death, a four years old child, death at home on night 30-31 March. He consult tree days ago and present any sign of gravity. This case take all measures to confirm aggressively of serotype and more then 180 diseased was recovered with 57% by strong non hemorrhagic form. Blood samples not obtained, the aim of post mortem investigations was taking tissues and liquids for Dengue fever confirmation from Pasteur Institute of Guiana. These demonstrate a typical hypovolemic Dengue shock syndrome with poorly hepatic hemorrhagic lesions but diffuses acute histological alterations of sweat acini's.

Miscellaneous

PP4-288
INTRODUCING PATHONET, A VIRTUAL HISTOLOGY LABORATORY

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BACKGROUND Virtual microscopy has become accepted, both as a new research tool and as an application used in education and in routine surgical pathology as well. After the problem of high-quality, whole-slide-digitizing was solved we are working to built up a holistic virtual histology laboratory. AIMS During the past few years the staff of the 1. Dept. of Pathology, Semmelweis University, in association with 3DHISTECH Ltd. were continuously developing a web portal, mainly for pathologists, aiming to provide a platform, to share and consultate problematic cases, and to built up an open access knowledge pool, where interesting cases are to be discussed and virtual slide seminars are organized. As an University institute we also emphasize to provide easy-to-handle and up-to-date web-based digital reference book for our students, thus we uploaded both undergraduate and postgraduate educational materials, for medics and for pathology residents as well. The aim of this presentation is to acquaint the features of this virtual meeting place: Pathonet RESULTS Till the submission of this abstract, about a hundred users have registered on the portal, we had about 9000 page loads from abut 900 unique and 500 returning visitors so far which is a doubling in the number of visitors in a year. Till 2006, the portal guarantees the background for the Hungarian Slide Seminars. Previewing and the discussion of the cases in our institute is no longer made with optical, but the virtual slides. Preparing for exams the students can use another feature on the portal. In an e-book form the uploaded slides of the educational material will be completed with texts, macroimages and comments. From the beginning of the next semester the whole concept of histology teaching will be changed in our Institute. Instead of the optical microscopes we use computers and via the portal the students could revise the slides after practices and before exams. Recently we have developed a new, completely free feature for our registered users on our portal for editing cases and uploading whole slides on Pathonet server to use it as reference to their publications. The server generates a unique link to the slide. Installing the MiraxViewer and typing the link to a webbrowser or clicking on it if it appears on the online form of a journal article, the slide will open in a MiraxViewer window. CONCLUSIONS Pathonet portal is a user-friendly meeting place for pathologist.

PP4-289
"I'AM STILL LEARNING" – INTRODUCING E-SCHOOL, A NEW WAY TO STUDY PATHOLOGY

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BACKGROUND During the past years the developments on the field of virtual microscopy have reached a professionally accepted higher level. Now the question is no longer weather the digital microscope could be applied equivalently to the conventional microscopes but what additional benefits can we gain using the digital slides. One of the most popular field of using digital slides is education. As a University Institute,

teaching pathology, we also encounter the most serious challenges, measuring up to the expectations of our prime privileged customers, the students. AIMS On the 8th European Congress on Telepathology and 2nd International Congress on Virtual Microscopy we reported our first experiences on building up our telepathological workstation and testing it for gradual education. After the success of virtual microscopy on pilot histology practices we decided to replace the conventional microscopes to computers and in strong cooperation with 3DHISTECH Ltd. to design and create an educational software: E-School. RESULTS The E-School software-package now contains 3 major parts: an encoder, a viewer and an E-School server software. With the encoder you can encrypt your document, written in html format, so Universities or publishers of any kind can protect their materials and can ask license fee for them. Of course these documents can be free as well. The viewer can decode the documents and in case of non-free materials, via the E-School server the product keys could be handled by the product owners. The major advantage of the system is that digital slides, stored on a slide server or on DVD can be linked to the document allowing entire slide access. There is a self-testing and an exam module in the software-package as well. The educational material of our Institute is now fully digitized and a histology textbook is written with the E-School softwer. On the beginning of the next semester we launch our digital histology lab in regular practice. CONCLUSIONS In the era of e-learning it is outmost important for Univerities to provide a streamlined platform for her students where they can benefit all the possibilities of the advances in computer sciences. The E-School system is a good solution for compiling educational material for students and as the documents could be encrypted as well, it is also appropriate for publishing e-books of histology with real rich-in-details images and entire slide access.

PP4-291
AUTOMATED FLUORESCENT SLIDE SCANNING

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Introductions: Today there are several whole slide imaging systems for bright field, but there is no product supporting fluorescence scanning. To digitize the daily workload of a routine histology laboratory walk away automation is needed. Our goal was to develop a system which is capable of scanning both bright field and fluorescent samples automatically. Materials: We used a Mirax Scan (Carl Zeiss MicroImaging, Thornwood, NY) whole slide imager with an optional filter changer with fluorescent filters for DAPI, FITC and Rhodamine. The light source was an X-Cite 120 metal halide lamp (EXFO, Vanier, QC). The Marlin F-146C (Allied Vision Technologies, Stadtroda, Germany) fire wire camera was applied for image capture. A Celsius PC (Fujitsu Siemens Computers, Maarssen, Netherlands) with dual 3.2 GHz CPU and 3 GB RAM was used for controlling the scanner microscope. We developed the software with Visual Studio (Microsoft, Redmond, WA) and C++ Builder (Borland Software, Scotts Valley, CA). Methods: Mirax Scan uses a preview camera to locate tissue samples on a slide. Fluorescent samples are usually invisible to such a low magnification camera in normal illumination. To overcomes this issue users are required to mark the sample with a marker pen. At fluorescent slides the exposure times can vary from few milliseconds to several seconds and the different channels have different exposure times. In the selected area Mirax Scan is auto focusing on grid points using a special algorithm developed for fluorescence, which is continuously adjusting the exposure time. On the focused field of views exposure times are measured for every selected fluorescent channel. The sample is then digitized using an interpolated focus map and the shortest exposure time in every channel respectively. Results: A 10 x 10 mm sample using a Plan-Apochromat 20x / 0.8NA objective with a resolution of 0.23 µm / pixel in 3 channels, with exposure times of 52, 100 and 48 ms was scanned in 40 minutes. The system was tested with success in a walk away scanning mode with 30 slides yielding focused images and slides. Field of view black and white image compensation was not necessary. Conclusions: Fully automated multichannel, fluorescent scanning is feasible. Longer scanning times compared to bright field are inevitable but acceptable since the required scanning volume is much lower on average. With further algorithm development and the use of cameras suited for fluorescence scanning can be sped up.

PP4-292
PARAGOMINUS WESTERMANI: CASE REPORT

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Male patient, 80 years old, history of pulmonary tuberculosis (class III, ATS) and diabetes type II. The patient started three days before hospital admission with cough and dyspnea. The day of his admission he presented fever and acute respiratory failure. Arterial blood gas analysis showed respiratory acidosis and blood sample with eosinophilia. He required orotracheal intubations and mechanical ventilatory support. Chest x-ray showed pulmonary bilateral reticulonodular infiltrates and bronchiectasis. The patient died 24 hours after hospital admission. At the post-mortem examination we found histological changes due to diabetes mellitus and tuberculosis. Lung tissue showed bronchiectasis with colonization by aspergillus sp. And paragominus westermani Paragonimiasis is a parasite disease with low frequency of presentation in Mexico, is acquired by eating row crab meat. The body sites affected are lungs and brain. Differential diagnosis is with tuberculosis and fasciolosis. Treatment is albendazol.

PP4-293
ADRENAL ADENOMATOID TUMOR

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Adenomatoid tumors are benign neoplasms of mesothelial origin more commonly observed in the paratesticular area of the testis and corpus uterus. Extragenital adenomatoid tumors have also been described in unusual sites such as heart, mesentery, pleura, lymph nodes and adrenal glands. We report on a case of adrenal adenomatoid tumor (AAT) incidentally discovered in a thirty two year-old woman. A surgical operation was performed. Grossly, a greyish-white, solid tumor measuring 6.5 x 5.2 x 3.5 cm occupied almost the entire adrenal gland while only a thin rim of adrenal tissue was preserved. Microscopically, the tumor was composed of anastomosing tubules lined by flat or cuboidal cells with scanty or moderate eosinophilic cytoplasm. An ensuing intricate tubular network resembled hemangioma. Cells with prominent cytoplasmic vacuoles as well as spindle cells were also present. The intervening stroma was scanty and was composed focally of smooth muscle fascicles. At the periphery of the tumor collections of lymphocytes with occasional follicle formation were seen. Immunohistochemically the neoplastic cells were strongly positive for calretinin, cytokeratin 5/6 and WT1, whereas they were negative for HMB-45, SMA, CD34, estrogen and progesterone receptors. In the reviewed literature 29 cases of AAT have been reported, all but one referring to men. As far as histogenesis is concerned, in our case there are no indications of hormonal influence on tumor progression while the development of the tumor within the adrenal gland may be attributed to the common mesodermal origin.

PP4-294

COMPARATIVE STUDY BETWEEN SEMI-AUTOMATED AND MANUAL CELL QUANTIFICATION IN DIGITAL IMAGES IN PATHOLOGY

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Background: Manual quantification of immunohistochemical stained cells is the most frequent method in the current practice in pathology. To avoid the manual subjectivity, a number of automated and semi-automated processes have been previously developed and described. In the present study, we have developed different semi-automated processes in order to quantify different immunohistochemical markers. To obtain a gold-standard reference, we have performed manual quantifications and we have also evaluated inter and intra-observer variability. Method: 196 digital color images were obtained from sections of Hodgkin's lymphoma biopsies and stained with various nuclear, cytoplasmatic and membrane markers. Each image was manually quantified twice by 3 different observers. To analyse the images, we developed a specific macro for each marker with a commercial analysis software. The global dataset obtained with the software, were dropped to an Excel Datasheet, where the number of cells was obtained after we introduced different corrector factors. To evaluate the agreement degree between manual and semi-automated method and to quantify our intra and inter-observers variability, a comparative statistical analysis was performed with SPSS 11.0. Results: Globally, the variability was higher for intra-observers than for inter-observers counts, except in nuclear stains were both types of variations have similar differences. The comparison between semi-automated and manual methods shows that both methods have the same way of cell quantification. Both manual and automatic quantification showed a higher variation when the images contained more than 100 positive nuclei. Discussion: This study quantifies variation observed in manual quantification and shows that the different semi-automated procedures developed for each marker in our laboratory represent a valid alternative to manual quantification. (PI 04/1440, 04/1467, 05/1527).

PP4-295

NEW APPROACH FOR AUTOMATIC QUANTIFICATION OF IMMUNOHISTOCHEMICAL NUCLEAR MARKERS IN DIGITAL IMAGES

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Background: Several procedures of digital image analysis have been developed for quantitative evaluation of nuclear immunohistochemical markers with prognostic, diagnostic and therapeutic significance in current medical practice. To our knowledge, do not exist effective algorithms that permit to obtain a precise cell quantification in digital images with a high grade of complexity. The aim of this study is to develop a new methodology capable to analyze correctly, images with high variability in their morphology, cellular density, stain intensity and cell distribution. Method: 118 digital images from 4 different immunohistochemical nuclear markers were captured with different grade of cell concentration and clusters composition. Two coordinated macros were elaborated to perform the automatic count of positive nuclei. The first macro was developed with a commercial software that allows the modification and segmentation of the images. All extracted information was dropped to an Excel datasheet, where we have developed a macro with Visual Basic and introduced different algorithms that manage the dataset obtaining a final number of positive nuclei on each image. All statistical analysis was performed with SPSS 11.0. Results: t-Student test, Spearman correlation and ICC, showed no significant differences between the manual and the automatic count, whatever the image complexity. Kaplan-Meier and Bland Altman graphic representations indicate that cluster composition and a high nuclei density, increase variability on both types of count, and globally in more than 90% of images, automatic count was similar to the manual. Conclusion: This study, describes a new methodology for the automatic count of digital images for different immunohistochemical nuclear markers. This method improves the quantification of images with a high complexity in their nuclear composition. Variability observed between human and automatic count are the same as inter or intra-observer variability, which is accepted in the current clinical practice. (PI 04/1440, 04/1467, 05/1527).

PP4-296

EFFECTS OF DIGITAL IMAGE COMPRESSION ON COMPUTER-ASSISTED IMAGE QUANTIFICATION OF IMMUNOHISTOCHEMICAL STAINED CELL NUCLEI

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Background: The analysis of digital images and standard compression algorithms in current clinical practice has been used since some years ago. Image compression may reduce the amount of computer memory required for store images. The consequences of image compression have been previously evaluated on different diagnostic techniques. The aim of this study is to analyse the consequences of computer-assisted quantification, between uncompressed Tiff format and different levels of image compression in Jpeg format of immunohistochemical stained cells. Method: Digital images were captured with the software Leica IM50 4.0, in Tiff format from tissue samples stained with immunohistochemical markers, Ki67 (n=24) and FOXP3 (n=24). In a second step Tiff images were converted with the software ACDSee 6.0 to Jpeg files with a compression factor of 0, 50 and 100%. All the captured and converted images (n=196), were analysed with two coordinated macros developed with an image analysis software and an Excel datasheet. Quantified parameters were the total stained positive area and the number of positive nuclei. Tiff images results were compared with the different compression factor in Jpeg format, with Kaplan-Meier and Bland Altman plots using SPSS 11.5 Statistical Software. Results: Globally, variations in the quantification of positive stained areas between Tiff and the different compressed files are similar. On the other hand, cell count differences are lower with a compression factor of 0% and higher with a 50 or 100% of compression. Nevertheless, all compressed formats have a similar variability when images have less than 100 nuclei, and in this case, this variability is lower than globally. Conclusion: This descriptive study shows that image compression is a source of variation in automatic count of digital images. In these conditions, image compression should be omitted for immunohistochemical digital image quantification, overall in images with more than 100 nuclei. (PI 04/1440, 04/1467, 05/1527)

PP4-298

MUCOEPIDERMOID PAPILLOMA OF CONJUNCTIVA

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Background: Mucoepidermoid papilloma of conjunctiva is a papillary lesion. Columnar cells and Goblet cells line these papillary structures. Exophytic mucoepidermoid papilloma of conjunctiva has not been reported in the literature previously. Case report: Forty-three year-old male patient presented with a cojunctival lesion. Excisional biopsy was performed. Macroscopically the greatest dimension of the tumor measured 0,9 cm. Microscopically, a papillary lesion was seen. Lining epithelium was columnar cells and Goblet cells. A diagnosis of mucoepidermoid papilloma of conjunctiva was given. Conclusion: Conjunctival mucoepidermoid carcinomas have been described but there is no evidence of the presence of any precursor lesions. Two separate case reports describe inverted mucoepidermoid papillomas. Present case had a papillary configuration and did not contain an inverted component. Although conjunctival papillomas show HPV effects in present case cytopathic HPV effects could not be detected. It can be predicted that these lesions do not carry a potential of malignancy but because there is no previous information about biological behavior potential of these lesions, strict clinical follow-up is recommended. Whether mucoepidermoid papilloma is a precursor lesion of mucoepidermoid carcinoma of conjunctiva is a problematic issue which should be solved in future reports.

PP4-299

OXALATE CRYSTAL DEPOSITION ASSOCIATED WITH MULTIPLE INFARCTION IN THE SPLEEN OF A PATIENT WITH NON-CIRRHOTIC PORTAL HYPERTENSION

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Splenic infarction is an unusual finding of cirrhotic or non-cirrhotic portal hypertension. Dystrophic oxalosis can be seen in thyroid, breast, and retina in many conditions. Oxalate deposition is well known in renal pathology, and had been reported in many organs in patients with primary and secondary oxalosis. We couldn't find oxalate deposition in the spleen in the literature. The presented case was a 59-year-old man with non-cirrhotic portal hypertension. Pathological examination of splenectomy revealed moderate splenomegali, multifocal infarctoid areas causing capsular retraction. Microscopic investigation revealed multifocal infarction of different ages. Gandy-gamma body formations were also present. Surprisingly, multifocal heavy oxalate crystal depositions around the necrotic area, and perivacular spaces in vessels nearest to infarction were seen. With the presentation of this case, spleen can be included in the oxalate depositing organs. The oxalate deposition in the spleen may be dystrophic due to infarction, or may also be the first sign of hyperoxaluria.

PP4-300

A CASE OF PARARENAL RETOPERITONEAL CASTLEMAN DISEASE: INCIDENTALY DETECTED AS AN ADRENAL GLAND MASS

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INTRODUCTION: Castleman's disease, or angiofollicular lymph node hyperplasia, is a relatively rare disorder characterized by benign proliferation of lymphoid tissue. Castleman's disease is usually related to chronic herpes virus infection and it is usually localized in the mediastinum. Retroperitoneal and especially pararenal localization is very rare. CASE REPORT: A retroperitoneal mass was incidentally found on the abdominal ultrasonographic imaging of a 51 year-old woman. Surgery was planned with a preoperative diagnosis of non-functioning adrenal gland neoplasia. The tumor was removed by retroperitoneal laparoscopic adrenalectomy. In gross examination the tumor was an encapsulated, homogenous pale colored mass measuring 4.0x5.5x5.5 cm. Microscopic examination showed a histologically normal adrenal gland, and a large lymph node rich in vascular structures. The nodal architecture was altered by an increased number of lymphoid follicles. These lymphoid follicles were unusually small and their germinal centers consisted of radially penetrating capillaries, some of which formed a characteristic "lollypop" structure. Many of these capillaries were surrounded by wide cuffs of hyaline substance. Multiple concentric layers of mature lymphocytes were surrounding the small germinal centers. Thus, the tumor was pathologically diagnosed as hyaline-vascular type angiofollicular lymph node hyperplasia (Castleman's disease) of a peri-adrenal gland lymph node. DISCUSSION: In this poster, we present a case of Castleman's disease localized in the retroperioneum next to the uninvolved adrenal and the kidney. Although it is an unusual localization Castleman's disease should be included in the differential diagnosis of retroperitoneal masses.

PP4-301

LEPROSY: REPORT OF THREE CASES AND CURRENT SITUATION IN TURKEY

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BACKGROUND: Diagnosis of leprosy is most commonly based on the clinical signs and symptoms. Laboratory confirmation of leprosy requires the demonstration of acid-fast bacilli in a skin biopsy. We report herein 3 cases which were diagnosed as borderline lepromatous (BL), lepromatous (LL) and borderline tuberculoid leprosy (BT) last year in our department. Among them, only one case was clinically suspected as leprosy before the histopathological examination. REPORT OF THE CASES: Case 1: A 26-year-old man had been complaining of spontaneously regressing and recurrent erythematous anesthetic macules and infiltrated lesions on his forearms and legs since 2 months. N. radialis and ulnaris were bilaterally palpable. Clinically vasculitis, Sweet syndrome and syphilis were suspected and a punch biopsy was done. The histopathological diagnosis was BL. Case 2: A 56-year-old man had been suffering from erythematous macules and pathches on the whole body and nasal congestion

since 3 years. N. ulnaris, radialis, poplitealis and auricularis were bilaterally palpable. Clinical diagnosis was LL, which was fully compatible with the histopathological examination of the punch biopsy. Case 3: A 45-year-old man had pale anesthetic macular lesions with erythematous border on his face, arms and back since 4 years. Clinically erythema annulare santrifugum and erythema dischromicum perstans were suspected and a punch biopsy was done. The histopathological diagnosis was BT. CONCLUSION: Leprosy is endemic in tropical and subtropical Asia, Africa, Central and South America and the Pacific regions. In Turkey, 2 595 cases were registered to our leprosy research center. Seven new cases were detected last year. Three of them, which were diagnosed in our department, were presented. As reported to WHO by 115 countries in 2006, 296 499 new cases were detected last year. Clinically, the skin lesions often resemble those of lupus erythematosus, sarcoidosis, syphilis, erythema nodosum, erythema multiforme and cutaneous tuberculosis. The most effective way of preventing disabilities in leprosy, as well as preventing further transmission of the disease, lies in early diagnosis and treatment with multi-drug therapy.

PP4-302

ULTRASTRUCTURAL STUDY IN A CASE OF PRIMARY BREAST CARCINOMA WITH OSTEOCLAST-LIKE GIANT CELLS

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Primary carcinoma with osteoclast-like multinucleated giant cells is a very rare tumor of the breast. We present the light and electronmicroscopic features of a case of invasive ductal carcinoma containing osteoclast-like multinucleated giant cells with regard to the histogenesis (origin) of giant cells. A 48-year-old woman with a 3 cm-mass in the middle part of her right breast was admitted to our hospital, and she underwent modified radical mastectomy. Macroscopically the tumor was well circumscribed with a grey-white cut surface. Microscopically the tumor was invasive ductal carcinoma with cribriform features and many osteoclast-like multinucleated giant cells between the neoplastic glands and within their lumina. Of the eleven axillary lymph nodes dissected, two were metastatic. The lymph-node metastasis also contained osteoclast-like multinucleated giant cells and stromal reactions. On immunohistochemical analysis, multinucleated giant cells were positive for CD68 and alpha-1-antitrypsin and negative for keratins, revealing their histiocytic origin. Neoplastic cells were positive for keratins and estrogen and progesteron receptors, and negative for c-erb B2. Ultrastructural analysis revealed multinucleated cells with many lysosomes, supporting their histiocytic origin, in vicinity of ordinary glandular cells, elsewhere intermixed with them. They contained neither cell junctions nor microvilli. We were able to detect cytoplasmic fusion of some mononuclear cells of the same character. We conclude that osteoclast-like giant cells are formed by fusion of mononuclear histiocytic cells.

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REVIEW AND PERSPECTIVE

Melanocytic nevi simulant of melanoma with medicolegal relevance

Guido Massi

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Abstract A group of melanocytic benign nevi are prone to be misdiagnosed as nodular or superficial spreading melanoma. This review illustrates the most frequent forms of these nevi in direct comparison with their malignant morphologic counterparts. The nevi are: hyper-cellular form of common nevus to be distinguished from nevoid melanoma, Spitz nevus (vs spitzoid melanoma), Reed nevus (vs melanoma with features of Reed nevus), cellular atypical blue nevus (vs melanoma on blue nevus), acral nevus (vs acral melanoma), Clark dysplastic nevus (vs superficial spreading melanoma), desmoplastic nevi (vs desmoplastic melanoma), benign proliferative nodules in congenital nevi (vs melanoma on congenital nevi), epithelioid blue nevus (vs animal type melanoma) and regressed nevus (vs regressed melanoma). For each single 'pair' of morphological look-alikes, a specific set of morphological, immunohistochemical and genetic criteria is provided.

Keywords Melanoma · Melanocytic nevi · Simulators · Medicolegal lawsuit

Today, melanoma is the most frequent cause of medicolegal lawsuit for pathologists [144, 145]. This is because melanoma frequently has a deceptively bland nevus-like histological appearance, and it is doomed to be overlooked as a nevus, especially by pathologists not familiar with dermatopathology. All too aware of this, pathologists today tend to overdiagnose a melanoma whenever they come across a lesion that even slightly deviates from the ordinary. This overdiagnosis, although less catastrophic than the underestimation of a melanoma, has ominous consequences for the patient. In fact, enlargement of the surgical excision, lymph node sentinel procedure and systemic therapy are incapacitating therapies, and they should be spared to anyone not bearing a true malignant melanoma. Presented here is a review of benign melanocytic lesions very similar to malignant melanoma from an architectural or cytological point of view. This study is restricted to lesions with an intra-dermal component that can be confused with an invasive form of melanoma eligible for aggressive therapy. The choice and hierarchy of the items are based on my own experience on cases of benign lesions most frequently misdiagnosed as invasive melanoma by myself and by other colleagues.

The different entities are here presented on the hypothesis that each form of nevus has its own morphological malignant counterpart (Table 1) so that the differential diagnosis must be done by comparing the characteristics of the two morphological look-alikes that have their own peculiar histological characteristics (Table 2).

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Nevi with high cellular density *vs* nevoid melanoma

A small but defined group of entirely benign lesions can be misdiagnosed as nevoid melanoma [127], a form of melanoma so named because of its striking cytological and architectural similarity with a common nevus. The lowest common denominator of such nevi is their growth in large solid sheets of cells without interposed collagen fibres, a pattern that is indeed one of the main clues for the diagnosis of nevoid melanoma [86].

The first type of nevus, which is likely to be confused with nevoid melanoma, is a cellular variant of a fairly common nevus popularized by Ackerman as Unna nevus

Table 1 Differential diagnosis between melanocytic nevi and their malignant simulators

Nevi		Melanomas
Common nevus	vs	Nevoid melanoma
Spitz nevus	vs	Spitzoid melanoma
Reed nevus	vs	Melanoma with features of Reed nevus
Common blue nevus	vs	Melanoma with features of blue nevus
Cellular Blue nevus	vs	Melanoma developed on blue nevus
Acral lentiginous nevus	vs	Acral lentiginous melanoma
Dysplastic Clark's nevus	vs	Superficial spreading melanoma ('lentiginous melanoma')
Desmoplastic nevus	vs	Desmoplastic melanoma
Spindle cell nevus	vs	Spindle cell melanoma
Proliferative nodules	vs	Intra-dermal melanoma in congenital nevus
Deep Penetrating Nevus	vs	Melanoma with features of deep penetrating nevus (plexiform melanoma)
Epithelioid blue nevus	vs	Animal-type melanoma
Regressing nevus	vs	Regressing melanoma

To minimize errors in diagnosing melanocytic neoplasms, one should always remember that for each single nevus, there is a melanomatous simulator, very close to its benign counterpart from an architectural and cytological point of view. The differential diagnosis has to be placed between the two look-alikes (i.e. between Spitz nevus and spitzoid melanoma) and not generically between nevus and melanoma. For each 'pair,' there is a specific set of characteristics and diagnostic criteria.

Table 2 Melanocytic nevi and their malignant simulators—main clues for the differential diagnosis

Nevi	Melanomas
Common nevus	Nevoid melanoma
Cellular monomorphism, dispersed nuclear chromatin, pinpoint nucleoli	Cellular pleomorphism, clumped nuclear chromatin, prominent nucleoli
Melanocytes interspersed among collagen fibres	Solid pattern of growth
Inconspicuous junctional component	Melanoma in situ-like pattern at the junction
No mitoses at the bottom of the lesion	Mitoses at the bottom of the lesion
Spitz nevus	Spitzoid melanoma
Small size and sharp lateral circumscription of the lesion	Large size, poor lateral circumscription of the lesion
Low mitotic rate, no mitoses at the bottom	High mitotic rate, mitoses at the bottom
Pigment regularly distributed	Pigment irregularly scattered, pigment at the base of the lesion
Large and confluent Kamino bodies	No or small Kamino bodies
Symmetric epidermal hyperplasia	Irregular epidermal alterations
Pagetoid spread only at the centre of the lesion	Pagetoid spread at the borders of the lesion
No expansive nodules in the dermis	Expansive nodules in the dermis and at the junction
FAS, P53, Bcl-2 scantly expressed, no chromosomal aberrations	FAS, P53, Bcl-2 expressed also at the bottom, chromosomal aberrations
Reed nevus	Melanoma with features of Reed nevus
Youngsters, lesion on the lower part of the body, no signs of solar damage	Old patients, on the face, signs of solar damage
Preserved and elongated epidermal rete ridges	Epidermal rete ridges 'consumed'
Strict nuclear monomorphism, no nuclear hyperchomasia, no mitoses	Cellular pleomorphism, nuclear hyperchromatism, mitoses
Superficial dermis only involved	Deep dermis involvement
Cellular atypical blue nevus	Melanoma on blue nevus
Random cellular atypia	Widespread cellular atypia
No nodules or solid masses of atypical cells	Nodules or large masses of highly atypical cells
No necrosis, only rare mitoses	Cellular necrosis, necrosis en masse, mitoses in number, atypical mitoses
Common blue nevus	Melanoma with features of blue nevus
No or inconspicuous junctional component	Melanoma in situ at the junction
No cellular atypia, no mitoses	Widespread atypia, mitoses
Acral lentiginous nevus	Acral lentiginous melanoma
Young age of the patient, small size of the lesion	Old age of the patient, large size of the lesion
In the superficial dermis only	Medium and deep dermis involved

Table 2 (continued)

Nevi	Melanomas
Mostly nested pattern at the junction	Lentiginous pattern prevalent at the junction
Eccrine ducts involvement in nests	Eccrine ducts involvement in a lentiginous array
No genomic alterations	Genomic alterations
Displastic Clark's nevus	Superficial spreading melanoma
Youngsters, no signs of solar damage	Old patients, occasionally signs of solar damage
Discontinuous cellular atypia	Diffuse cellular and architectural atypia
No significant pagetoid spread	Significant pagetoid spread, also present at the shoulders of the lesion
Cells mostly in nests both at the junction and in the dermis	Prevalent lentiginous array at the junction
Desmoplastic nevus	Desmoplastic melanoma
In young adults, small and well circumscribed lesion	In elderly patients, large and poorly circumscribed lesion
Presence of component of ordinary nevus or Spitz nevus or blue nevus	No nevus remnants
No signs of solar damage	Solar elastosis inside, around and below the lesion (in sun exposed areas)
Rare or no lymphocytic follicles	Lymphocytic follicles inside and around the lesion
Spindle cell nevus	Spindle cell melanoma
Vertically oriented, elongated fascicles	Roundish nests at the junction and in the dermis
Regular epidermal hyperplasia	Irregular epidermal alterations
No solid sheets of cells	Solid growth pattern (large nodules or solid sheets of cells)
Sparse and superficial mitoses	Mitoses at the base of the lesion
Proliferative nodules in congenital nevus	Intra-dermal melanoma on congenital nevus
Frequent	Rare
Cellular monomorphism, low mitotic rate	Cellular pleomorphism, high mitotic rate
Roundish silhouette	Irregularly shaped silhouette
Collagen fibres interposed	Growth in solid pattern, collagen fibres obliterated
Numerical chromosomal aberration	Structural chromosomal aberrations
Deep penetrating nevus	Melanoma with features of deep penetrating nevus
Random cytological atypia	Subtle, but widespread cytological atypia
Inconspicuous or absent junctional component	Melanoma in situ at the junction
Epithelioid blue nevus	Animal-type melanoma
Young age of the patient, small size of the lesion	Usually in elders, large size of the lesion
Sharp circumscription of the borders	Poor lateral and deep circumscription
Uniform cytology, occasionally presence of other type of nevus	Cellular pleomorphism, no nevus remnants
Regressing nevus	Regressing melanoma
Young age of the patient, small size of the lesion, multiple lesions	Old age of the patient, large size of the lesion, single lesion
No nodules of atypical cells, only sparse mitoses	Nodules of atypical cells, combined with mitoses
Rete ridges thinned but preserved	Rete ridges annihilated

None of the above reported criteria is specific. Criteria should be always used in clusters, and clinic–pathologic correlation is mandatory. Cases with conflicting criteria are possible and have to be diagnosed on individual base.

(named after the scholar who first described it) but also known as papillated or verrucoid common nevus [2, 3]. Unna nevus is typically located on the trunk and usually does not pose any diagnostic challenge whatsoever. On rare occasions, however, as said above, especially in young adults, the lesion is hyper-cellular, and cells appear overcrowded and they seem to overlap each other (Fig. 1). Collagen fibres are absent in these large masses or solid sheets of cells. Because the differences from a nevoid melanoma can be subtle [27, 95, 107, 112], it is indeed wise to study these lesions carefully and through serial sections:

– The main clue is, in our opinion, the cytological details [109, 110, 146, 158]. In nevoid melanoma, there is, with few exceptions, an evident hyperchromatism throughout the neoplasm and the nuclear features are atypical: The nuclear membranes are irregularly thickened with clumps of chromatin stuck to it, chromatin is coarse, and the nucleolus is prominent. Nuclei and nucleoli do not modify in size, colour and characteristics as cells go deeper in the dermis. In many cells, a sort of nuclear groove is also present. These aspects are absent in hyper-cellular nevi: Nuclei are small and not hyperchromatic, the chromatin is finely dispersed and the nucleolus is pinpoint.
– Although in most of the lesion, the growth pattern of hyper-cellular nevus and nevoid melanoma is indistinguishable, in nevus, the cells at the bottom of the lesion are dispersed among collagen fibres as in ordinary nevi, while in nevoid melanoma, pushing margins are most

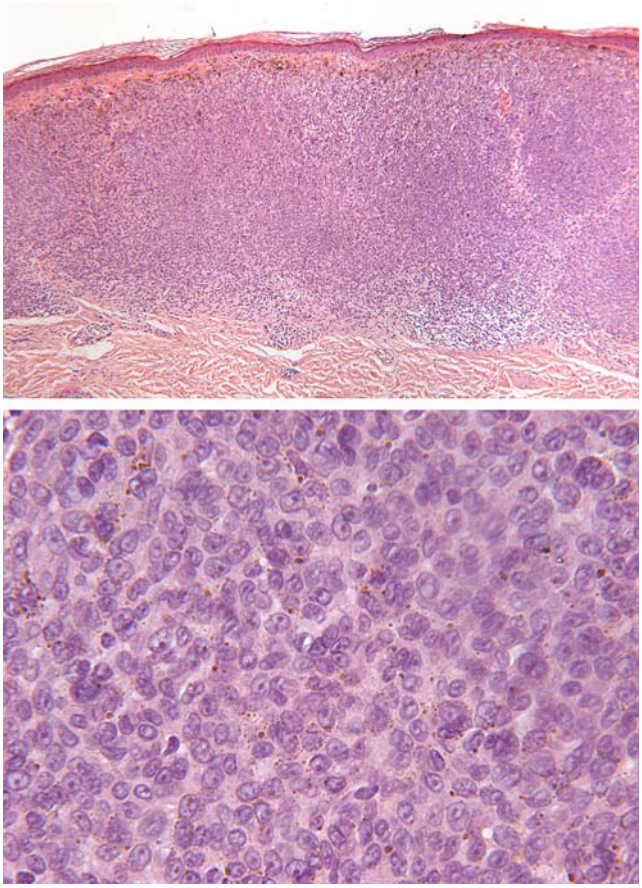

Fig. 1 This nevus shows a solid growth pattern similar to nevoid melanoma. In addition, cells seem overcrowded, and pigment is present at the base of the lesion. However, mitotic activity is nil, cells are monomorphous and have innocent looking nuclear details. The pigment is in coarse granules

frequently present. In melanoma, at the junction, large masses of cells seem to be directly connected with the epidermis or the epithelium of elongated or distorted hair follicles.

- In hyper-cellular nevi and nevoid melanomas, the junctional component can be identical; that is, it can be inconspicuous with only rare unremarkable melanocytes scattered at the basal layer [100]. However, melanoma frequently presents a melanoma in situ-like pattern at the junction: Atypical cells are aligned back-to-back substituting the keratinocytes of the basal layer. Atypical cells also involve the eccrine glands and follicular infundibulum. A subtle pagetoid spread (especially in the infundibulum) can be occasionally useful for the diagnosis of nevoid melanoma [154].
- Mitotic figures can be absent or undetectable both in highly cellular nevus and in nevoid melanomas. However, when present in large amounts (i.e. "easily found") or at the bottom of the lesion, they are a strong suggestion of melanoma, especially in a background of hyperchromatic cells.
- Pigment mostly present at the base always indicates a melanoma (this aspect regards the differential diagnosis between a common hyper-cellular nevus and a nevoid melanoma. Pigment at the base can be present in other forms of nevi). However, one should remember that nevoid melanoma can be entirely achromic.
- Immunohistochemistry could be helpful [73, 89, 90, 105, 121, 122]: In melanoma, HMB45 is positive in a number of cells at the deeper front of the lesion, a detail absent in nevi. The same can be said about Ki67, which, however, I use only at times. Authors report that p53 and cyclin D1 immunostain are useful, but also these immunostains seem to work much better in published papers than in everyday routine work [31]. It is clear, in my own opinion, that such ancillary techniques only provide an additional criterion that must be interpreted in the histological (and clinical) context.

Another form of cellular nevus similar to nevoid melanoma is a sub-set of congenital or acquired nevus termed neurocristic hamartoma, a melanocytic neoplasm present both in children and in adults, as a rule, in the head and neck regions [37, 92, 133]. Histologically, this entity has been reported in the literature in quite heterogeneous forms. However, in most cases, it is described as composed, at least in part, by solid sheets of round cells containing a spherical nucleus and regularly dispersed chromatin (Fig. 2). Cytoplasm is reduced to an almost undetectable pale rim, and cells have a lymphocytic appearance. The most striking aspect that can lead to the misdiagnosis of melanoma is the complete obliteration of the dermis, which is entirely occupied by a thick, patternless sheet of uniform nevus cells. The sub-cutis is also involved, on occasion massively, with cells infiltrating the adipose tissue. A few mitoses are also seen in these cases. These lesions are indeed rare but are likely to be diagnosed as melanomas (and to further complicate the matter, melanoma has been reported to develop in neurocristic hamartoma [102, 103]). To avoid this overdiagnosis of melanoma, the following criteria are adopted for the diagnosis of neurocristic hamartoma:

- Besides the large sheets of melanocytes, neurocristic hamartoma usually has other peculiarities namely, masses of spindle cells with collagen fibres interposed, features of neural differentiation [120] as well as aspects similar to cellular blue nevus, an entity to which neurocristic hamartoma is probably related [18, 65].
- In a few cases, the involvement of the pilar follicles is evident, and small dysmorphic follicles may be also present as the result of follicular induction (the so-called pilar neurocristic hamarthoma).

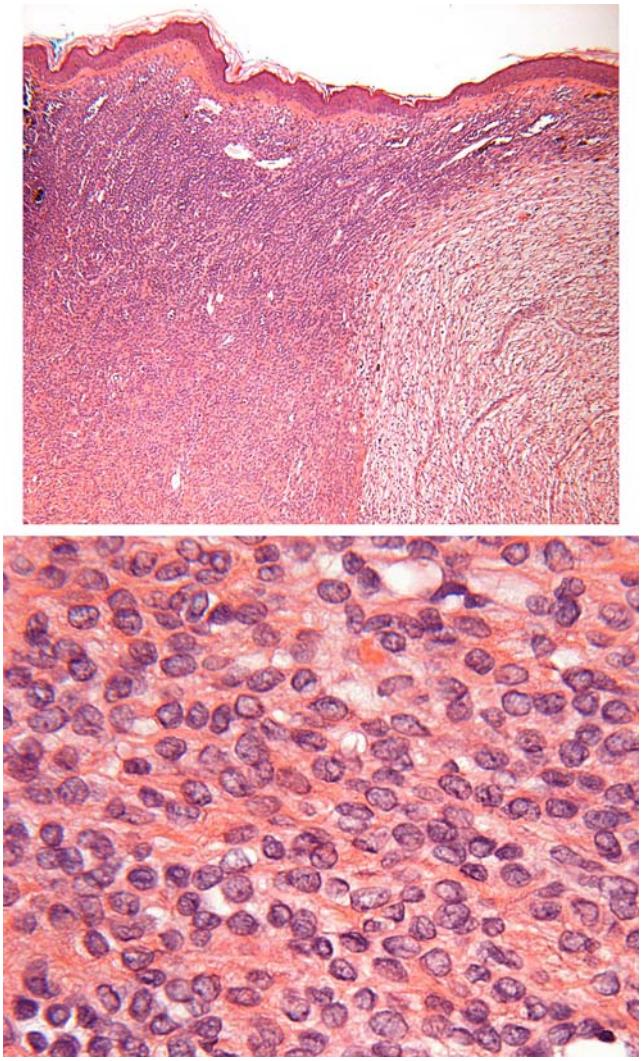

Fig. 2 This form of neurocristic hamartoma is composed on the left by a solid sheet of melanocytes, which occupies the entire dermis and the sub-cutis. The similarities with nevoid melanoma are striking, but cells have obvious benign features and no mitotic activity. On the right of the picture, at scanning magnification, maturation in spindle cell elements is seen

– In nevoid melanoma, a melanoma in situ pattern is occasionally seen at the junction, whereas neurocristic hamarthoma is entirely intra-dermal; in some cases, a grenz zone separates the epidermis from the nevus.

A third group of cellular melanocytic nevi, which can be confused with nevoid melanoma, includes nevi with large nodules of tightly packed small melanocytes (Fig. 3). These huge, irregularly shaped nodules are indeed very similar to the expansile nodules with selective advantage of growth, which is indicative of nevoid melanoma. Another misleading detail is the presence of pigment in these deep-sited nodules (and indeed pigment in the deep dermis is always a strong indication of malignancy).

In these cases, the differences from a nevoid melanoma are the following:

– These nevi have the general features of congenital nevi or of the so-called tardy congenital nevi (acquired nevi with congenital features) namely, periadnexal disposition of the melanocytes, Indian file alignment along the collagen fibres and large junctional nests.
– A benign looking cytological feature is present in the cellular intra-dermal or junctional nodules, where cells are small and uniformly distributed inside the nodule. Chromatin is evenly scattered within the nucleus, and the nucleolus is undetectable or pinpoint. Mitotic activity is virtually nil throughout the lesion. Most significantly, mitoses are absent at the bottom of the neoplasm.
– At the dermoepidermal junction, there is an innocent-looking proliferation of melanocytes arranged in nests.

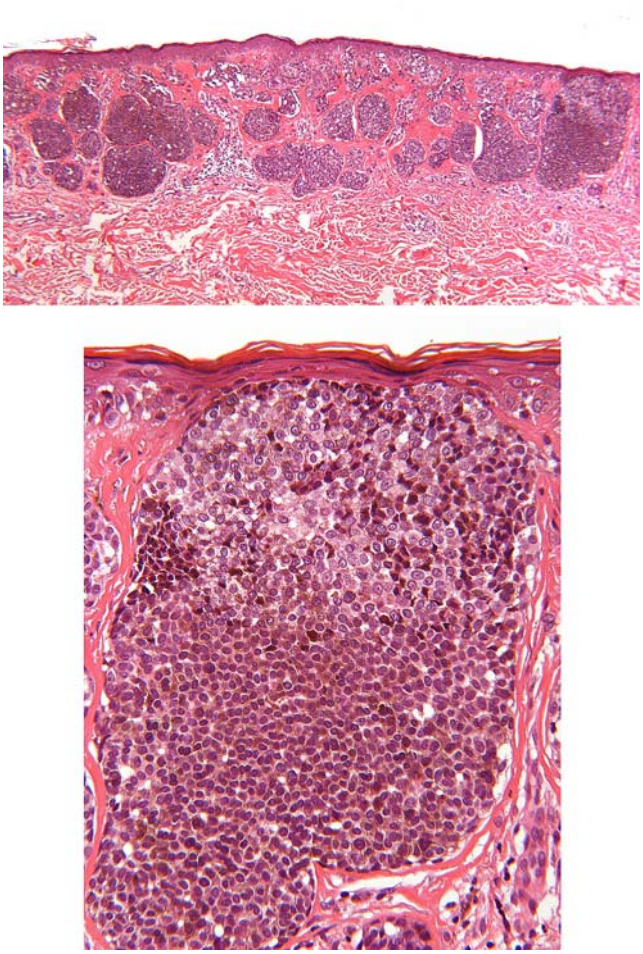

Fig. 3 In this nevus, the nests at the base of the lesion are larger than those at the top. Moreover, nests are confluent and bizarrely shaped. Pigment is mostly synthesized at the bottom of the neoplasm. Despite these alarming details, the bland cytology indicates a nevus (probably congenital). When regressing, traumatized or recurrent, this type of nevus becomes almost indistinguishable from a melanoma

Cells are typical, and a melanoma in situ type proliferation is absent. The epidermis is usually hyperplasic with elongated rete ridges.

Spitz nevus vs spitzoid melanoma

Spitz nevus is a benign melanocytic lesion composed of epithelioid or spindle cells. Cytological details can be highly atypical, and the lesion is regularly confused with a type of melanoma with spindle or epithelioid cells, called for this reason spitzoid melanoma. The similarities between a Spitz nevus and a spitzoid melanoma are so striking as to question the former's very nature and to suggest a sort of grey zone for lesions in which the resemblances with a melanoma are far more numerous than the differences. These lesions, in which a diagnosis does not seem to be based on solid ground, are called by some scholars atypical Spitz nevus, Spitz tumour or melanocytic neoplasms of indeterminate biological potential [11–14, 111, 132, 153]. For the time being, I prefer to maintain a clear-cut difference between benign and malignant lesions with Spitzian features [59, 96, 97].

In dealing with the differential diagnosis of Spitz nevus vs spitzoid melanoma, the following steps could be suggested:

- First of all, it is necessary to ascertain that the tumour in question is a melanocytic neoplasm with large spindle or epithelioid cells. To consider the nevus as a Spitz nevus, cells must be large and have a peculiar morphology. Prevalence of small cells or of cells with other features always questions the diagnosis of Spitz nevus. A neoplasm in which a combination of large and small elements is present is most likely another form of nevus (combined nevus, clonal nevus and so on) or most probably, a melanoma [7, 10].
- The presence at the dermoepidermal junction of numerous, large-sized and confluent Kamino bodies (eosinophilic, pink, periodic acid-Schiff-positive globules) always questions the diagnosis of melanoma [150]. Cases of melanoma with such bodies have been indeed reported, but the few photos presented in the papers failed to convince me.
- A number of spitzoid melanoma is found on sun-damaged skin: The absence of severe solar elastosis should promote further investigation [147]. Unfortunately, not all spitzoid melanoma arise on severely sun-damaged skin.
- The most common forms of Spitz nevus has a distinctive wedge-shaped silhouette, which is at least slightly distorted in most spitzoid melanoma.
- In melanoma, the intra-epidermal margins of the lesion are rarely sharp, even in its spitzoid variant. As a rule, a melanoma is surrounded by a lentiginous proliferation at the borders. In the dermis, at the bottom, melanoma usually has a compressive ('pushing') border. The dispersion of the cells among the collagen fibres is more consonant with a Spitz nevus.
- Although mitoses are not an indispensable requisite for the diagnosis of spitzoid melanoma, their absence, especially at the bottom of the neoplasm, should always challenge such a diagnosis [139]. Furthermore, the presence of mitoses should be sensibly evaluated: Numerous mitoses in fact can be present in rapidly growing Spitz nevus and in recurrent, regressing or traumatized Spitz nevus.
- In spitzoid melanomas, as in most melanomas, pigment is unevenly scattered and present also at the bottom of the lesion. Symmetrically distributed melanin deposits and the absence of pigment at the deepest front of the neoplasm are more indicative of a Spitz nevus. A most ominous indication of melanoma is the uneven distribution of the pigment inside the nests and between them.
- A 'perfectly' symmetrical epidermal hyperplasia, with the epidermis more expanded at the centre and gradually thinning at the periphery, is characteristic of a Spitz nevus. Usually, a melanoma with spitzoid features has a more irregular epidermal hyperplasia; moreover, areas of epidermal thinning are present: Such aspects are called 'consumption' of the epidermis and are indeed a useful indication of melanoma [23, 55, 64].
- Pagetoid spread of melanocytes above the junction is an important indication of melanoma but can be also present in Spitz nevus. However, in melanoma the pagetoid cells are more numerous, and the spread of cells involves the uppermost reaches of the epidermis and the lateral borders of the tumour.
- A symmetrical scattering of cells among undisturbed collagen fibres in the dermis ('dispersion') is indicative of a Spitz nevus. Melanomas grow in large expansile and compressing nodules with high cellular density.
- Pleomorphism is a characteristic of spitzoid melanoma, whereas cells with uniform nuclear details throughout the neoplasm or at the same level in the dermis ('zonation') are most probably benign.
- The presence of large nuclei is not tantamount of melanoma. In the epithelioid form of Spitz nevus (especially in youngsters), large vesicular nuclei with round nucleolus are possible: A more worrying detail is the presence of large nuclei with scant cytoplasm or large nuclei with large, amphophilic and angulated nucleolus.
- A general architectural disorder of the lesion does indicate a melanoma but at times is difficult to evaluate. A common indication of melanoma is the presence in the deep dermis of nests larger and more atypical than those in the superficial dermis. Large,

confluent, irregularly shaped, highly cellular masses always are indicative of melanoma.

- HMB45 immunostain decorates the melanoma cells unevenly throughout the lesion, whereas a sort of symmetric top-to-bottom zonal gradient is more indicative of Spitz nevus. Ki67 is positive in the intraepidermal and junctional component of Spitz nevus; on the contrary, melanoma-positive nuclei are found scattered irregularly all over the lesion [17, 122, 148]. A gradient is to be found also with cyclin D1, fatty acid synthase, P53 and bcl-2 and are more expressed in melanoma than in Spitz nevus. On the contrary, p21 seems more expressed in Spitz nevus than in melanoma. All these immunohistochemical results are at times contradictory, variable and difficult to interpret, and for the time being, final diagnosis cannot be trusted exclusively upon them.
- Bastian reported marked differences in the pattern of chromosomal aberration between Spitz nevi and melanoma: Cases with no aberration or an isolated gain on chromosome 11p were interpreted as benign; multiple aberrations (besides gain on chromosome 11p) indicate melanoma. However, 5% of melanoma do not have any chromosomal aberration. A serious limitation to the technique is that it may be confidently used only in pure melanocytic proliferation: Lymphocytic infiltrate can hamper the procedure.
- As regards to the age of the patient, one must always remember that as illustrated by a number of publications on so-called atypical, metastasizing and malignant Spitz nevi, spitzoid melanoma do occur in youngsters and even small children, and they may eventuate into metastatic disease with lethal outcome [10, 14, 96, 138, 139]. The criteria that allow a confident distinction between benign and malignant spitzoid lesions in such cases are basically the same as in adults.

However, in very young patients (less than 1 or 2 years old), striking cellular pleomorphism or occasional mitotic figures (also at the bottom of the lesion) or a growth pattern in solid sheets are possible in lesions with benign outcome. In these cases, however, one should consider:

- Only one of the above mentioned aspects is present; that is, cells are either atypical or pleomorph but not atypical and pleomorph. Cells grow occasionally in solid sheets, but mitoses must be scant. Cases of spitzoid melanoma under the age of 2 (which metastasized) had a striking combination of atypia, mitoses, pleomorphism and growth in solid sheets.
- As a rule, benign lesions are small and confined in the dermis, whereas spitzoid melanoma in this age group usually reach the sub-cutis [10, 96].

In synthesis, it is always wise to reconsider a diagnosis of spitzoid melanoma when the following are found in a suspected lesion with large epithelioid or spindle cells: many Kamino bodies, symmetrical epidermal hyperplasia, cellular monomorphism and no significant mitotic figures. Being cautious in diagnosing a melanoma in very young children is also important.

Rare variants of Spitz nevus are even more likely to be misdiagnosed as melanoma. In our own experience, these variants are: Spitz nevus with prominent dendrites, Spitz nevus with superficial growth pattern, angiomatoid Spitz nevus and Spitz nevus on volar skin.

A Spitz nevus, especially in dark skinned people, can be composed of melanocytes with elongated dendrites [46, 135]. In such cases, cells are even more atypical than in ordinary Spitz nevus and are mostly arranged at the junction in solitary units and in a lentiginous pattern (Fig. 4). Moreover, the lesion is quite large and does not have the wedge-shaped silhouette of a common form of Spitz nevus. This form is

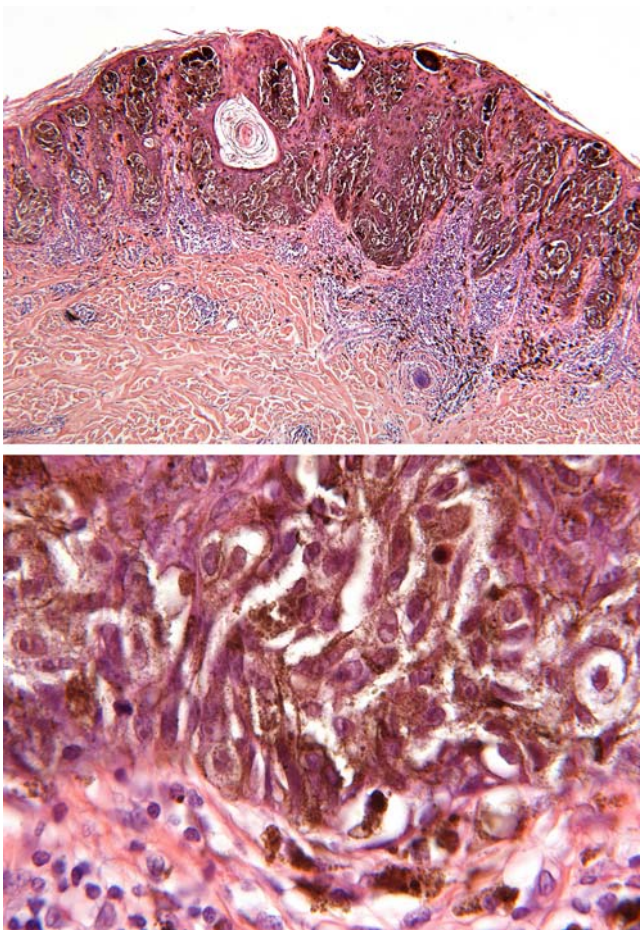

Fig. 4 In dark-skinned people, this form of heavily pigmented dendritic Spitz nevus is quite common and regularly confused as melanoma. The uniform epidermal hyperplasia and the symmetrical distribution of the melanin are both indications, at scanning magnification, of the benign nature of the lesion

unusual and badly categorized in the literature and is frequently confused with melanoma, with Clark's atypical nevus or with an atypical form of Reed nevus. The symmetrical silhouette of the hyperplastic lesional epidermis and the symmetrical distribution of the pigment both are hints of the lesion's benign nature. Cellular monomorphism is also a useful clue for not overdiagnosing this lesion.

A flat 'superficial spreading' silhouette is seen in rare cases of Spitz nevus with conventional cytological features [80]. These lesions are occasionally very worrisome because the dermal component is impressive, nodular and situated only in the centre of the lesion (Fig. 5). In these cases, the Spitz nevus resembles a superficial spreading melanoma with central invasion of the dermis (so-called early vertical growth phase). However, all the other criteria for the diagnosis of a benign lesion are present; in particular, the cytological features of a Spitz nevus are evident, especially at the dermo-epidermal junction: The diagnosis would not be difficult if it were to be based only on the junctional component where nests are discrete with an artificial cleft between them and the overlying hyperplastic epidermis ('capping'). Even in the intra-dermal component, which is apparently worrisome, the cells are large but monomorphous, organized in discrete nests separated by collagen fibres (and not in expansile nodules compressing dermal structures) and mitoticaly quiescent. The nucleus/cytoplasm ratio is that of a Spitz nevus (large nuclei with abundant, granular or ground glass cytoplasm with distinct borders).

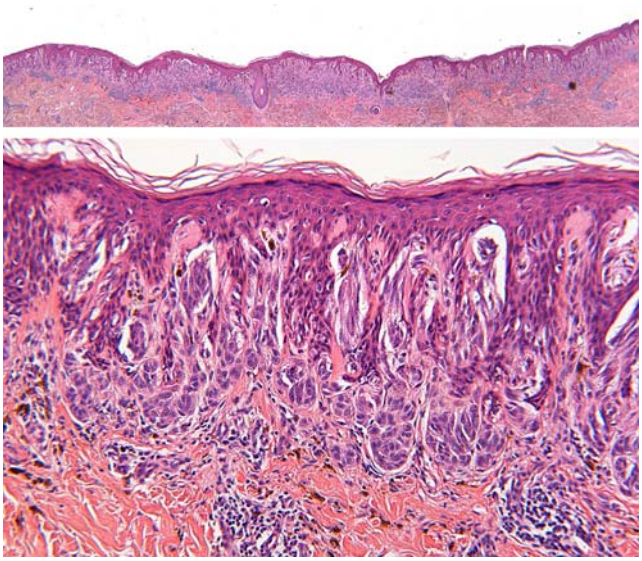

Fig. 5 Spitz nevus usually grows in the dermis with a wedge-shaped silhouette. In this case, the growth pattern is flat and resembles a form of superficial spreading melanoma early invasive of the dermis. At higher magnification, however, the cells appear monomorphous, nests are discrete and similar to each other in shape and size. 'Capping' above the nests and a few Kamino bodies are further reassuring features of the benign nature of the lesion

The angiomatoid Spitz nevus [40] is a form of nevus in which the dermis is rich with newly formed capillary vessels. A sparse infiltrate of epithelioid or spindle cells is present among the vessels. A scant inflammatory component is also seen. All these findings are easily confused with the granulation tissue of a regressing melanoma. This happens quite frequently because although the angiomatoid variant of Spitz nevus is rare, the finding of an area with vascular proliferation in a Spitz nevus is not so uncommon. Cellular characteristics are the major clue in ruling out a melanoma. In fact, in angiomatoid Spitz nevus, cells are epithelioid and spindle with abundant cytoplasm and are usually scattered among collagen fibres without forming nodules. Mitoses are virtually nil. Moreover, the lesion is usually small and well circumscribed, just the opposite of a regressing melanoma.

Spitz nevus in volar skin can have striking details indicating melanoma: Cells are large, and there is an evident pagetoid spread of melanocytes above the junction. However, cells are gathered in discrete nests, with a minimal lentiginous component. In the dermis, they are organized in small discrete nests, cords or single cells with no evident mitotic activity (these aspects are better seen at the lateral borders of the neoplasm). Finally, the lesion is restricted almost always to very young patients, in whom a form of volar invasive melanoma is exceedingly rare.

Reed nevus vs melanoma with features of Reed nevus

Reed nevus (pigmented spindle cell nevus) is a variant of Spitz nevus that, at the beginning, is characterized by heavily pigmented elements, which, in the course of time, first become dendritic and then elongated spindle melanocytes. In each step of its evolution, the Reed nevus can be misdiagnosed as melanoma [87, 101, 125, 134].

In its early stage, the Reed nevus (Fig. 6) is rich in pigment, poor of melanocytes and with an inflammatory dermal infiltrate, and in this setting, it can be confused with a regressing melanoma with tumoural (nodular) melanosis [28]. The following features could indicate the correct diagnosis:

- Reed nevus, especially in this early stage, is a small lesion, while a regressing melanoma is a large one.
- The nevus is located on the limbs (mostly on the legs) and lower trunk and abdomen. A melanoma with regression occurs everywhere in the body, mostly on the trunk.
- In the nevus, the cells at the junction are dendritic or spindle but typical, while, on the contrary, in melanoma, melanocytes have hyperchromatic nuclei.
- In regressed melanoma, the cells of nodular melanosis are readily identified as macrophages; if residual

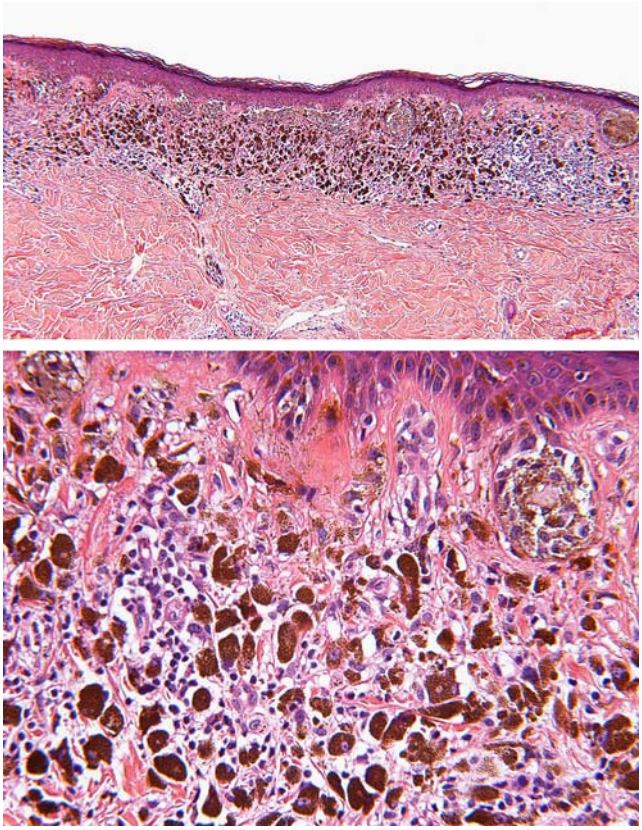

Fig. 6 At first glance, this heavily pigmented melanocytic nevus can be misinterpreted as a form of regressing melanoma. The small size of the lesion, the absence of atypia and the presence of small regular nests at the junction will confirm its benign nature

tumour is present, it consists of clustered atypical cells in the dermis and, occasionally, of epithelioid atypical junctional melanocytes in a lentiginous array.
– Rete ridges are preserved or even elongated in the nevus, annihilated in the regressing melanoma.

Serial sections in both entities usually reveal further evident details indicating the nature of the lesion, like expansive nodules in the melanoma and regular junctional nests in the nevus.

When rich in dendritic cells, the Reed nevus can be misdiagnosed as a melanoma in severely sun-damaged skin. The clue for the correct diagnosis is indeed the presence of signs of sun damage, mostly represented by abundant fibroelastosis. Such indications of chronic and severe solar damage rule out the Reed nevus. Moreover, a melanoma of this type, at the shoulders of the lesion, has lentiginous proliferation of melanocytes at the junction and above it.

In mature forms of Reed nevi, cells are arranged in elegant elongated fascicles parallel to the epidermis [8, 9, 114]. Cells are mostly spindle and rich in pigment, which is also present in the epidermis in the keratinocytes and in the dermis inside numerous melanophages. A dermal inflammatory infiltrate is also frequent. A few melanocytes are scattered above the junction, and epithelioid cells are regularly found among the spindle ones. With this morphology, the Reed nevus has the tendency of being confused with a spindle cell melanoma [78].

The similarities between the Reed nevus and spindle cell melanoma are indeed numerous, but a few details will lead to the correct diagnosis:

– Reed nevus has a peculiar clinical setting. It develops in youngsters usually on the lower part of the body, below the waist. A ‘Reed nevus’ on the face or on severely sun-damaged skin is most probably a form of spindle cell melanoma.
– The elongated cells, which compose the nevus, are always remarkably typical, while hyperchromatic nuclei or prominent nucleoli are consonant with a spindle cell melanoma.
– In Reed nevus, the pigment is overwhelmingly abundant but regularly and symmetrically distributed. Uneven distribution of the pigment or nodules of melanophages restricted in one focus belongs to a melanoma.
– Reed nevus is restricted to the papillary dermis, whereas infiltration of the reticular dermis is indicative of a melanoma.
– Large nodules of epithelioid cells are absent in the Reed nevus; hence, they always must be considered as diagnostic of melanoma.
– As said above, the presence in the skin of signs of severe sun damage (mostly represented by solar elastosis) rules out the Reed nevus.
– In Reeds nevi, horizontally oriented fascicles of melanocytes are found in the papillary dermis, whereas the junctional/intra-epidermal component consists of vertically oriented nests with spindle-shaped cells arranged in the fashion of hanging bananas. This regular architectural pattern is absent or distorted in melanoma-simulating Reed nevus.
– Melanoma with features of Reeds nevus often presents with skip areas, whereas the cells of Reeds nevus are evenly distributed.

Cellular atypical blue nevus vs melanoma on a blue nevus

Among the many forms of cellular blue nevus, a few may be confused with a melanoma developed on a blue nevus or with other forms of melanoma [25]. The most frequently misdiagnosed form of cellular blue nevus is the so-called atypical cellular blue nevus (an unfortunate but entrenched

term). The atypical cellular blue nevus [143] is characterized by large size and by deep infiltration of the subcutaneous fat. Cells show random cytological atypia with large hyperchromatic or vesicular nuclei (giant or bizarre cells are also possible), mild pleomorphism and scattered mitoses. Despite its worrying cytological details, the atypical cellular blue nevus retains a constant benign conduct and must not be diagnosed as a melanoma developed on a cellular blue nevus [52, 97, 141, 160].

In the distinction between these two entities, one must consider:

- A melanoma on a cellular blue nevus is exceedingly rare, whereas cytological and architectural atypia in a large cellular blue nevus is much more common.
- A melanoma on a blue nevus usually consists in large hypopigmented nodules of atypical epithelioid cells, which are indistinguishable from a common form of melanoma [76]. In these nodules, necrosis en masse can supervene, and cytology is obviously malignant. In other cases, melanoma develops in blue nevus as large sheets of sarcomatous spindle cells with marked nuclear pleomorphism and numerous mitoses. A third morphologic aspect of melanoma on a blue nevus is represented by masses of highly hyperchromatic branching dendritic elements, which are rich in melanin [50]. In summary, melanoma on a blue nevus always betrays itself with marked cytological atypia and a growth pattern in large solid sheets, elongated fascicles or nodules, destructive of the surrounding nevus or the dermal structures.
- The atypical blue nevus is characterized by the preservation of the structure of a cellular blue nevus. In this benign-looking background, a few large melanocytes with hyperchromatic nuclei are randomly scattered. These cells have nuclei with evenly dispersed chromatin, reminding alteration induced by senescence. On occasion, however, nuclei are vesicular with a prominent nucleolus. These atypical cells never aggregate in sheets or nodules and are neither associated with mitoses in number nor with large areas of necrosis.
- Mitoses are possible in both entities, but they are more numerous in a melanoma. Atypical mitoses are indicative of melanoma.

Another variant of cellular blue nevus, which may be confused with a melanoma (or with clear cell sarcoma), is the recently reported form named by Mihm known as amelanotic cellular blue nevus [19, 159]. A variation on the theme is probably the entity described by Deyrup et al. [39] as paraganglioma-like melanocytic tumour because of its peculiar nested architecture and solid pattern of growth (Fig. 7). In these tumours, cells are very large, epithelioid, with pale or clear cytoplasm. Cells aggregate in nests or cords, which are separated by mature, occasionally sclerotic, collagen fibres. Melanin is absent or very scarce. Mitotic figures are possible in these lesions, as in all forms of cellular blue nevus, even in the deep portion of the tumour. Cells can be very large and nuclei hyperchromatic (but nucleoli are inconspicuous). In cases like this, the diagnosis of cellular blue nevus (or of a variation on the theme) is based on the presence of recognizable areas of cellular or common blue nevus (serial sections may be necessary). Moreover, the masses of clear cells show a monotonous cytology, while a solid growth pattern is absent. Necrosis and pleomorphism are also absent. Finally, in this entity (at variance with melanoma), collagen fibres among the nests are undisturbed and sclerotic. The lesion is divided in lobules, cords and alveolar structures and has a sort of organoid or paraganglioma-like architecture.

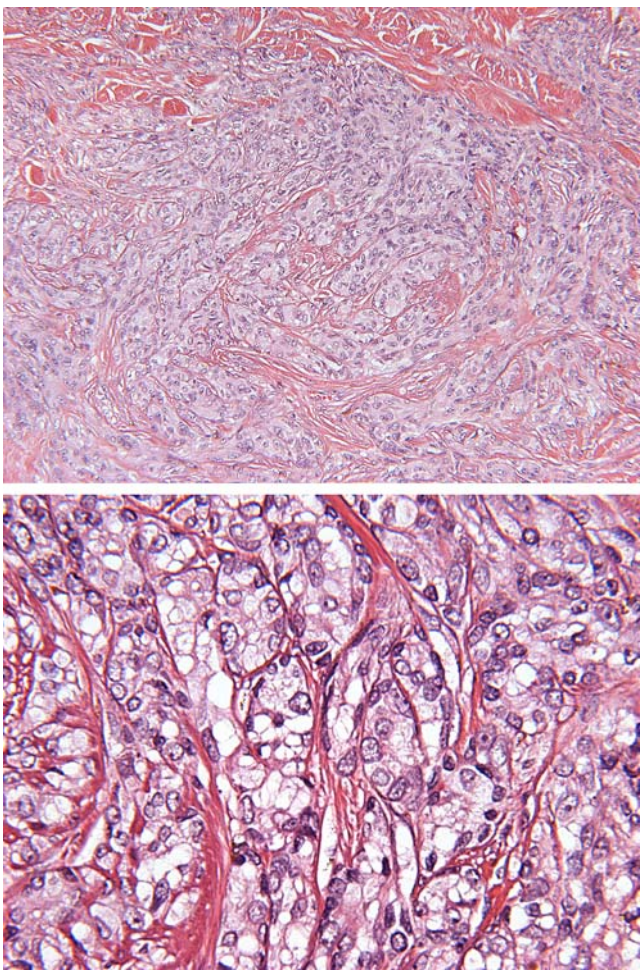

Fig. 7 In other parts of this large lesion, the features were that of a cellular blue nevus. In these images, we illustrate a more bizarre architecture: Cells gather in small anastomotic nests and cords and are utterly devoid of melanin. The differential diagnosis with a melanoma on blue nevus, intradermal melanoma and clear cell sarcoma is based on the absence of pleomorphism, of large solid nodules, and atypical cells with many mitotic figures

Other cases in which an innocent cellular blue nevus can be misdiagnosed as a melanoma are those in which the cellular density is very high [26] or in which the lesion is scattered with densely packed alveolar structures (the so-called microalveolar cellular blue nevus) [58, 94]. In these cases, however, the high cellular density is the only worrying sign; cells are typical, and mitoses are low in number.

Acral nevi vs acral melanoma

Nevi on palms and soles (volar or acral skin) have a tendency to be confused with melanoma [44]. The two most important sources of confusion are the so-called acral lentiginous nevus and the Spitz nevus on volar skin.

In acral lentiginous nevus [33], cells are organized at the junction in small nests, alternated with melanocytes in single units. The cells, especially the lentiginous ones, have dendrites. A scattering of the typical elements above the junction is the rule. Occasionally, this aspect is so overwhelming that the acronym MANIAC (melanocytic acral nevi with intra-epidermal ascent of cells) is ironically suggested. The epidermis is hyperplastic with elongated rete ridges. In the dermis, melanocytes are arranged in small nests or cords or also in single units scattered among undisturbed collagen fibres.

Occasionally, the acral lentiginous nevus is so similar to the so-called acral lentiginous melanoma that a distinction between these two entities is almost impossible. This happens because this form of melanoma has a junctional pattern in nests and single dendritic units similar to that seen in the acral lentiginous nevus. Moreover, this form of melanoma occasionally develops an intra-dermal growth of small, loosely aggregated melanocytes that is deceptively similar to that of a nevus. Furthermore, the epidermal alterations (elongation of rete ridges and thickened cornified layer) are similar in both entities as is the poor lateral circumscription of the lesions.

The following observations could be of help to the diagnostic pathway:

- Being that the histological similarity between acral nevi and acral melanoma is so great, it would be wiser to base the diagnosis mostly on clinical grounds [75, 106]. With due caution and acknowledging exceptions, one could say that a lesion with dendritic melanocytes on volar skin less than 6–8 mm in lateral diameter in a young patient is most probably a nevus. On the contrary, a melanocytic neoplasm bigger than 6–8 mm in a patient older than 50 is almost certainly a melanoma with the only exception being the congenital nevus.
- Histologically, the depth of the lesion is another useful indication for the diagnosis, the only exception being the congenital nevus (and the Spitz nevus, as discussed below); benign lesions on volar skin do not infiltrate deep dermis. Any other widely invasive form of melanocytic neoplasm is to be considered a melanoma unless the contrary is convincingly and exhaustively proved. Invasion of the sub-cutis is highly indicative of melanoma, even if the cytological features of the neoplasm are bland and mitotic activity is undetectable.
- At the junction, in a nevus, nests are prevalent in relation to single cells aligned in a lentiginous array, while the exact opposite is the rule in a melanoma [22, 77]. In a nevus, the cells gather in nests quite soon when the lesion is still small, while melanomas form discrete nests only after having reached a large lateral extension.
- In an acral lentiginous nevus, there is a scattering of melanocytes above the junction as in a melanoma. In melanoma, however, this aspect is much more evident and involves the lateral shoulders of the lesion. Most importantly in melanoma, pagetoid melanocytes have large and hyperchromatic nuclei, with a clear halo and possess prominent dendrites.
- Both in nevi and in melanoma, cells are frequently dendritic at the junction, and dendrites contain melanin [129]. However, in melanoma, dendrites are much thicker and elongated up to the highest part of the spinous layer and bizarrely oriented in all directions. Usually, in melanoma, dendrites are heavier pigmented than in nevus.
- In melanoma, a striking involvement of eccrine ducts is present in most cases [71]. The infiltration of the ducts is in single units, usually the melanocytes are highly hyperchromatic with scant or no detectable cytoplasm. These melanoma cells aligned in a lentiginous array displace the myoepithelial cells around the eccrine intra-dermal ducts. This peculiar eccrine involvement interests the ducts to their deepest reaches. In nevi, eccrine ducts can also be involved by melanocytic proliferation, but benign melanocytes in the eccrine ducts are usually gathered in discrete oval nests and not aligned in single units, and the involvement is restricted to the uppermost portion of the ducts.
- In melanoma, cells are atypical and enlarged at the dermoepidermal junction [116, 131] and above it and can be stellate or spindle, with finely granular cytoplasm. Nevus cells are usually less conspicuous, and atypia is less evident. These aspects are, however, difficult to evaluate, and one must remember that atypia can be slight in some cases of melanoma.
- The dermal component in the nevus is usually typical. Cells are small and symmetrically distributed in the dermis. In many melanomas, the intra-dermal compo-

nent is atypical, but cases in which the intra-dermal component in melanoma is nevoid can occur [137]. We have seen a few cases in which the intra-dermal component of melanoma was barely distinguishable from a nevus. In these cases, the presence of mitoses in the deepest portion of the lesion is always an indication of melanoma (but their absence does not rule it out). Furhtermore, hyperchromatic nuclei and their pleomorphism are indication of melanoma.

– Although not decisive for the final diagnosis, ancillary techniques can also be useful. HMB45 often may stain cells in the deepest portion of acral melanoma, which is rarely the case in acral nevi [69]. As for other techniques, Bastian noted a set of genomic alterations in acral melanoma, aspects that could be decisive in the near future [140].

Another source of error in the diagnosis of acral melanocytic neoplasms is the Spitz nevus. The entity is rare on acral skin, but the similarity with melanoma is striking. In fact, the Spitz nevus frequently has a centrally sited invasive and nodular component in the acral location. In the Spitz nevus, a pagetoid spread of melanocytes is present in the centre of the lesion, frequently accompanied by transepidermal elimination of entire well-circumscribed nests. In the nodule, the cells are large and with vesicular nuclei, and they are very similar to melanoma.

In this differential diagnosis between Spitz nevus and melanoma on volar skin, the most important clues for the diagnosis of a Spitz nevus are on the two lateral shoulders (that is, the two lateral borders beyond the centrally sited intra-dermal mass) of the lesion, where more clear-cut histological features of the Spitz nevus are present. Cells are epithelioid or spindle but monomorphous here. Moreover, they are gathered in comfortingly discrete regular nests, and a clear crescent-shaped cleft (‘capping’) is frequently present between the nests and the epidermis. Mitotic activity is scant or nil, both at the junction and—most importantly—in the dermis. Finally, Spitz nevus involves youngsters, whereas melanoma on volar skin is a neoplasm of old patients.

At the end of this section dedicated to acral nevi, it should be stressed one more time that a great care should always be used in the diagnosis of a melanocytic lesion in this area, and an overdiagnosis of melanoma must be avoided. In fact, an erroneous diagnosis of melanoma can cause an undue and debilitating surgical procedure, especially in weight-bearing areas. In my opinion, a consultation should be sought in all lesions on volar skin where the diagnosis is not immediately obvious. Moreover, clinic pathological correlation in this field is a mandatory procedure: The combination of the age of the patient, the lateral and vertical diameters of the lesion and the extension of the lentiginous and pagetoid spread are together the most important indications for the diagnosis [124].

Dysplastic nevus vs superficial spreading melanoma

The dysplastic nevus, first popularized by Clark, is a peculiar form of nevus, which has never found a clear-cut set of diagnostic criteria, this despite (or because of) the hundreds of articles dedicated to the subject. In general [15, 34, 42, 43, 123], the dysplastic nevus is considered a lesion with irregularly shaped and confluent nests at the junction, where nests are unevenly mingled with melanocytes in single units (so-called lentiginous array). Rete ridges are unevenly spaced and sized but always present. In the dermis, the melanocytes are restricted to the most central area of the lesion. Peculiar host response alterations, such as lamellar fibroplasia and mild inflammatory infiltrate are also present. Clark’s dysplastic nevus (also called atypical nevus) is only rarely a forerunner of malignant melanoma, but per se, before the actual degeneration in melanoma occurs, there is a general consensus that it is a benign lesion unable to metastasize. Clark’s dysplastic nevus can be morphologically very similar to some sub-types of superficial spreading melanoma, especially the form recently labelled by Mihm as *lentiginous melanoma* [70]. Similar lesions have been also presented as dysplastic nevus-like lentigo maligna and nevoid lentigo maligna [45, 74]. In this form of melanoma, melanocytes are only mildly atypical, and the epidermal rete ridges are mostly preserved.These two entities, the dysplastic nevus and the so-called lentiginous melanoma, are indeed so similar that most probably, in the past, an unpredictable number of diagnosed dysplastic nevi were in fact superficial spreading melanoma, lentiginous melanoma variant. We recently saw two cases of this form of melanoma that had been diagnosed as dysplastic nevi years ago. Both were later associated with metastatic disease. Melanoma misdiagnosed as dysplastic nevus range high in the list of medicolegal claims [144, 145].

Although our knowledge on the matter is still insufficient, we can propose the following criteria for the diagnosis:

– Patients with this peculiar form of superficial spreading melanoma simulating the Clark’s nevus (lentiginous melanoma) are usually older than those bearing a real Clark’s nevus. The clinical picture is always worrying, and frequently, areas of regression can be seen.
– Histologically, the Clark nevus has only few melanocytes above the junction. Indeed, unless located on special sites like the genitalia or the mammary line, it would be better that no significant pagetoid spread were present. In any case, these aspects should be closely restricted on the central portion of the lesion and on the lowermost part of

the epidermis. In a melanoma simulating a dysplastic nevus, the pagetoid spread is prominent with cells reaching the granular layer. Most importantly, melanocytes above the junction are also found in the 'shoulders' of melanoma (beyond the intra-dermal component of the lesion). The presence of hyperchromatic nuclei, cellular necrosis or mitoses in the pagetoid cells is also indicative of melanoma.

- A crucial element in the lentiginous melanoma is the presence of numerous slightly atypical elements. Occasional atypical melanocytes are actually necessary for a diagnosis of dysplastic nevus (so-called discontinuous or discontiguous cytological atypia), but a large prevalence of these atypical cells is a clear indication of melanoma. The cells have enlarged vesicular nuclei, irregularly thickened nuclear membranes, plump asymmetrically marginated chromatin and prominent nucleoli. Moreover, nuclei are also pleomorph. Many cells are epithelioid, and the cytoplasm/nuclear ratio is altered, and the cytoplasm contains dusty melanin. In other cases of melanoma with the architectural aspect of the dysplastic nevus, the melanocytes on the contrary are small, lymphocytic like, highly hyperchromatic with barely distinguishable cytoplasm, and nucleoli are not seen.
- Although in Clark's dysplastic nevus, a lentiginous component is present, in this lesion, most of the cells are gathered in bizarrely elongated nests. On the contrary, a large prevalence of the lentiginous organization throughout the lesion is indicative of melanoma.
- In melanoma, cells involve the adnexa: In an appropriate setting (that is, with most of the criteria being present), a pagetoid scattering of cells in the infundibular epithelium is indeed diagnostic of melanoma.
- Melanoma presents itself as a large lesion: Most cases are larger than 0.8–1 cm. All the 'dysplastic nevi' of that size should be closely scrutinized.
- Although the architectural features of Clark's nevus are not always regular, nests are mostly at the tips of rete ridges in a dysplastic nevus, whereas they are much more chaotically scattered in melanoma. Indeed, in melanoma, the nests are widely confluent to each other and seem to form one single aggregate of cells at the junction. This pattern brings to mind the acral lentiginous melanoma of the volar skin and of the mucous membranes or the lentigo maligna.
- The epidermal rete ridges are preserved or elongated both in the dysplastic nevus and in the superficial spreading melanoma that simulates it (lentiginous melanoma). However, the rete ridges are more irregularly shaped and outlined in melanoma. Effacement of rete ridges in large tracts and focal epidermal atrophy both indicate melanoma.
- The presence in the dermis of well-structured, widely represented, concentric or lamellar fibroplasia suggests a benign lesion. The pattern of the inflammatory infiltrate is similar in dysplastic nevus and melanoma that simulates it.
- A warning should be issued regarding the intra-dermal component. In the subset of melanoma prone to be misdiagnosed as Clark's nevus, the melanoma cells in the dermis can be only slightly atypical and thus easily misinterpreted as benign. Mitoses are not usually found, and cells seem to mature. Furthermore, from an architectural point of view, the dermal component simulates a nevus: Cells are gathered in regular nests and cords distributed among undisturbed collagen fibres.
- So far, immunohistochemistry and molecular genetic techniques provided only minor contribution to the routine diagnosis of these entities. In general, Ki67-positive cells are said to be less numerous in dysplastic nevus than in melanoma. This notwithstanding, it is wise not to rule out a melanoma upon Ki67-negative results. The same applies to other immunostains.

Indeed, Clark's dysplastic nevus and the sub-set of superficial spreading melanoma that most simulates it are very similar. The main detail indicating a melanoma is mild, but widespread, nuclear atypia, lentiginous-prevalent architecture at the junction and pagetoid spread above it. Whether these morphologically similar neoplasms are also biologically related (that is, one being the precursor of the other) is a matter of speculation [63, 68] and beyond the aim of this paper.

Desmoplastic nevi vs desmoplastic melanoma

Melanocytic nevi can have a large collagen component. The most important entities of this group of collagen-burdened lesions are the desmoplastic Spitz nevus [86], the desmoplastic common nevus [56] and the sclerotic blue nevus [93]. All of the above and particularly the desmoplastic Spitz nevus are frequently confused with desmoplastic melanoma.

Desmoplastic Spitz nevus (Fig. 8) is an entity that usually develops in adults but is occasionally seen in elderly patients, usually on the limbs [85]. The lesion is mostly intra-dermal, but a junctional component is frequently present. Cells are those usually found in a Spitz nevus namely, spindle and epithelioid melanocytes with abundant eosinophilic cytoplasm. Abundant mature hyper-eosinophilic collagen fibres surround the cells. On rare occasions, the entire dermal span is occupied by the neoplasm, which reaches the sub-cutis. The Desmoplastic Spitz nevus is frequently confused with a melanoma with desmoplasia [24, 29, 142, 149], mostly because of its cytological

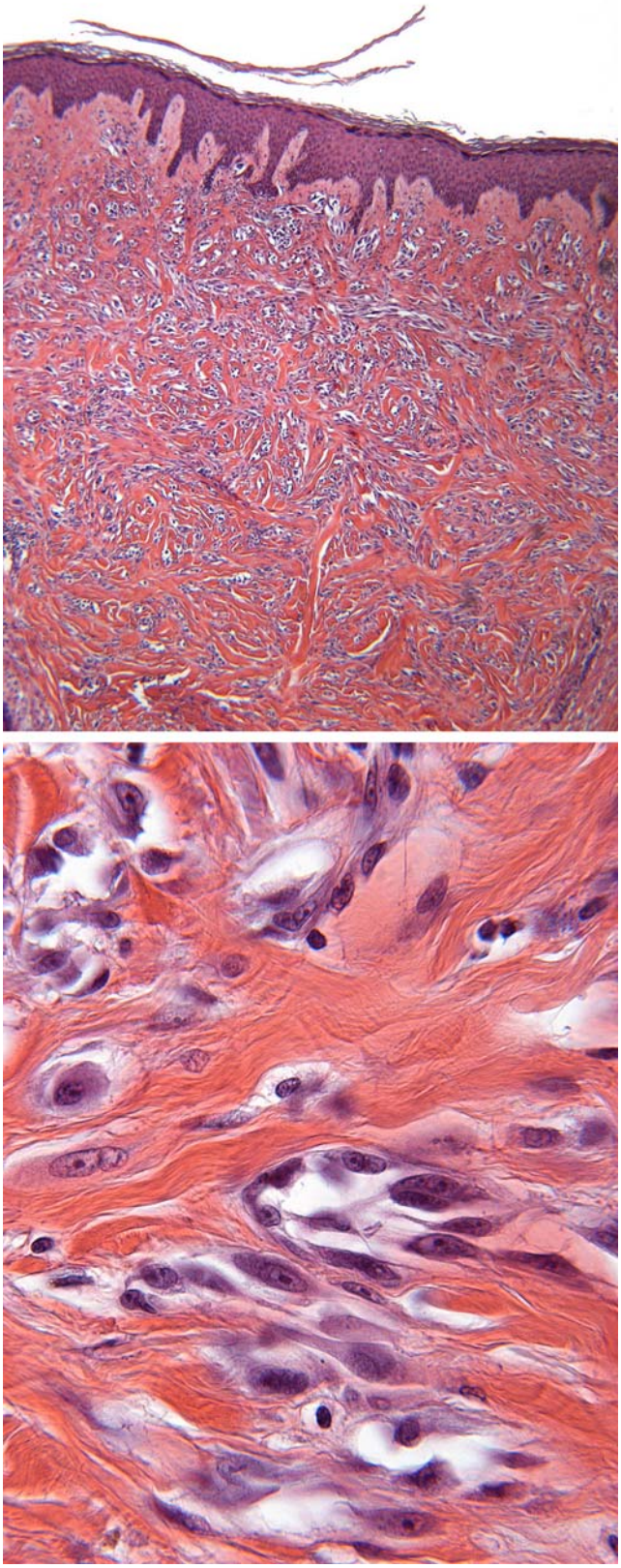

Fig. 8 Desmoplastic Spitz nevus closely simulates a desmoplastic melanoma. The large epithelioid cells with abundant cytoplasm of this lesion are the most important clue for the diagnosis. Note also the hyperplasia of the epidermis, a feature frequent in all forms of Spitz nevus

atypical features. Other misleading aspects could be neurotropism and poor circumscription. The following criteria for a distinction between desmoplastic Spitz nevus and desmoplastic melanoma could be suggested:

- Desmoplastic melanoma is exceedingly rare outside the classic sites namely, head, hands, feet and mucous membranes.
- Desmoplastic melanoma usually derives either from a lentigo maligna on sun-exposed areas or from a lentiginous melanoma on acral skin or on mucous membranes. Remnants (even scant) of these lesions are found at the junction in most of the desmoplastic melanomas. On the contrary, in some cases of Spitz nevus, there are at least a few well-circumscribed, round nests of epithelioid cells at the junction, which are very similar to those of an ordinary non-desmoplastic Spitz nevus. These epithelioid cells and discrete nests are also present in the papillary dermis, where these features indicating a Spitz nevus can indeed be found. However, one must remember that many desmoplastic Spitz nevi are entirely intra-dermal, so that junctional nests may be altogether absent.
- The size of the lesion is crucial for the diagnosis. A desmoplastic melanoma entirely removed at the first attempt of excision is rare: Strands of the neoplasia almost always infiltrate the margins. The opposite is true for the Spitz nevus, which an excisional biopsy almost always successfully removes in toto. This depends on the melanoma's large dimension and also on the fact that clinically, the Spitz nevus (even in its desmoplastic variant) is always much better circumscribed than the desmoplastic melanoma.
- In the cases sited on the head, masses of solar elastosis are usually present in desmoplastic melanoma. Tangles of elastotic fibres are above, inside and all around the neoplasm: features not to be found in any form of Spitz nevus.
- If the lesion has a junctional component, the epidermis is thinned in melanoma (on cases not sited on acral skin) and thickened in Spitz nevus.
- Paradoxically, the presence of isolated, large atypical cells with abundant sharply delineated cytoplasm in the lesion is an indication of its benign nature. In fact, the elongated melanocytes of desmoplastic melanoma most frequently seem like plump innocent-looking fibroblasts with scant or barely discernible cytoplasm. The presence of large triangular or polygonally shaped melanocytes (either multi-nucleate) with ground glass cytoplasm indicate a Spitz nevus.
- Both in Spitz nevus and in melanoma, the desmoplasia is accompanied by lymphocytic infiltrate. However, in melanoma, the lymphocytes are characteristically ag-

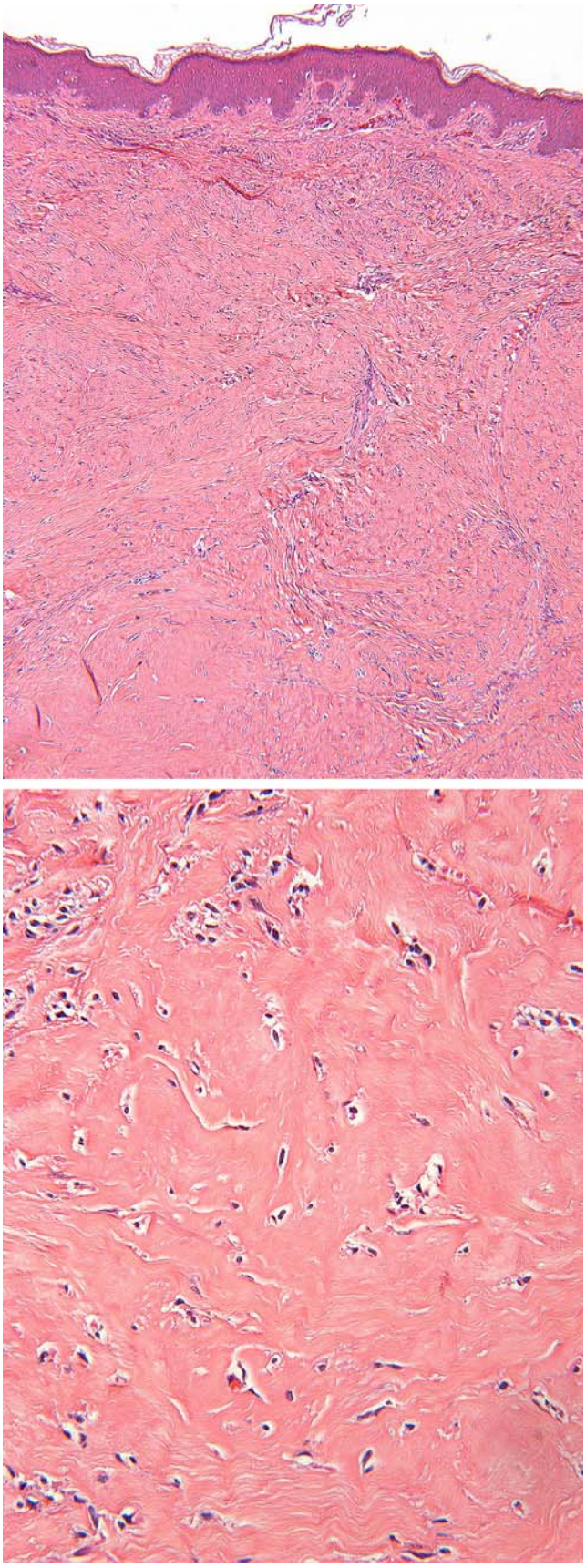

Fig. 9 This fibrous variant of blue nevus seem almost undistinguishable from a desmoplastic melanoma. However, at the periphery of the lesion, a clear pattern of blue nevus was present. Moreover, cells are delicately dendritic and pigmented, aspects not to be found in desmoplastic melanoma. Immunostain for HMB45 will contribute to the diagnosis

gregated in round follicles scattered all around the neoplasia, even at the bottom. Moreover, in melanoma, the lymphocytes aggregates are present not only inside and at the periphery of the tumour but also in the dermis and sub-cutis at a distance from the neoplasm.

– In melanoma, collagen fibres are fibrillary or dense and are inter-woven chaotically with the fibroblast-like melanocytes. The overall aspect is that of a mesenchymal neoplasia, with a whorled or storiform pattern or a scar (with which desmoplastic melanoma is, in fact, frequently confused). In desmoplastic Spitz nevus, fibres are thick and uniform, hyper-eosinophilic and oriented parallel to the cells in a quite regular fashion.
– Mitoses are usually rare in both entities, when present in number, they indicate a melanoma. In a few cases, in melanoma, there are small areas of conventional nodular melanoma, which can be found through serial sections.
– In the differential diagnosis of these entities, immunohistochemistry [30] can be useful but never decisive: Desmoplastic melanoma is usually HMB45 negative, whereas desmoplastic Spitz nevus is positive especially in the uppermost portion of the neoplasm [83, 152]. However, one must keep in mind that areas of more conventional melanoma (epithelioid or spindle) are possible in desmoplastic melanoma, and the cells in these areas are HMB45 positive. In one third of desmoplastic melanoma, a significant number of smooth actin-positive cells are found, a finding not reported in any desmoplastic nevus.

A second less frequent type of nevus with desmoplasia is a common ordinary nevus with an overwhelming production of collagen fibres. This entity, however, is rarely confused with melanoma because a plain nevus tissue is always present at the periphery of the lesion.

The distinction with a sclerotic blue nevus (Fig. 9) is more complex, especially if this neoplasm has been only partially removed. Indeed, despite their deeply different biological nature, desmoplastic melanoma and sclerotic blue nevus share numerous clinico-pathological characteristics. Patient's age and the site of the lesion (head and extremities) are, in fact, the same. Both can be very large and are composed of elongated fibroblast-like melanocytes embedded in collagen fibres. The distinction is occasionally problematic and is mainly based on the following:

- Although the blue nevus can be mostly sclerotic and achromic, a few spindle and dendritic melanocytes containing melanin can be found (with serial sections) at the periphery of the tumour. If these cells are along few melanophages, the picture is characteristic of an ordinary common blue nevus.
- Blue nevus, especially when sclerotic, does not have a junctional component but only melanocytic hyperplasia in the epidermal basal layer. Most desmoplastic melanomas have a melanoma in situ-like proliferation at the junction, or at least, a few nests are recognizable (an aspect not to be found in a common or cellular blue nevus).
- A useful clue to sclerotic blue nevus is a stromal hyalinosis with sharp circumscription and often a wedge-shaped silhouette.
- In blue nevus, the lymphocytic follicles that are scattered inside and all around the desmoplastic melanoma are absent.
- Neurotropism is not a feature of sclerotic blue nevus as it is of desmoplastic melanoma. The same applies to invasion of follicular adventitia, more consonant with melanoma.
- Blue nevus is HMB45 positive, whereas, with few exceptions, desmoplastic melanoma is negative or with only few positive cells.

Proliferative nodules on nevus vs melanoma on congenital nevus

A large nodular growth of atypical melanocytes can arise in the core of a congenital nevus. This usually occurs at birth but may also happen later in childhood. Nodules excised in adulthood are rarer but still possible. In children, such nodules are polypoid and can be ulcerated or bleeding. The clinical diagnosis is often that of a melanoma, but over time, most of these stiff and large nodules evolve in soft and smaller lesions and can also completely disappear. These nodular lesions in the midst of a congenital nevus are called proliferative nodules and are considered benign and must be distinguished from true nodular melanoma developed in the core of the intra-dermal portion of a congenital nevus [126]. Such benign proliferative nodules can have disparate histological features reminding one of any possible nevus variants. Consequently, they can have features of blue nevus [119], Spitz nevus [35, 155], Deep Penetrating nevus and so on [103]. Occasionally, nodules have cytological or architectural aspects that do not fit with any form of nevus and are composed of immature round melanocytes with scant cytoplasm. The latter are called melanoblasts. On rare occasion, nodules have no melanocytic features at all but show, instead, aspects or neural, adipocytic or myoblastic differentiation [49, 82].

As a rule, the nevus hosting these nodules is a large congenital nevus, but occasionally, small proliferative nodular or plaque-like foci are present in medium or even small nevi, which can be not only congenital but also acquired.

In a few cases, especially in very young patients, an atypical melanocytic proliferation can also be present in the epidermis with important pagetoid spread of melanocytes above the junction. Such proliferation is unrelated to the nodule itself, but these aspects can contribute to mislead the diagnosis towards a melanomatous nature of the entire lesion.

Because of their worrying aspects, these nodules can be misdiagnosed as melanoma. Those with Spitz nevus features are the most frequently confused because of the striking cytological details of the nodule. In the differential diagnosis between these Spitz nevus-like nodules and nodular melanoma developed in the dermal portion of a congenital nevus, the following aspects must be taken into account:

- The age of the patient can be crucial. Melanoma in the intra-dermal portion of a congenital nevus usually develops between 2 years of age and puberty. Before this age range, the lesion is most likely benign. In neonates, although the histological features can be highly atypical, the prognosis can still remain good. Congenital melanomas and melanomas in the first 2 years of life are, in fact, exceedingly rare. In adulthood, melanomas on congenital nevi usually develop at the junction and not in the intra-dermal core of the lesion. In an adult, a melanoma on the dermal component of an acquired or congenital nevus is very rare.
- Large benign proliferative nodules arising in the midst of a large congenital nevus are much more frequent than a melanoma in the same site. Therefore, based on pure statistical considerations, a nodule in the core of a congenital nevus is most likely benign, in both children and in adults. This is even truer in very young patients (1 or 2 years old) where benign nodules can have remarkable cytological atypia and a very high mitotic rate. In this group of very young patients, pathologists should always be reluctant in giving a straightforward diagnosis of malignancy despite the striking cellular features.
- Benign proliferative nodules in patients older than 1 or 2 years usually have low mitotic activity, whereas melanomas developed in the dermal portion of the nevus show evident mitotic activity. Finally, in melanomas, mitoses are often atypical, a finding not to be seen in benign nodules.
- In nodules with features of Spitz nevus, cytological details are monomorphous. Cells have atypical characteristics but are uniform in size, shape and cytological features. Per se, the nodules closely resemble a Spitz

nevus. On the contrary, in melanomas developed in the dermal component of a congenital nevus, cells are heterogeneous in size, shape and cytological details. In melanomatous masses, atypia is striking with large-sized nuclei and huge amphopilic macronucleoli and nuclear pseudo-inclusions. In melanomas, the cytoplasm is variable, ranging from cells with abundant cytoplasm to cells with only a rim of pale cytoplasm or 'naked nuclei' [104]. Inside the benign nodules, collagen fibres may be present on the contrary melanoma growths as solid masses of compactly packed cells without interposed collagen. Pathologists should be cautious in judging cytological details when only part of the lesion is available. Frequently, the uppermost portion of the nodule is the most atypical, whereas at the bottom cells mature and blend into the surrounding benign nevus cells.

- At scanning magnification, benign proliferative nodules have a quite clear-cut border; however, at higher magnification, a gradual maturation is seen between the large Spitzian cells of the nodule and the small benign-looking melanocytes of the rest of the nevus [156]. In melanoma, there is a more abrupt border between the atypical proliferation and the rest of the bland nevus tissue without a progressive blending of one cell type in the other. Infiltrative or blurred margins of the nodule are also more consonant with a melanoma arisen in a congenital nevus.
- As regards to the proliferation of melanocytes in the epidermis, which is frequently seen in the area above the benign proliferative nodule (but is unrelated to the nodule itself), the pattern is similar to the pseudo-melanomatous intra-epidermal proliferation seen in congenital and acquired nevi in young subjects. Cells are gathered in irregular confluent nests at the junction and scattered throughout the epidermis but are typical, and they mature (become smaller) when they descend into the papillary dermis.
- Immunohistochemical studies are contraddictory. Herron et al. [60] found that proliferative nodules and the surrounding nevus cells have similar expression for Ki67, p16, p27, bcl-2 and CD95. The cells in the nodules overexpress P53 and c-Kit. The most cytologically atypical nodules overexpress Ki67 and bcl-2.
- Comparative genomic hybridization performed by Bastian et al. [16] showed chromosomal aberration in most of the nodules. The type of these alterations was different from those found on melanoma. In nodules, there were numerical aberrations (i.e. gain and loss of entire chomosomes) without structural aberration (i.e. one or several gains or losses of fragment of chromososme). Structural aberrations were typical of melanoma. Chomosome 7 is more involved in nodules, whereas 9 and 10 are the mutant chromosomes in melanoma. However, the chromosome pattern of melanoma was found also in a lesion that had a benign outcome.

A tightly packed population of small sized, nevus-like melanocytes composes the second most frequent type of benign proliferative nodules that, in our own experience, are likely to be confused with a melanoma in young patients [5]. These lesions are diagnosed as melanoma because of the impressive cellular density; cells have only scant cytoplasm and show occasional mitoses. The nature of these cases is more difficult to be assessed. Judging from the literature, the most objective clues indicating malignancy are the high mitotic rate, the presence of atypical mitoses and the age of the patient [38]. In short, a nodule of these melanoblasts, as such cells are called, is probably benign if it is small, monomorphous, mitotically quiescent and developed in a congenital nevus in a very young patient [21]. Criteria indicating a melanoma are mostly pleomorphism and high mitotic rate with atypical mitoses. Other criteria are the presence of a spindle cell component, a palisading nuclear pattern or other forms of neural differentiation with the presence of hyperchromatic cells. Necrosis is also an ominous sign. Moreover, in melanomas, a few foci of large epithelioid, plainly malignant cells can appear among the uniform population of small melanocytes. Finally, the size of the nodule should be considered: Large and deeply sited nodules are most probably melanoma, whereas, on the contrary, a small, circumscribed and superficial collection of melanoblasts is most likely benign.

As said above, many other histological types of nodules can develop in a congenital nevus. These nodules usually have the features of blue nevi (both common and cellular), deep penetrating nevi or other forms of not easily classifiable nevi. These nodules, having a plain benign-looking architectural or cytological characteristic, are usually easily diagnosed.

The distinction between a nodule with neuromesenchymal differentiation and a melanoma with similar features is more complicated. These cases are very rare and not clearly described in the literature. The presence of neurosarcomatous-like features with atypia and mitoses seems to be the best indication of a malignant neoplasm. Benign nodules with similar differentiation are usually cytologically typical and mitotically quiescent.

Epithelioid blue nevus vs animal-type melanoma

In a few rare cases, melanocytic neoplasms are overwhelmingly hyper-pigmented (Fig. 10). Cells are mostly epithelioid and similar to melanophages, but spindle or dendritic elements are present as well [62, 79, 113, 161]. In these

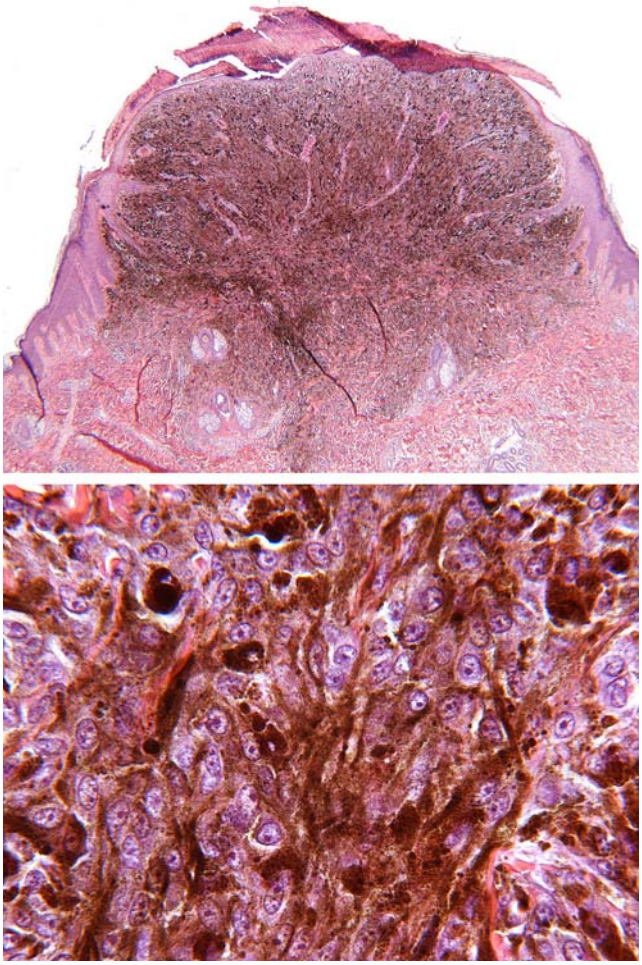

Fig. 10 A diagnosis of pigmented epithelioid melanocytoma is usually provided for this type of neoplasm. Often, the sentinel lymph node contains a small nest of pigmented dentritic melanocytes similar to those illustrated here. Whether this finding represents a true metastasis or a nodal nevus is yet to be established

cases, the differential diagnosis is between heavily pigmented compound (or intra-dermal) nevi and similarly heavily pigmented melanoma. The former has been reported under disparate denominations as epithelioid blue nevus, pigmented epithelioid Spitz nevus, combined nevus with features of blue and Spitz nevus and pigmented benign melanocytoma [6, 151]. Heavily pigmented melanoma is frequently reported as animal-type melanoma or pigment-synthesizing melanoma (probably also melanoma with features of blue nevus have been included in this group) [66, 86, 115, 128]. This group of heterogeneous entities is complex and have in common similar histological features, for these reasons, Mihm proposed to lump them all together under the uncommitted term of pigmented epithelioid melanocytoma, without further indication of the benign or the malignant nature of the neoplasm [161]. I find that pigmented epithelioid (Spitz) nevus, epithelioid blue nevus, and animal-type melanoma deserve to be considered as separate entities that can be distinguished by silhouette and cytology.

Certainly, our knowledge on the matter is incomplete, and studies on large groups of patients with prolonged follow-up is mandatory; however, we can say that in a heavily pigmented epithelioid and dendritic melanocytic neoplasm, a melanoma is very doubtful when the following aspects are present:

- The lesion is small and histologically well circumscribed or wedge shaped.
- Collagen fibres are present among the cells.
- There is a regular epidermal hyperplasia on the lesional epidermis.
- The distribution of the pigment is symmetrical.
- Cells are uniform, and nuclei are vesicular with inconspicuous nucleolus.
- No significant mitotic activity is found.
- Details of a congenital nevus or common blue nevus are present.
- There is an association with Carney syndrome.

In the malignant counterpart (animal-type melanoma or other forms of heavily pigmented melanoma), the cytological features can be deceptively bland, and mitoses are often undetectable, but in the cases reported as metastatic, the following aspects were present:

- The lesion is large: as a rule, more than 1 cm and occasionally of several centimetres in its lateral diameter (although animal-type melanomas inevitably start as small lesions and may be excised at that time [130]).
- The lesion is also many millimetres deep, occupying the entire dermis reaching the sub-cutis.
- The growth is solid with large sheets or nodules of cells without collagen interposed.
- Foci of necrosis are present.

Presence of brisk mitotic activity, nuclear atypia and strong P53 positivity all indicate melanoma, but these findings can be absent.

In this group of lesions, a sentinel node procedure for diagnostic purposes would be advisable. However, one should remember that the presence of melanocytes in the node is not tantamount to metastatic melanoma: Melanocytes gathered in small nests inside the capsule or the septa of the node could be an intra-nodal nevus (with the same features as the cutaneous lesion) and not a metastatic melanoma.

Regressed nevus vs regressed melanoma

Both nevi and melanomas can undergo partial or total regression. Regression implies a dense inflammatory infiltrate causing melanocytic necrosis followed by a

fibrous granulation tissue. The latter partially or completely replaces the regressed melanocytic neoplasm.

In its first phase of dense inflammatory infiltrate, a regressing nevus can be very similar to a regressing melanoma [48, 98, 108], especially when the involved nevus is congenital with large nests. The architecture of the nevus is altered by the inflammatory infiltrate, which gives the lesion an asymmetrical aspect. The presence of large cells with a hyperchromatic nucleus among the melanocytes is most worrisome. Such cells can be easily confused with melanomatous elements. Few mitotic figures are frequent in this setting (although they may belong to the lymphocytes), and few melanocytes are epithelioid. Regressing nevi might have architectural alterations as well: Large confluent nests are present at the junction, and irregularly shaped unusually large and pigmented nests occupy the dermis. However, the differential diagnosis with melanomas [20, 36] is possible considering that:

- In its usual presentation, a regressing nevus has its own clinical setting. It appears in youngsters, usually teenagers, and it is frequently multiple and can be clinically surrounded by a clear halo. A melanoma with extensive regression is a lesion of the elderly, is irregular from a clinical point of view and no clear-cut perilesional whitish halo is evident. A large regressing melanoma is, as a rule, an isolated lesion [91].
- A regressing nevus is a small lesion, while regressing melanoma is large, at least when regression is significant.
- Atypical melanocytes present in a regressing nevus are scattered among typical ones, whereas in melanoma, irregular nodules or masses are composed only of atypical elements. In these masses or nodules, cells have a high nucleus/cytoplasmatic ratio and nuclei are hyperchromatic.
- Mitoses are usually easily found in melanomas but are sparse in nevi. Presence of mitoses in the nodules of atypical cells at the bottom of the lesion is an indication of melanoma (mitoses on the core of inflammatory infiltrate can belong to non–melanocytic cells).
- In a nevus, the inflammatory infiltrate is quite homogeneous throughout the lesion and is sharply circumscribed, with a flat bottom in the deep dermis; on the contrary, a much more disordered infiltrate is present in melanoma, where the peripheral border of the infiltrate is blurred. In a regressing melanoma, plasma cells are more abundant than in nevus.
- A clearer aspect of the lesion usually appears with serial sections, especially in cases of melanomas. In fact, in melanoma, nodules or masses of atypical cells not altered by the inflammatory infiltrate are possible. A pagetoid spread of cells above the junction is indicative of a melanoma.
- The presence of tumoural (nodular) melanosis, i.e. of intra-dermal nodular aggregates of melanophages, is always an indication of melanoma [54].

When the inflammatory process has burnt out and granulation and fibrous tissues supervene, the regressed nevus can be misinterpreted as a regressed melanoma. The nevus in this case shows an area of fibrillary collagen, which occupies the superficial dermis along a line of melanophages. Only residual melanocytes are present. In cases with sub-total or total regression and cicatricial substitution, an ‘absent’ nevus can be reasonably misinterpreted as an ‘absent’ melanoma [61, 91, 99]. In such cases, it is possible to pursue the following diagnostic pathway:

- First of all, ruling out other non-melanocytic-pigmented neoplasms that are also likely to regress or to cause a scarring process rich of melanophages is necessary: Basal cell carcinoma, seborrheic keratosis, lichenoid keratosis, postinflammatory pigmentary alteration from lichen planus in dark-skinned people and mycosis fungoides are all lesions that can leave a fibrillary scar scattered with melanophages [81]. Serial sections and clinical correlation will resolve at least a few of these cases. However, one should never forget that in extreme cases, a definitive distinction from a completely regressed melanoma, a regressed nevus and a regressed lichenoid keratosis is utterly impossible.
- The size of the lesion is crucial: A regressed nevus is usually a small lesion, while a regressed melanoma is a large one (usually larger than 1, 5 cm). Furthermore, the age of the patient is important: An entirely regressed melanoma is exceedingly rare in youngsters.
- Nevus retains, at least for a while, a gently papillated silhouette, whereas melanoma is mostly flat or dome shaped.
- In the case of a regressed nevus, rete ridges are still present or even hyperplasic. In melanoma, the rete ridges are entirely erased.
- The presence of tumoural (nodular) melanosis is indicative of melanoma.
- Small residual melanocytes at the junction or in the dermis are possible in both conditions, while atypical melanocytes indicate a melanoma.
- Severe solar elastosis is an indirect, although precious, hint of melanoma.
- Unlike nevi, where collagen is more delicate and fibrillary with sparse mucin deposits, an entirely regressed melanoma occasionally has a fibrotic dermis.
- Residual plasma cells indicate a melanoma.

There is a very peculiar condition, which, on occasion, can be seen in both nevi and melanomas: the recurrence of the neoplasm on the scar [1]. In melanoma, this phenom-

enon is well known and has been described by McGovern et al. [88] many years ago. Malignant cells surviving the regression reoccupy the epidermis and the dermis. In the epidermis, a lentiginous array of atypical melanocytes substitutes the junction, and atypical melanocytes are scattered in the spinous layer, while in the dermis, large expansive nodules of atypical melanocytes appear. This sort of recurrent melanoma developed on the site of a previously regressed melanoma can be very similar to a regressed nevus in which junctional and dermal proliferation re-appear after regression. In fact, in such nevi, the junctional proliferation has a striking architectural feature with large confluent nests, pagetoid spread and irregular architecture of the dermal component. This sort of regressed nevus with re-grown atypical proliferation is similar to the so-called recurrent nevus with pseudomelanomatous features described by Ackerman and Kornberg years ago [72].

The distinction between this regressed nevus with atypical proliferation from its melanomatous counterpart is mostly based on the size of the lesion and on the cytological details. Nevus is small and cytologically inconspicuous, whereas regressed melanoma with new proliferations is rich with large nodules of atypical cells, which are often in mitosis. Immunohistochemistry can be effective in this quandary: In the nevus, a strong HMB45 positivity is indeed present, but it is restricted to the uppermost portion of the lesion; in melanoma, HMB45-positive cells are scattered all over the area, even in the deepest portion of the lesion.

A careful survey is required in distinguishing between regressing Spitz nevus and regressing melanoma [4, 57, 157]. In these cases of regressing Spitz nevus, the cytological features could be impressive: Cells are large, epithelioid and contain hyperchromatic nuclei. The distinction from melanoma is trusted on the absence of large expansile nodules, the lack of mitotic figures and peculiarly symmetrical distribution of lymphocytes in the lesion. In regressing Spitz nevus, the lymphocytes seem evenly scattered among the Spitzian cells.

Other forms of nevi simulant melanoma

Nevus on special sites

It is now very well known that nevi developed on special sites can have important architectural alterations evoking a form of melanoma [43, 118]. The ‘special sites’ are mostly along the mammary line (axilla, breast, genital area). Other sites are the umbilicus, the ear canal, the knee and the elbow. Recently, this type of nevus has been also seen by us and by other scholars on the scalp of adolescents.

The knowledge of such lesions is today widespread, and they are rarely diagnosed as a melanoma; in fact, the only criterion for the diagnosis of melanoma is peculiar architecture: irregular, large nests haphazardly scattered in the epidermis and in the dermis. Cells, both in the epithelium and in the dermis, show only moderate atypia. These aspects of cytological and architectural atypia reach the climax in the genital skin of young women [32]: Nests are large, confluent and elongated vertically and are scattered in a disordered pattern at the junction and in the corium (Fig. 11). A few nests are also confluent or oriented parallel to the epidermal surface, forming a sort of elongated festoon that can substitute the lowermost portion

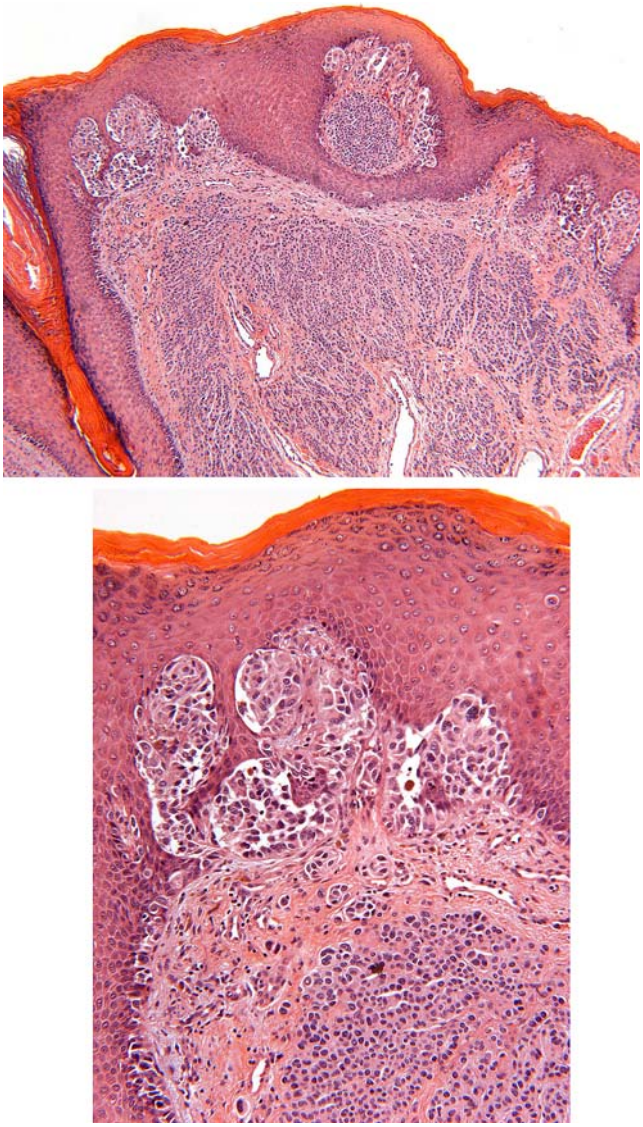

Fig. 11 In genital nevi, a striking cytological and architectural dysplasia is possible, as in this case. Note that the melanoma-like alterations are restricted to the junction, and the intradermal component has obvious benign looking features

of the epidermis. Cells can be large and have hyperchromatic nuclei, but they are monomorphous. In the past, these entirely benign lesions have been diagnosed as melanomas with subsequent vulvectomy and extensive lymph-node dissection. Fortunately, today, pathologists are well aware of these peculiar entities, and catastrophic errors of this kind are no more a part of our profession's medicolegal record.

The main criterion for the differential diagnosis of a melanoma is the fact that melanomas on the genitalia are highly atypical lesions with numerous mitoses, plain nuclear pleomorphism and cellular necrosis.

With the exception of architectural anomalies and mild nuclear hyperchromasia, there are no further indications of the malignant nature of the lesion in atypical nevi of the genitalia (and of other special sites): Cells are, in fact, typical (although they can be hyperchromic) and monomorphous, pagetoid spread is limited in the central portion of the lesion (or altogether absent) and there is no significant mitotic activity.

Congenital nevi with unusual features

A few dramatic cases of congenital nevi or of nevi with histological features of congenital nevi (occasionally called 'tardy' congenital nevi) have been misdiagnosed as a melanoma. Indeed, when arising on special sites, in youngsters or in pregnant women, these nevi can be very atypical and can contain unusual, alarming features.

These lesions are usually taken as invasive melanoma for the following three aspects: pagetoid scattering of cells above the junction, presence of large and confluent nests in the dermis and at the junction and presence of pigment at the bottom of the lesion. The most worrisome aspect is certainly represented by the large intra-dermal nests, which are much larger than the junctional ones and mimic the tumorigenic vertical proliferation (the so-called vertical growth phase) of a melanoma. Moreover, these nodules are scattered asymmetrically in the neoplasm giving a quite inordinate appearance. In fact, these congenital nevi are considered unusual because of their silhouette and of the infiltration's depth, while other aspects are indicative of an entirely benign congenital lesion:

- These bizarre nevi frequently present nests inside the follicular structures or in the eccrine ducts. Cells are occasionally found in and around the vascular and nervous structures. A perivascular cuffing of melanocytes is highly characteristic of these lesions.
- Cells are small and lymphocyte like, with barely detectable mitotic activity. Significant cellular atypia is absent.
- The large intra-dermal nodules, which are particularly worrisome, are roundish, symmetrical and homogeneous in cellular density. They neither compress nor destroy the surrounding nevus nor the dermal structures.
- Mitotic activity is virtually absent.

Deep penetrating nevus

Deep penetrating nevus [47, 117] is a form of melanocytic nevus with peculiar architecture and cytological features. Cells are gathered in nests and cords in a vaguely anastomotic, plexiform pattern, for this reason, Barnhill proposed the term plexiform spindle cell nevus. The entire dermis may be occupied by the nevus, and frequently, the sub-cutis is involved. Although usually wedge shaped and symmetrical, many cases of deep penetrating nevus have architectural asymmetry. Cells are plump, oval or dendritic. Among these cells, a few epithelioid elements are present as well. These large epithelioid cells have abundant clear cytoplasm and a large vesicular nucleus. In a few elements, nuclei can be hyperchromatic or with prominent nucleolus. Mitoses are possible even in the deep portion of the neoplasm. In the lesion, there is no maturation of the melanocytes as they go deeper in the dermis.

Deep penetrating nevus has some architectural similarities with forms of melanoma growing in a plexiform or nested pattern [136]. In this type of melanoma, cells aggregate in small nests scattered throughout the dermis; normal collagen fibres separate the nests. These nests are connected to each other forming a sort of plexiform structure. Despite the fact that the architecture of this form of melanoma is similar to that of deep penetrating nevus, the cytological aspects are completely different. In fact, melanoma cells show a uniform cellular atypia and nuclear pleomorphism; moreover, mitoses are easily found. In doubtful cases, one can consider that in melanoma, there is presence of atypical melanomatous proliferation at the junction, an aspect absent in deep penetrating nevus.

Ancient nevi

Pathologists, which are not familiar with a melanocytic neoplasm, may have some perplexity when facing compound or intra-dermal nevi containing some cells with large, hyperchromatic nuclei and blurred chromatin [67]. Occasionally, these cells are numerous or in clusters. The over diagnosis of these nevi as melanomas can be avoided considering that the atypical cells have indeed hyperchromatic, irregularly shaped nuclei, but no prominent nucleoli and no mitotic figures are seen. Cells are similar to those seen in ancient schwannomas (from the term ancient nevus). In fact, other degenerative or

senescent details are present, i.e. ectasic vessels, dermal sclerosis and fibrin deposits. Moreover, no expansile nodules are present, and the junctional melanocytic component is inconspicuous.

Nevi with occasional intra-dermal mitoses

It has been described decades ago that completely innocent lesions may have a few scattered mitotic figures in their midst [51]. The presence of a background of a common Miescher or Unna nevus easily rules out melanoma. Abundant collagen fibres and large portions of small nevus cells separate the cells in mitosis. Besides, melanoma developed in the core of the intra-dermal portion of a nevus is exceedingly rare in adults.

Polipoyd nevus of pregnancy

During pregnancy, a few alterations are occasionally seen in a compound nevi [41, 53, 84]. In a few cases, cells are large and epithelioid with pale cytoplasm and vesicular clear nuclei. Melanocytic nests become large and confluent. In other cases, nevi show a remarkable or irregularly distributed pigmentation. Sparse mitoses are possible. However, these nevi have no other indication of melanoma, no pagetoid spreading is seen and melanocytes, although large, are not atypical.

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ORIGINAL ARTICLE

Prognostic factors for ampullary adenocarcinomas: tumor stage, tumor histology, tumor location, immunohistochemistry and microsatellite instability

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Abstract Prognostic factors for ampullary carcinomas (ACs) are poorly defined. Fifty three resected ACs were analyzed for CDX2, MUC1, MUC5AC, MUC6, MUC2, and for mismatch repair proteins (hMLH1, hMSH2, PMS2, hMSH6) using immunohistochemistry. Microsatellite instability (MSI) status was evaluated by fluorescently labeled PCR using an automated sequencer. Univariate and multivariate analysis was performed for clinicopathological, immunohistochemical and molecular parameters. CDX2 was found in 32 out of 53 (60%) ACs with a significantly higher frequency among intestinal ACs compared with biliopancreatic (BP) ACs. The MUC1, MUC5AC, MUC6, MUC2 apomucins were expressed in 75, 43, 39, and 28% of ACs, respectively, with a significantly higher coexpression of MUC1/MUC5AC in BP ACs. MSI and loss of expression of hMLH1/PMS2 or hMSH2/hMSH6 proteins were observed only in intestinal ACs. Factors significantly correlated with improved survival in the univariate analysis were: low stage, absence of lymph nodes metastases, negative surgical margins (*R*0 status), and presence of MSI. In the multivariate analysis, stage was the only independent prognostic factor of survival. We conclude that stage is the only independent prognostic factor of survival in the multivariate analysis, whereas histological criteria and the immunohistochemical expression of apomucins and CDX2 are helpful in the classification and understanding of the histogenesis of ACs.

Keywords Ampulla of Vater · Cancer · Prognostic factor · Microsatellite instability · CDX2

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Introduction

There are different histological classifications of ampullary carcinomas (ACs) which [2, 3, 20] makes comparison of clinical, therapeutic, and prognostic studies of these tumors difficult. So far, two major histological types of these tumors have been recognized: an intestinal type, arising from intestinal mucosa of the papilla, and a biliopancreatic (BP) type, deriving from the BP ductal epithelium. Nevertheless, conflicting data have been reported about the frequency of these two histological types due to the absence of reliable and consistent histomorphological criteria for differential diagnosis [2, 20, 31]. As patients with intestinal-type ampullary ACs have been reported to have a significantly better prognosis than patients with BP-type ACs [2, 20], the clear differentiation between the two tumor types would have clinical implications.

Although immunohistochemistry has had only limited value to date in unequivocally differentiating BP ACs from intestinal-type ACs, several authors have suggested that knowledge of CDX2 and apomucin expression may be

useful for this purpose and may have a prognostic value [10, 12, 16, 18, 21, 23, 26, 31]. In particular, adenocarcinomas of the ampulla of Vater show positive reactivity for MUC1, MUC2, MUC5AC, and MUC6 [16], and some authors suggested that the prognosis of AC is worse with stronger staining for MUC1 and weaker staining for MUC2 [21, 26].

Human CDX2 is a homeobox gene encoding for an intestine-specific transcription factor [7, 14]. The CDX2 protein regulates normal development and differentiation of the small and large intestine and is expressed in the nuclei of epithelial cells throughout the intestinal tract both in embryonic and in postnatal life. Some authors have suggested that the immunohistochemical analysis of CDX2 expression may be a useful marker for identifying intestinal-type carcinomas with a sensitivity and specificity higher than 90% [5, 30]. In particular, very recently, Hansel et al. [18] reported that CDX2 expression may identify intestinal-type ACs with a favourable prognostic outcome.

Finally, few studies assessed the role of DNA mismatch repair (MMR) in ACs, and discrepant data are available about the frequency of a widespread microsatellite instability (MSI) in these cancers [1, 25, 27]. To date, the largest series of ACs was investigated for MSI status by Scarpa et al. [27] demonstrating the occurrence of MSI in 15% of cases as well as a significant correlation between MSI and a better 5 year survival in these patients compared with the remaining cases.

The aim of this investigation was to evaluate in a large series of ampullary cancers the prognostic value of clinicopathological, immunohistochemical, and molecular parameters such as location, histology, and stage of tumors, MSI status as well as the immunohistochemical expression of the MUC1, MUC2, MUC5AC, MUC6 apomucins and CDX2 protein.

Materials and methods

Histological and immunohistochemical study

Fifty-three cases of surgically resected primary carcinomas of the ampulla of Vater (ACs) were retrieved from the surgical pathology files at the Department of Pathology (University of Insubria, Varese, Italy) and the Ospedale di Circolo (Busto Arsizio, Varese, Italy). The selection of the cases was based on the availability of sufficient tumor samples for histopathological, immunohistochemical, and molecular analysis. Moreover, only patients harboring sporadic tumors were eligible for the study (two cases of ampullary cancers were excluded because of their association with familial adenomatous polyposis and with hereditary nonpolyposis colorectal cancer syndrome, respectively). The ethical consent of each patient was collected according with the rules of the local ethical committee.

Formalin-fixed and paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E) and Alcian Blue periodic acid Schiff stain (AB-PAS). The slides were reviewed to confirm the diagnosis according to the WHO classification (2001) and to study the extension of the neoplastic disease [17]. In each tumor, we considered both the main histological pattern (intestinal or BP) and a second or a third pattern when present in more than 10% of neoplastic tissue according to the criteria reported by Albores-Saavedra et al. [2]. The macroscopic classification as described by Cubilla and Fitzgerald [11] was used to distinguish the tumors as periampullary (PA), intra-ampullary (IA), and mixed types (MT). Follow-up data were collected by contacting clinicians and/or by consulting the Tumor Registry of the Lombardy region (Italy).

Immunoperoxidase studies were performed on sections prepared from formalin-fixed and paraffin-embedded specimens that were dewaxed and rehydrated using Bio-clear (Bio-Optica, Milan, Italy) and graded alcohols. Endogenous peroxidase was blocked by dipping sections in 3% aqueous hydrogen peroxide for 10′ and antigen retrieval was performed with 10′ microwave treatment in 10-mM citrate buffer, pH 6. The immunostaining was performed with the avidin–biotin–peroxidase complex technique using diaminobenzidine as a chromogen. The sections were incubated overnight at 4°C with mouse monoclonal antibodies against full-length hMLH1 protein (G168-15, PharMingen, San Diego, CA) at 1:100 dilution; carboxy-terminal fragment of hMSH2 protein (FE11, Oncogene Research Products, Cambridge, MA) at 1:200 dilution; hMSH6 protein (44, BD Transduction Laboratories, Europe) at 1:200 dilution; PMS2 (A16-4, BD Biosciences, Europe) at 1:200 dilution. Sections were lightly counterstained with hematoxylin. The normal staining pattern for hMLH1, hMSH2, hMSH6, and PMS2 was nuclear, and a case was considered positive only in the presence of nuclear staining of neoplastic cells. A case was considered negative for expression of hMLH1, hMSH2, hMSH6, and PMS2 when there was a complete absence of nuclear staining of neoplastic cells in the presence of an unquestionable internal positive control, represented by normal epithelial cells, stromal cells, muscle cells, or lymphocytes [8]. Immunoreactive intraepithelial lymphocytes were detected by using a mouse monoclonal antibody anti-CD3 (Biogenex, San Ramon, CA, USA) at 1:100 dilution.

The immunohistochemical study of intestinal transcriptional factor CDX2 was done with monoclonal antibody CDX2-88 (Biogenex) raised against a full-length CDX2 recombinant protein at 1:100 dilution.

Apomucins were studied using mouse monoclonal antibodies against MUC1 glycoprotein (MA695, Novacastra

Lab., Newcastle, UK) at 1:100 dilution, MUC2 glycoprotein (CCP58, Novacastra Lab) at 1:200 dilution, MUC5AC glycoprotein (CLH2, Novacastra Lab) at 1:100 dilution, and MUC6 glycoprotein (CLH5, Novacastra Lab) at 1:100 dilution.

All immunostaining results were evaluated according to the percentage of immunopositive tumor cells ranging from less than 10% of positive cells (+), between 10 and 50% of positive cells (++) and more than 50% of positive neoplastic cells (+++). Cases with no immunoreactive cells were indicated as negative (−).

DNA extraction and microsatellite analysis

The MSI status of all 53 ampullary cancers was determined. Tumor DNA was extracted from archival paraffin-embedded tissues using DNeasy Tissue kit (Qiagen, Hilden, Germany) after a manual microdissection to enrich the neoplastic cellularity of at least 80% in all tumor samples [15]. MSI status was assessed by a fluorescent pentaplex PCR of five quasi-monomorphic mononucleotide repeats (Bat-26, Bat-25, NR-21, NR-22, NR-24) according to the amplification conditions reported by Suraweera et al. [28]. The fluorescently labeled PCR products were electrophoresed on an Applied Biosystems 310 automated DNA sequencer (Applied Biosystems, Milan, Italy). This panel of quasi-monomorphic mononucleotide markers can identify the MSI status of tumors without the need for matching normal DNA. However, the size of all PCR products from tumor DNA were always aligned with normal-sized alleles from a germline DNA sample. A tumor was classified as MSI when allelic size variations were observed in ≥3 out of five markers and as MSS when allelic size variations were observed in ≤2 out of the five markers.

Statistical analysis

The statistical analysis was performed using Fisher's exact test and the χ^2 test with Yates' correction (SPSS 7.5 software). The survival analysis was performed by employing the Kaplan-Meier product limit estimate of probability of survival against time, producing a product limit survival curve for each of the following variables: tumor size, macroscopic localization, histological type, grade, stage, presence of lymph node metastases, immunohistochemical expression of CDX2, MUC1, MUC2, MUC5AC, MUC6, and MSI status.

Multivariate analysis was carried out using the Cox proportional hazard model. Variables with $p<0.15$ in the univariate analysis were entered into the multivariate analysis.

For all the survival analyses, the Survan XL program, version 1.14 (copyright© 1995–1997), was employed.

Results

Histopathological study

The series of patients with ACs included 30 men and 23 women with a mean age at diagnosis of 65.8 years (range 44–82 years). The comprehensive results of the clinicopathological and immunohistochemical study of these tumors are reported in Tables 1 and 2, respectively. According to Cubilla and Fitzgerald's criteria, 11 of 53 tumors (21%) were classified as PA, 35 of 53 (66%) as IA and 7 of 53 (13%) as MT ACs. Histologically, 23 ACs (43.4%) were classified as intestinal (I) type and 30 (56.6%) as BP type (Fig. 1). A second histological pattern (intestinal or mucinous) was detected in eight cases (26.6%)

Table 1 Clinicopathological, molecular, and outcome data for ampullary carcinoma

Variable	*N*°	Percent
Number of cases	53	
Mean age (years±SD)	65.8±8.67	
Gender		
Male	30	(56.6)
Female	23	(43.4)
Macroscopic classification		
PA	11	(20.7)
IA	35	(66)
MT	7	(13.3)
Histotype		
BP	30	(56.6)
I	23	(43.4)
Grade of differentiation		
G2	33	(62.2)
G3	20	(37.8)
Staging		
I	18	(36.7)
II	26	(53)
III	5	(10.3)
Lymph nodal metastasis		
N0	20	(40.8)
N+	29	(59.2)
Resection margin		
R0	42	(80.7)
R+	10	(19.3)
MSI status		
MSI+	5	(9.4)
MSI−	48	(90.4)
Follow-up		
ANED	14	(27)
DOD	29	(55.7)
POD	9	(17.3)

IA Intra-ampullary, *PA* periampullary, *MT* mixed type, *I* intestinal type, *BP* biliopancreatic type, *M* mucinous type, *Med*, medullary type, *S* solid type, *na* not available, *DOD* died of disease, *ANED* alive with no evidence of disease, *POD* postoperative died

Table 2 Summary of immunohistochemical staining results of MUC1, MUC2, MUC5AC, MUC6, CDX2 in ampulla of Vater adenocarcinomas

Ampullary carcinomas	Total cases	Positive cases (%)				
		MUC1	MUC5AC	MUC6	MUC2	CDX2
	53	40 (75)	23 (43)	21 (39)	15 (28)	32 (60)
Macroscopic classification						
PA	11	7 (63)	4 (36)	5 (45)	4 (36)	10 (90)
IA	35	30 (86)	15 (43)	15 (43)	8 (23)	17 (48)
MT	7	3 (42)	4 (43)	1 (14)	3 (42)	5 (71)
Histological type						
BP	30	29 (97)	17 (57)	13 (43)	4 (13)	9 (30)
I	23	11 (47)	6 (26)	8 (35)	11 (47)	23 (100)

PA Periampullary, *IA* intra-ampullary, *MT* mixed type, *BP* biliopancreatic type, *I* intestinal type

out of the 30 BP-type ACs (Table 1), whereas seven cases (30.4%) out of 23 intestinal-type ACs were associated with a BP pattern as a second component. Thirty-three tumors were moderately differentiated (grade 2), and 20 were poorly differentiated (grade 3). The T stage distribution was: 11 cases (21%) at T1, 21 (40%) at T2, 16 (30%) at T3, and 5 (9%) at T4. The pancreatic and choledochus resection margins were negative in all the cases. The posterior fat tissue was macroscopically negative in all cases but residual neoplastic disease (microscopically positive margin of resection: R1) was found in ten cases (19.2%). A total of 924 regional lymph nodes were examined, (range 5–49, mean 18.8; median 14). Lymph node metastases were observed in 28 out of the 49 (57%) cases for which the information was available (range 1–32, mean 4.7, median 2). The stage distribution was: 18 cases (37%) at stage 1, 26 (53%) at stage 2, 5 (10%) at stage 3.

Immunohistochemical and molecular study

The immunohistochemical expression of CDX2 and MUC1, MUC2, MUC5AC, and MUC6 apomucins was significantly different in intestinal and BP ACs. In particular, all intestinal ACs showed a nuclear immunoreactivity for CDX2 with 19 out of 23 cases demonstrating a high level of expression (score 3+ in seven cases and 2+ in 12 cases) compared with BP ACs exhibiting CDX2 immunoreactivity in nine out of 30 (30%) of cases (score

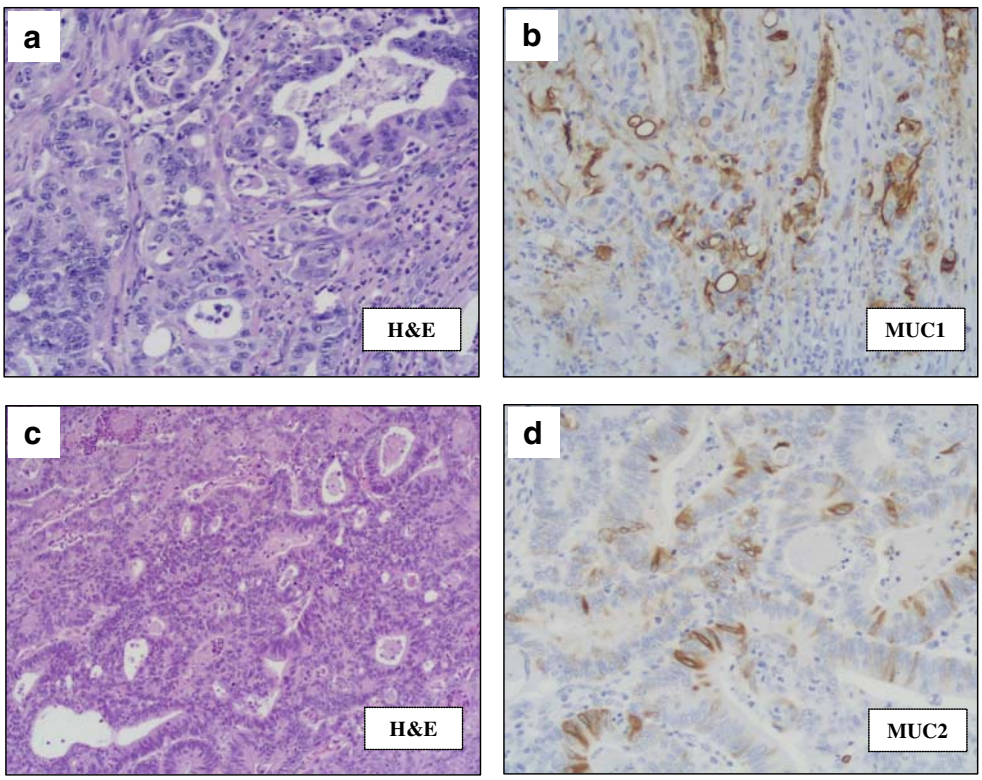

Fig. 1 Histology and immunohistochemistry of grade II ampullary adenocarcinoma of biliopancreatic type (**a**) with MUC1 expression (**b**) and of grade II ampullary carcinoma of intestinal type (**c**) with MUC2 immunoreactivity (**d**). Original magnification ×200 in (**a**), (**b**), (**d**); ×100 in (**c**)

Table 3 Immunohistochemical expression of MMR proteins and mononucleotide instability in ampullary carcinomas with MSI in at least one locus

N°	Histology	Immunohistochemical analysis				MSI analysis				
		hMLH1	hMSH2	hMSH6	hPMS2	Bat-26	Bat-25	NR-21	NR-22	NR-24
1	AC-I	−	+	+	−	+	+	+	+	+
2	AC-I, Muc	−	+	+	−	+	+	−	−	+
3	AC-I, Muc	+	−	−	+	+	+	+	+	+
4	AC-I, Med	+	−	−	+	+	+	+	+	+
5	AC-I, Med	+	−	−	+	+	+	+	**na**	**na**
6	AC-I, BP	+	+	+	+	+	+	−	−	−
7	AC-I	+	+	+	+	−	+	−	−	−
8	AC-I	+	+	+	+	−	+	−	−	−

AC-I Ampullary carcinoma of intestinal type, *Muc* mucinous, *Med* medullary type, *BP* biliopancreatic type, *M* methylated, *U* unmethylated, *na* not available

2+ in 3 cases and 1+ in 6 cases; $p<0.000001$). Notably, all but one of the BP ACs which were positive for CDX2 showed an intestinal and/or a mucinous component.

A nuclear immunoreactivity for CDX2 was found in 32 out of 53 (60%) ACs with a significantly higher percentage of positive cases among PA and MT ACs compared with IA ACs ($p=0.028$). Considering the grading of the tumors, CDX2 staining was found in 18 of 33 (54.5%) moderately differentiated and, in 13 of 20 (65%), poorly differentiated adenocarcinomas, but no significant correlation was observed.

The MUC1, MUC2, MUC5AC, MUC6 apomucins appeared to be expressed in 75, 43, 39, and 28% of ACs, respectively. A significantly higher frequency of MUC1 and MUC5AC expression was detected in BP than in

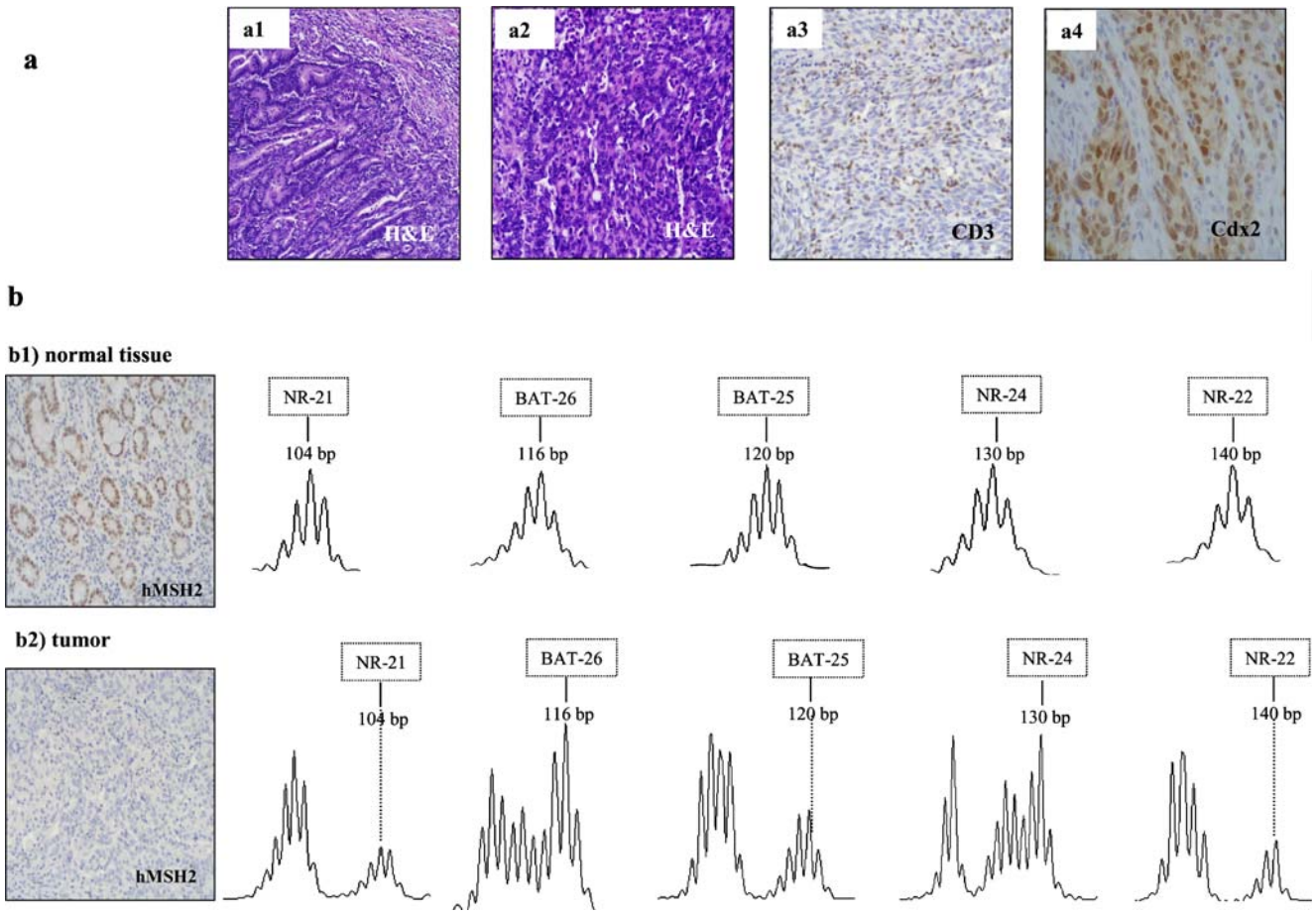

Fig. 2 Example of an intestinal-type AC (case 40) showing CD3 and CDX2 immunoreactivity (**a**) and loss of hMSH2 immunohistochemical expression and presence of MSI at all five mononucleotide markers examined (**b**)

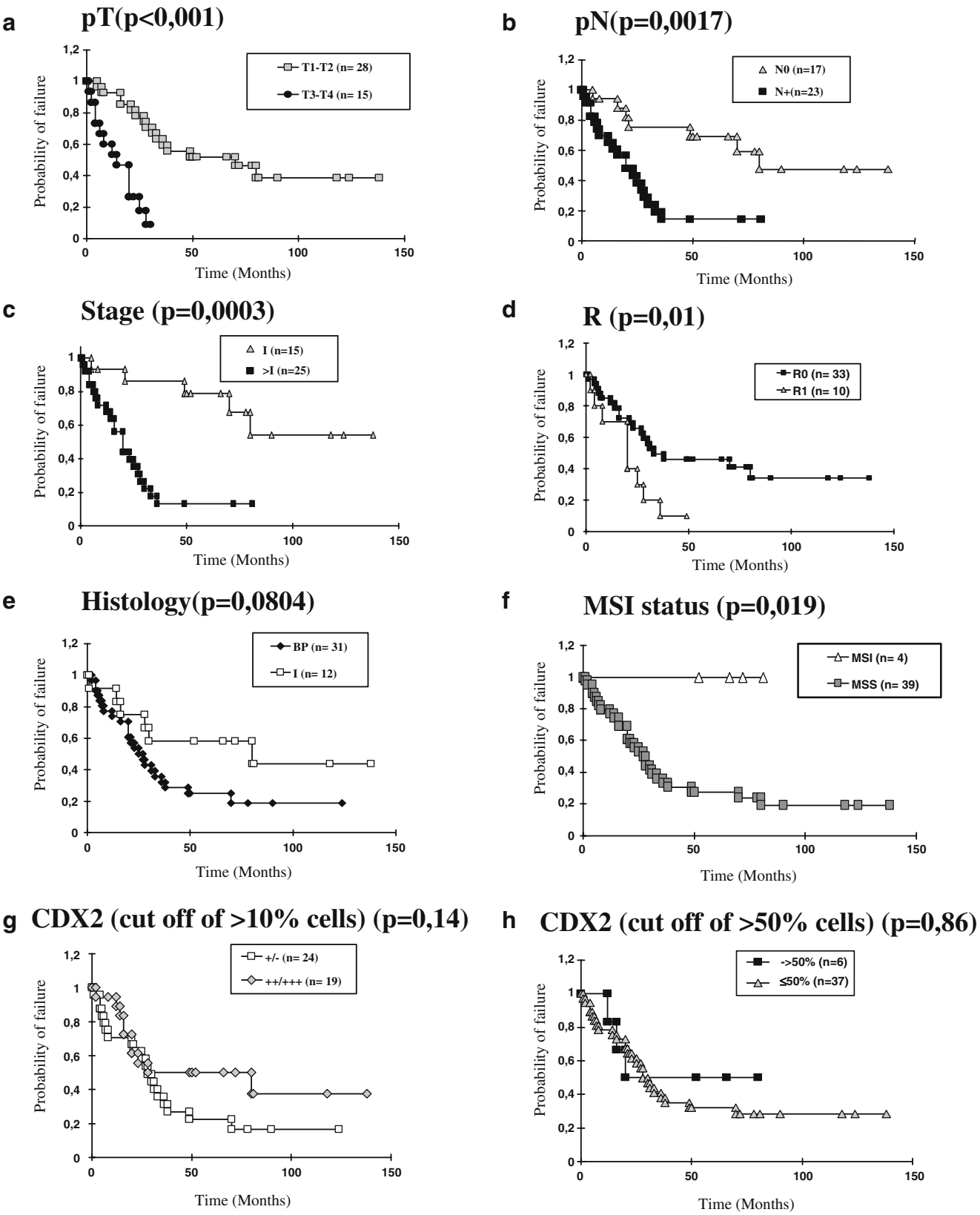

Fig. 3 Factors influencing survival in the univariate analysis were presence of microsatellite instability (**f**), clinical and pathological stage (**c** and **a**, respectively), lymph node metastases (**b**), *R* status (**d**), histology (**e**), CDX2 immunoreactivity (**g**–**h**)

Table 4 Results of the multivariate analysis

Variable	HR	95% CI	SE	*p* value
Histology	0.73	0.2335–2.3	0.58	0.5943
CDX2	1.00	0.4143–2.4568	0.45	0.9845
MSI status	0.00	0–1.7 E34	45.77	0.8117
R status	0.63	0.2528–1.5805	0.46	0.3266
Stage	6.84	1.8756–25.0107	0.66	0.0036

HR Hazard ratio, *CI* confidence interval, *SE* standard error

intestinal ACs ($p<0.0001$ and $p<0.05$, respectively, for the two antibodies), whereas a significantly higher percentage of positive cases for MUC2 was found among intestinal ACs compared with BP ACs ($p<0.023$). Moreover, a higher frequency of MUC1 immunoreactivity was detected in the IA cancers (86% of tumors) than in PA and MT ACs (63 and 42% of tumors, respectively). No other significant correlations were detected between the immunostaining results for the apomucins and macroscopic classification, histological type, or grading of ACs. MUC6 was rarely expressed in the ACs included in this study, and positive cases showed a focal immunoreactivity.

MSI was observed in five out of 53 ampullary ACs (9.5%) showing allelic size variations in at least three of the five mononucleotide markers (Tables 1 and 3). The remaining tumors were classified as MSS, exhibiting an absence of MSI in all markers or the presence of allelic size variations at two loci (case *N*° 6 in Table 3) or at one locus (cases *N*° 7 and *N*° 8 in Table 3). All five MSI tumors were intestinal-type ACs showing a mucinous component in two cases, a medullary component in two cases and a rich lymphoid infiltrate around the tumor in one case (Fig. 1). Moreover, all five MSI tumors showed a CDX2 immunoreactivity and a variable pattern of immunohistochemical expression of apomucins (Table 1).

To corroborate the MSI data, all eight ACs showing allelic size variations in at least one locus were analyzed for hMLH1, hMSH2, hMSH6, and PMS2 immunostaining (Table 3). All the five MSI ACs showed lack of the immunohistochemical expression of hMLH1/PMS2 proteins (cases 1 and 2) or of hMSH2/hMSH6 proteins (cases 3, 4, 5). By contrast, the three cases classified as MSS displayed an intense nuclear immunoreactivity for all MMR proteins. Figure 2 shows an example of the histological, immunohistochemical, and molecular profile of an AC with MSI.

Survival analysis

Follow-up data were available for 52 of the 53 patients with ACs. Nine patients were excluded from the survival analysis because they died within the first 60 days after tumor resection. Twenty-nine patients (67% of cases) died of disease after a median time of 24.8 months (range: 4–80), whereas 14 patients (33% of cases) are still alive after a median follow-up time of 71.2 months (range: 8–138).

Factors influencing survival in the univariate analysis were (Fig. 3): tumor stage (median survival >49 months for T1–T2 vs 7 months for T3–T4, $p<0.001$), lymph node metastases (median survival >52 months for lymph node negative tumors vs 20 months for lymph node positive tumors, $p=0.0017$), *R* status (median survival >31 months for complete tumor resection with grossly and microscopically negative margins vs 20 months for grossly negative but microscopically positive margins of resections), and presence of MSI (all patients with MSI tumors were alive with no evidence of disease after a median follow-up time of 69 months, $p=0.019$). Although the results were not statistically significant, a longer survival was correlated with the immunohistochemical expression of CDX2, using a nuclear labeling cut-off of >10% cells ($p=0.14$) as well as with an intestinal type of AC ($p=0.08$). No correlation with survival was observed even when considering different cut-offs for CDX2, including ≥25, 30, 40, and 50% nuclear labeling. Finally, no other correlation was observed between patient outcome and clinicopathological parameters including macroscopic localization, size, and grade of the tumor.

A multivariate analysis was performed using the Cox proportional hazard model for all variables which were significant or near to statistical significance in the univariate analysis, and only stage was an independent prognostic factor (Table 4).

Discussion

ACs demonstrate a wide spectrum of biological behavior that probably reflects their different histogenesis, arising from one of the three types of epithelium that converge at this site (duodenal mucosa, pancreatic ductal epithelium, and biliary ductal epithelium). Currently, conflicting data have been reported about the frequency of the two major histological types of ACs (intestinal and BP type) due to the absence of reliable histomorphological or immunohistochemical markers for differential diagnosis [2, 20, 31]. Very recently, Hansel et al. [18] examined a large series of ACs demonstrating that CDX2 expression alone may identify intestinal-type tumors with good prognosis, suggesting that it may be used as an independent marker of outcome at this site.

In this work we evaluated the correlation between the histomorphological classification and the immunohisto-

chemical expression of CDX2 and found a high specificity of the protein for intestinal-type ACs and for the minor intestinal and/or mucinous components of the BP-type ACs (Table 1). Intriguingly, a significantly higher percentage of CDX2 immunoreactive cases was observed among PA and MT ACs compared with IA ACs (p=0.028) in line with the possible origin of these cancers in duodenal mucosa.

As regards the prognostic role of CDX2 expression, our results are in contrast with previously reported data by Hansel et al. [18] showing that CDX2 expression in ACs was the only independent predictor of survival in the multivariate analysis of their AC cases. In the present work, although CDX2 expression showed a trend towards a longer survival in the univariate analysis, no significant prognostic role as an independent marker was demonstrated for this variable in the multivariate analysis.

The univariate analysis showed that, microscopically positive margins of resection (R1), advanced clinical stages, and lymph node metastases were significantly related to a shorter survival, whereas the presence of MSI was a marker of a better outcome. In the multivariate analysis, stage group remained the only significant and independent prognostic factor of survival, as reported in other studies [4, 22, 24].

A widespread MSI as well as loss of the immunohistochemical expression of hMLH1/PMS2 or hMSH2/hMSH6 proteins were observed in five ACs (9.5% of all cases, 22% of the intestinal ACs). All MSI ACs were of an intestinal type, and half were associated with a mucinous or with a medullary component. In one case, a rich lymphoid infiltrate around the tumor was observed. In addition, all MSI ACs were intensely CDX2-positive. This histopathological and immunohistochemical profile appears to be similar to that of gastric and colorectal MSI cancers [6, 9, 13, 19] and confirms the notion that MSI is a peculiar feature of a consistent percentage of intestinal-type ACs [27]. Until now, few and controversial data have been reported about the frequency of a MSI in ACs [1, 25, 29] probably due to different criteria for tumor selection, including cases of not unequivocally ampullary origin and ACs of different types. Our results regarding MSI ACs are comparable with those reported by Achille et al. [1] that indicated a 20% rate of MSI ACs in a series of 25 cases and reported a significant association between MSI and longer patient survival in the univariate analysis.

Finally, differences in the mucin distribution between BP and intestinal ACs were observed. The coexpression of MUC1 and MUC5AC appears to be a peculiar feature of BP ACs, whereas a strong production of MUC2 is associated with an intestinal histology and a CDX-2 positive immunophenotype. These data are consistent with the findings reported by Chu et al. [10] and suggest that the two main immunoprofiles mentioned above are helpful in the differential diagnosis between intestinal and BP types of ACs. However, the composite pattern of apomucin expression often observed in ACs does not allow a clear-cut classification of the two major histological types and suggests only an ancillary use of apomucins in this differential diagnosis. Furthermore, no significant correlation between mucin immunoprofile and survival was observed. Similar results have been reported by using other immunohistochemical markers such as cytokeratin 7 and cytokeratin 20 [10, 31].

In conclusion, this study shows a comprehensive analysis of the main clinicopathological, immunohistochemical, and molecular parameters suggested to have an impact on survival of patients with ACs and proves that a precise stage assessment remains the only independent predictor of survival. The extent of resection appears to be prognostically of great significance, although it is not part of the TNM staging as well as negative lymph nodes, and presence of MSI are significantly correlated with improved outcome for the patients.

Although precise histological criteria and the immunohistochemical expression of apomucines and CDX2 are helpful in the classification of the two major histological types of ACs, no significant correlation between intestinal profiles and improved survival is observed.

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ORIGINAL ARTICLE

Increased epithelial cell proliferation in the ileal pouch mucosa of patients with familial adenomatous polyposis

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Abstract To eliminate the risk of colorectal cancer in patients with familial adenomatous polyposis (FAP), reconstructive proctocolectomy is performed. Although most colonic mucosa is resected during the ileal pouch anal anastomosis, adenomas and carcinomas may develop in the pouch. This may be caused by altered cell kinetics due to intraluminal changes in the pouch. In 32 patients with FAP, biopsy specimens from the mucosa of the pouch and also of the afferent ileal loop were taken. Tissue sections were immunohistochemically processed with the monoclonal antibodies M30 and MIB-1 to assess apoptotic and proliferative indices, respectively. Cell proliferation was also assessed by a modified sign test. There were no significant differences in apoptotic rates between the mucosa of the pouch and the mucosa of the afferent ileal loop. However, cell proliferation was significantly higher in the mucosa of the pouch vs afferent ileal loop, both by using the quantitative (68.3% vs 61.6%, $p=0.001$) and semiquantitative methods ($p<0.05$). Our newly developed semiquantitative approach outperformed previously described methods. The higher cell proliferation in the pouch as compared to the afferent ileal loop may contribute to the increased risk for adenomas and carcinomas in the pouch of patients with FAP and emphasizes the need for regular endoscopic surveillance.

Keywords Familial adenomatous polyposis · Restorative proctocolectomy · Cell proliferation · Apoptosis and carcinogenesis

Introduction

Familial adenomatous polyposis (FAP) is an autosomal-dominant disease with an estimated prevalence of 1 in 5,000 to 1 in 7,500 [5]. It is characterized by the development of hundreds of adenomas in the large intestine. Without surgical intervention, virtually all patients will develop colorectal cancer at relatively young age.

To eliminate the risk of colorectal cancer, a restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) is accepted as one of the surgical treatments of choice in these patients.

In the IPAA procedure, almost all colonic mucosa is resected, thus reducing cancer risk significantly. However in patients with FAP, adenomas are also present in the upper gastrointestinal tract; approximately 5% of the patients having gastric, 60 to 90% duodenal [4], 50% jejunal [14], and 9 to 25% ileal adenomas [14, 23, 34]. Occurrence of ileal adenomas is of special interest because the terminal ileum, which is used to construct the pouch, may already contain adenomas. There is accumulating evidence that adenomas develop in the pouch. The reported incidence after a follow-up of at least 5 years is 8 to 60%, increasing up to 75% in certain subgroups [14, 26, 33, 38], which is much higher as

The experiments performed for this study comply with the current laws of The Netherlands.

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compared to the reported incidence of 9–25% in the preoperative ileum of patients with FAP [14, 23, 34]. Therefore, it seems that in the ileal pouch of patients with FAP, the development of adenomas is accelerated. At present, the malignant potential of the pouch adenomas is unclear. However, 13 patients with a carcinoma in the pouch have been recently described in the literature [2, 3, 7, 9, 13, 24, 25, 29, 35–37].

Stasis in the pouch causes a change in the luminal content, which is in close contact with the ileal mucosa. There is an increase in the concentration of short-chain fatty acids to colonic levels [10], an increase in anaerobic bacterial counts resulting in a more colonic type of flora, and an increased deconjugation and dehydroxylation of bile acids by anaerobic bacteria [20, 21]. The intestinal flora could play a role in initiation and promotion of colon cancer by activation of various classes of carcinogens, such as nitrosamines, polycyclic aromatic hydrocarbons, and glycosides [17]. A direct link between intestinal bacterial flora and tumor promotion in the $APC^{Min/+}$ mouse model for FAP has already been established [22].

Therefore, we hypothesize that the intraluminal ecological changes in the pouch are responsible for enhanced tumor formation in the mucosa of the pouch. Alteration of cell kinetics in the mucosa, including a lower rate of apoptosis and a higher rate of cell proliferation, is associated with tumor initiation and promotion. In colorectal cancer, the balance between apoptosis, cell proliferation, and cell shedding is disturbed, and such changes may favor the development of adenomas [30]. Up to now, no data are available on changes in proliferation and apoptosis rates of the ileal mucosa of the pouch after IPAA in patients with FAP.

The aim of this study is to investigate changes in apoptosis and cell proliferation rates, occurring in the mucosa of the pouch of patients with FAP, in comparison with the ileum of the afferent loop. The results may contribute to a better understanding of the enhanced adenoma formation in the pouch compared to normal ileum.

Materials and methods

Patients and tissues

The study was approved by the regional medical ethical commission, and informed consent was obtained from all patients.

Patients with FAP and an IPAA, who were under surveillance in the Radboud University Nijmegen Medical Centre or regional affiliated hospitals, were invited to participate in this study. Thirty-two patients with FAP were included. The diagnosis FAP was based on either a clinical presentation of at least 100 colonic adenomas or a mutation in the APC gene. Data concerning the surgical procedures were obtained from medical records.

From each patient, mucosal biopsy specimens of both the pouch and the afferent ileal loop were obtained during a regular surveillance endoscopy, in the period January 2002 until April 2004. Patients were fasted overnight. On the day of examination, patients were encouraged to drink liberally. No laxatives or cathartic enemas were given. To clear the pouch of fecal ruminants, two 250-ml water enemas were given before the endoscopy.

The endoscopy was performed with an Olympus GIF-1T140 video endoscope. From January 2002 until August 2003, a 2.8-mm diameter biopsy forceps (FB 13K-1 Olympus, Tokyo, Japan) was used, and from September 2003 until April 2004, a 3.0-mm diameter biopsy forceps (B102-C1-30.160 MedWork/Treier Endoscopie GA, Beromünster, Switzerland) was used. The afferent loop was introduced up to 20 cm proximal of the pouch. The mucosa was sprayed with 1% indigo carmine dye (Laboratoires SERB, Paris) at 1:1 dilution with water, where after photographs were taken to evaluate number and size of adenomas present.

For pathological examination, at least four biopsies were taken at random locations from the afferent ileal loop (10 to 20 cm proximal from the pouch), four biopsies from the pouch mucosa (at least 5 cm proximal from the anal verge), and four biopsies from adenomas if present. The biopsies from adenomas were used only for pathological evaluation, i.e., to exclude serious dysplasia in pouch adenomas, and were not used for research purposes. The biopsies were stretched on filter paper to maintain correct orientation of crypts, fixated in formalin, and embedded in paraffin.

Immunohistochemistry

Cell proliferation and apoptosis were measured in the stretched formalin-fixed nonadenomatous tissue. The crypt cell proliferation activity was assessed after staining with the monoclonal antibody MIB-1 (Dako A/S, Glostrup, Denmark), which recognizes the Ki-67 nuclear antigen of dividing cells in formalin-fixed paraffin-embedded tissue [8]. Apoptosis was assessed by staining with the monoclonal antibody M30 (Roche Diagnostics, Mannheim, Germany), which recognizes cleaved cytokeratin 18. Immunoreactivity of M30 is confined to the cytoplasm of apoptotic epithelial cells and is expressed during early apoptosis [18].

Tissue sections of 4-μm thickness were cut from paraffin blocks, mounted on electrostatic slides (Super Frost Plus, Menzel-Gläser, Germany), and dried overnight, followed by drying in a stove at 50°C for 15 min.

Sections were put in xylol for 10 min and taken from xylol through 100% alcohol to water. After deparaffinization, endogenous peroxidase was blocked by treatment with 3% hydrogen peroxide in phosphate-buffered saline (PBS) for 30 min. Pretreatment was performed by heating the tissue sections in citrate buffer (10 mmol/l, pH 6.0) at 180-W power in a microwave oven for 10 min. After cooling at room temperature for 1.5 h, sections were rinsed with PBS. Then, 20% normal horse serum (Vector Laboratories, Burlingame, CA, USA) was applied for 10 min. The sections were then incubated overnight at 4°C with either the mouse monoclonal antibodies MIB-1 at 1:1,000 dilution or M30 at 1:100 dilution.

Thereafter, the sections were rinsed in PBS for 10 min and incubated with biotinylated horse anti-mouse IgG (Vector) for 30 min. After rinsing for 10 min in PBS, incubation with 'avidin and biotinylated horseradish peroxidase macromolecular complex' (Vector) was performed for 45 min. Finally, 5 mg/10 ml diaminobenzidine was used as chromogen, and the sections were put in 0.5% $CuSO_4$/0.9% NaCl for 5 min to intensify the staining. Mayer hematoxylin counterstaining was applied. Sections were dehydrated through 100% alcohol, cleared in xylol, and coverslipped with permount (Fisher Scientific, Fiar Lawn, NJ, USA).

Tissue sections of rectal carcinoma were used as positive controls.

Evaluation of immunostaining results

Investigators were blinded for the origin of the tissue sections, regarding patient and biopsy location.

For evaluation of M30 staining, tissue sections were examined by light microscopy. M30 positivity was identified as brown cytoplasmic staining. M30-positive cells were marked by a first investigator (BvH) and reevaluated by an expert pathologist (IN). In all cases, the complete section was evaluated, and all M30-positive epithelial cells were counted. The apoptotic index was expressed as the number of M30-positive cells per tissue area in square millimeters. Tissue area was assessed by using a Zeiss KS400 computer-aided system.

In each MIB-1-immunostained tissue section, crypts whose entire length could be visualized were photographed under ×400 magnification using a Zeiss KS400 computer-aided system. Crypts were excluded when they did not reach the muscularis mucosae or had multilayered bases.

MIB-1 positivity was identified as brown nuclear staining. The number of MIB-1-positive epithelial cells and the total number of epithelial cells in up to five crypts per tissue section were counted from screen. The labeling index for each crypt was given by the ratio of MIB-1-positive cells and the total number of crypt epithelial cells and is expressed as percentage of total. For each patient, the labeling indices of pouch and afferent ileal loop were expressed as means of three to five counted crypts. If less than three crypts could be photographed for either pouch or ileal loop, the patient was excluded from analysis. The photographed crypts of five randomly selected patients were counted twice by one investigator (BvH) to determine intraobserver variability.

Although great effort was made to obtain well-orientated mucosal crypts when using the quantitative method, however, not all biopsies reached the criteria mentioned above, and therefore, could not be examined. This problem mainly occurred in the biopsies taken from the pouch and might be due to friability of the pouch mucosa. We therefore developed a new semiquantitative scoring system. A representative part of the biopsies showing several complete crypt/villous axes was photographed under ×100 magnification. The photographs were judged pair-wise (pouch vs afferent loop) during which the investigators had to choose from four possible outcomes; one of the two locations showed most MIB-1 positivity. MIB-1 positivity did not differ or no judgement could be made. Judgement was based on relative length of the area of positive cells and the relative size of the stem cell compartment. Five investigators, two pathologists (IN, HvK), two gastroenterologists (PF, FN), and one junior investigator (BvH) independently compared the paired photographs of biopsies of pouch and afferent ileal loop of all patients. When three or more observers agreed in their judgement, this judgement was denoted as consensus judgement. If this criterion was not met, no consensus was reached. If the quality of the tissue sections was poor, no judgment was made. One investigator (BvH) judged the whole series twice for evaluation of intraobserver reliability.

Statistical analyses

Values for apoptosis and cell proliferation in the quantitative study were not expected to be normally distributed; therefore, they were presented as median and range. The Wilcoxon matched-pairs signed-ranks test was used to compare the paired observations in the apoptosis staining and the paired mean labeling indices in the cell proliferation study.

Consensus judgements on the semiquantitative assessment of cell proliferation, favoring either pouch or ileal afferent loop, were compared with a Sign test. To evaluate the reliability of this semiquantitative method to assess cell proliferation, Cohen's kappa was calculated for the first and second series of judgements by the prime investigator to determine intraobserver reliability. Also, for each pair of investigators, a Cohen's kappa was calculated. The mean Cohen's kappa was taken as value for interobserver reliability.

Table 1 Patient characteristics

	Apoptosis	Cell proliferation
Number of patients studied	32	20
Male/Female	19/13	12/8
Median age in years (range)	32 (16–72)	29 (16–62)
Median age at surgery in years (range)	24 (10–55)	20 (10–52)
Median age pouch in months (range)	96 (9–216)	105 (9–216)
IPAA: hand-sewn/double-stapled/ unknown	9/21/2	5/14/1
Carcinoma at surgery	4	0
Patients with adenomas at biopsy: yes/no	24/8	15/5

For quantitative cell proliferation analysis, 12 patients had to be excluded from analysis (right column) due to absence of sufficient evaluable crypts (see under "Materials and methods").

Consensus judgments were compared to the difference in mean labeling index between pouch and ileal afferent loop for each evaluable patient.

A p value less than 0.05 was considered as significant (SPSS for Windows 11.0.1, 2001).

Results

Patient characteristics

Patient characteristics are given in Table 1. The median age of the 32 patients (19 men, 13 women) included in the study was 32 (range 16–72) years.

Twenty-three patients were operated in the Radboud University Nijmegen Medical Centre. The median age at the time of reconstructive colectomy was 24 (range 10–55) years. A mucosectomy with hand-sewn IPAA was performed in 9 patients, and a double-stapled IPAA was performed in 21 patients. For two patients, the information about the performed technical procedure could not be retrieved. At the moment of colectomy, four patients had a colorectal adenocarcinoma localized in the rectum, sigmoid, hepatic flexure, or appendix, respectively.

At the time of endoscopy, the median age of the pouch was 96 (range 9–216) months. The medication used was loperamide by 18 patients, psyllium fibres by 3 patients, and iron, metoclopramide, colestyramine, omeprazole, sulindac, tramadol, tamoxifen, gosereline, nifedipine, metoprolol, furosemide, or losartan each by 1 patient. Thirteen patients were not on medication 3 months before endoscopy.

One patient used sulindac, a nonspecific cyclooxygenase inhibitor, which is thought to influence cell proliferation and especially apoptosis [15, 27]. Exclusion of this patient from analyses had no effect on the results.

Histological examination revealed pouch adenomas in 24 patients (75%).

Apoptosis

In both pouch and afferent ileal loop, M30-positive cells were predominantly detected in the mucosal villi (Fig. 1).

In the 32 pairs of biopsies from pouch and afferent ileal tissue investigated, the median absolute number of apoptotic cells in the pouch mucosa was two per tissue section (range 0–9), which was identical to the values in the afferent ileal loop (2, range 0–19). The median apoptotic index (expressed as number of M30-positive cells per mm^2 tissue section area) did not differ between pouch (median 0.4/mm^2, range 0–2.9) and afferent ileal tissue (median 0.3, range 0–2.7; see Fig. 2).

Cell proliferation

Quantitative comparison

The intraobserver reliability for counting of the photographed crypts (Fig. 3) was r_s=0.855, p=0.002.

In 12 pouch tissue sections, less than three crypts were available for counting, and these samples were therefore excluded from analysis. For the same reason, two afferent ileal tissue sections were excluded. In the 20 pairs of pouch and afferent ileum tissue sections left for comparison, median labeling index (expressed as percentage of MIB-1-positive epithelial crypt cells) was significantly higher in

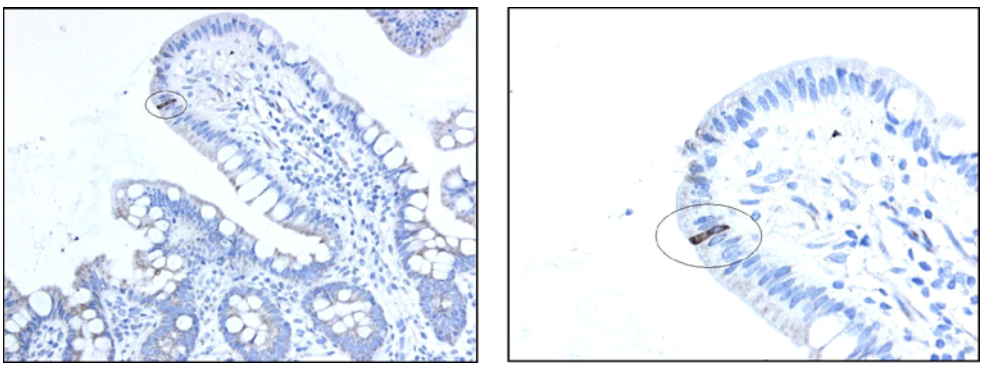

Fig. 1 Apoptotic epithelial cells (*encircled*) using M30 immunohistochemistry. Original magnification ×200 (*left*) and ×400 (*right*)

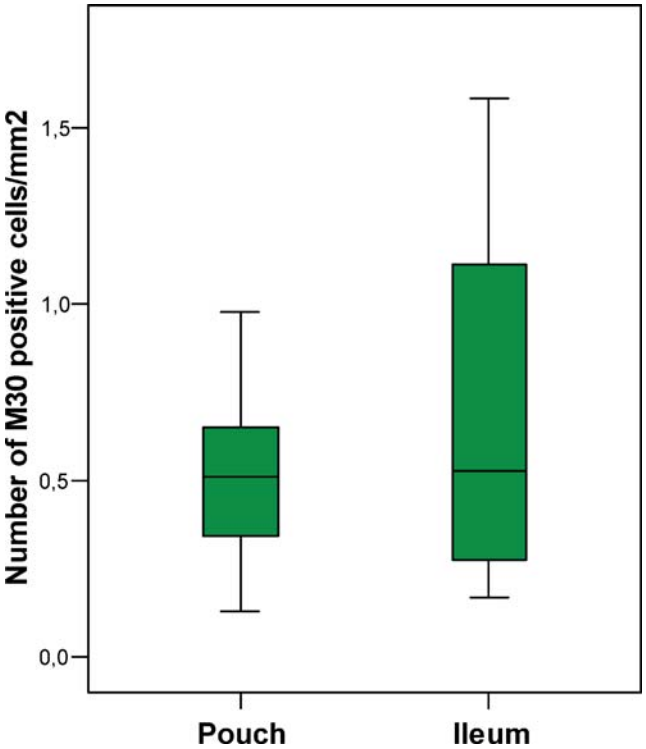

Fig. 2 Box-Whisker plots of apoptosis expressed as number of M30-positive epithelial cells per mm^2 tissue section area of pouch and afferent ileum of 32 patients with FAP. Difference between groups was not significant. Values are given as median (*fat line*), lower to upper quartile (*green box*), and minimum and maximum values (*error bars*)

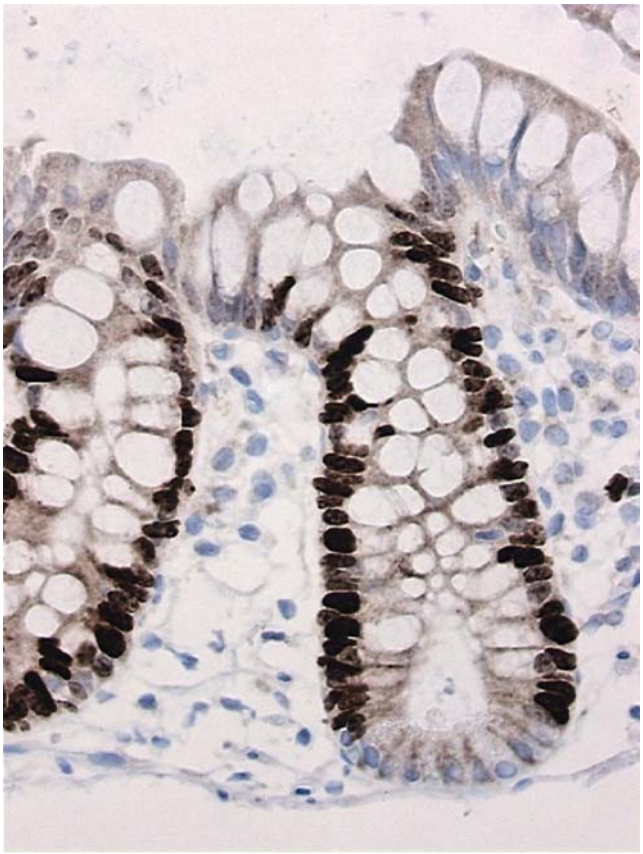

Fig. 3 Detection of proliferating crypt cells with MIB-1 immunohistochemistry. *Brown stained nuclei* are positive. Nonproliferative crypt stem cells are visualized at the base of the crypt. Original magnification ×400

the pouch as compared to the afferent ileum (median 68.3%, range 52.9–79.6% vs median 61.6%, range 38.0–73.9%; Wilcoxon signed-ranks test, $p=0.001$; Fig. 4).

Semiquantitative comparison

The results of the semiquantitative comparison of the photographed tissue sections from the pouch and afferent ileum mucosae (Fig. 5) are visualized in Fig. 6. In 15 patients (47%), the proliferation was higher in the pouch; in 7 patients (22%), there was no difference; in 5 patients (16%), proliferation was higher in the afferent ileal loop; in 2 patients (6%), no consensus could be achieved; and in 3 patients (9%), no judgement was possible due to poor quality of the tissue sections. These differences were significant ($p<0.05$).

Intraobserver reliability was $\kappa=0.87$. Cohen's kappa for each pair of observers ranged from 0.26 to 0.59 with a mean Cohen's kappa of 0.38, which corresponds with "fair agreement" following Byrt's guidelines [6]. Thus, interobserver reliability was acceptable.

In cases in which a labeling index could be assigned to both pouch and afferent ileal loop, consensus judgment was available in 18 cases (Fig. 7). In all cases in which the semiquantitative analyses showed more proliferation in the pouch ($n=11$), this was confirmed by the quantitative method; the median value of the difference in proliferation between pouch and ileum was 0.107 (range 0.032–0.175). In the five cases where no difference was observed with the semiquantitative approach, the median value of the difference in proliferation between pouch and ileum was 0.023 (range −0.096 to 0.120). There were only three cases in which the semiquantitative method showed more proliferation in the afferent loop, and the median value of the difference in proliferation between pouch and ileum in these cases was 0.098 (range −0.021 to 0.262). The Spearman's correlation coefficient between the semiquantitative and the quantitative methods was 0.273 ($p=0.244$).

Discussion

Although both the pouch and its afferent loop contain the same preexisting ileal mucosa, adenomas occur more frequently in the pouch of patients with FAP than in the afferent ileal loop, suggesting an accelerated adenoma formation in the pouch [14, 26, 33, 38]. To investigate the

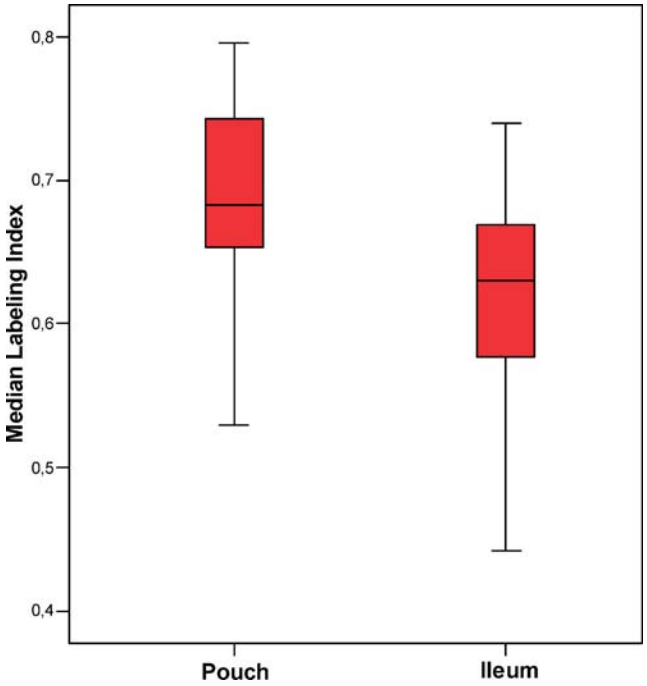

Fig. 4 Box-Whisker plots of cell proliferation expressed as median labeling index (MIB-1-positive crypt cells per total number of crypt cells) of 3–5 crypts in tissue sections of pouch and afferent ileum of 20 patients with FAP. Difference between groups was significant (p= 0.001). Values are given as median (*fat line*), lower to upper quartile (*red box*), and minimum and maximum values (*error bars*)

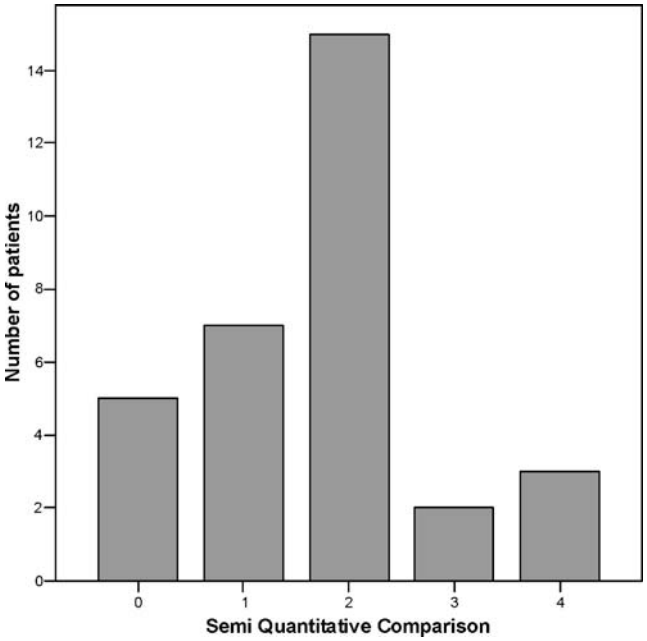

Fig. 6 Histogram of semiquantitative comparison; *0* ileal mucosa shows more cell proliferation than pouch mucosa, *1* no difference in cell proliferation between pouch and ileal mucosae, *2* pouch mucosa shows more cell proliferation than ileal mucosa, *3* no judgement possible, *4* no consensus could be reached

role of cell kinetics as a possible explanation for this observation, apoptosis and cell proliferation rates in the mucosa of the pouch were compared with those of the afferent ileal loop from the same patient to eliminate bias caused by interindividual differences. Cell proliferation was significantly higher in the pouch mucosa in comparison to mucosa of the afferent ileal loop. No significant difference in apoptosis was found in the mucosa of the pouch and afferent ileal loop.

A low amount of apoptotic cells in the pouch as well as in the ileal mucosa was found. This might be caused by the APC mutation-induced apoptotic resistance [16]. Only a trend but no significant difference in apoptosis was found. Taking into account the broad range of apoptotic rates as found in our study samples, a significant difference might be found when a larger group of patients could be studied. There are no clear guidelines for estimation of ileal proliferation. For estimation of colonic proliferation, an accepted method is to count proliferating cells in five colonic crypts. Although most biopsy specimens were stretched and orientated directly after endoscopy, only a lower number of crypts could be counted completely along the longitudinal axis in most patients. For this reason, we accepted three full crypts as the minimal number to assess proliferation. Using this criterion, tissue sections from 12 patients still could not be used to determine the cell proliferation and had to be excluded from the study. Especially in the tissue sections of the pouch, this problem was evident and is possibly caused by a higher fragility of this tissue. In the remaining 20 pairs of tissue sections left

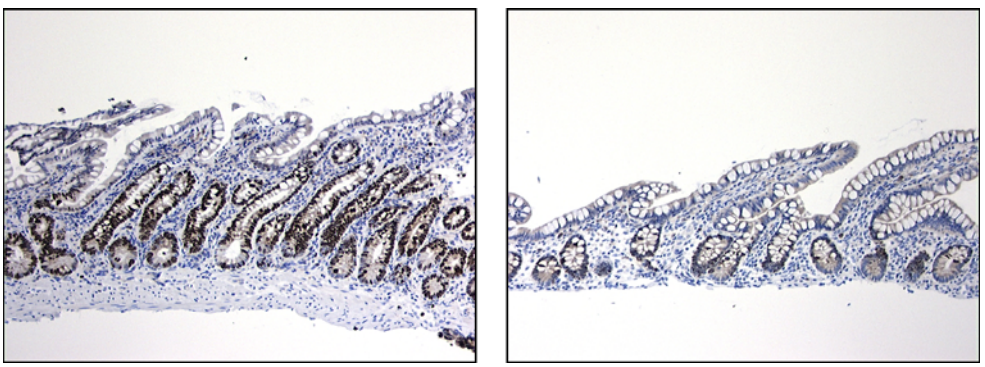

Fig. 5 Photographs used for semiquantitative comparison of cell proliferation in MIB-1 immunohistochemically stained tissue sections of pouch (*left*) and afferent ileal mucosae (*right*; original magnification ×100)

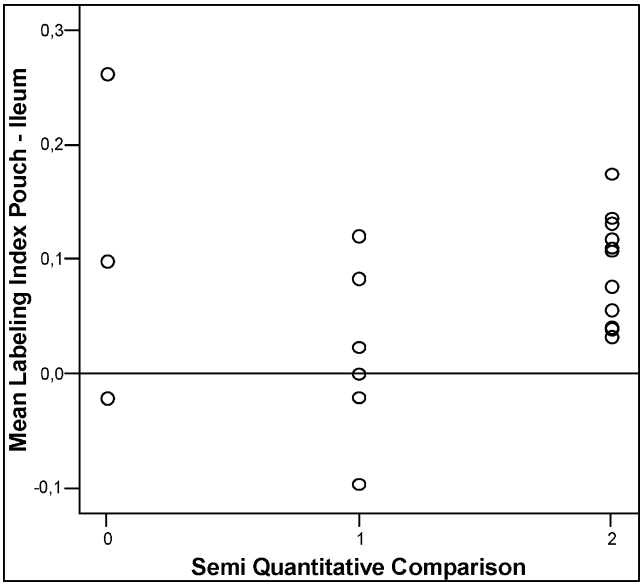

Fig. 7 Semiquantitative comparison vs difference in mean labeling index of pouch minus afferent ileal mucosa; *0* ileal mucosa shows more cell proliferation compared to the pouch mucosa, *1* no difference in cell proliferation between pouch and ileal mucosae, *2* pouch mucosa shows more cell proliferation compared to the ileal mucosa

for comparison, we found a significantly higher median labeling index in the pouch compared to the ileal afferent loop.

To overcome the problem of the relative low number of assessable crypts, another quicker but less quantitative method was applied in which five investigators compared photographs of tissue sections of pouch and ileal mucosae. In accordance with the results of the first method, we found significantly higher cell proliferation in the mucosa of the pouch compared to that of the ileal mucosa. Although no significant correlation could be found in a case-by-case comparison between both methods, in all cases in which the semiquantitative analyses showed more proliferation in the pouch, this was confirmed by the quantitative analyses of crypts. Furthermore, the inter- and intraobserver variability was good.

In addition, the semiquantitative method is far less time-consuming and can therefore give a relatively fast and easy impression of eventual differences in cell proliferation. Evaluation of this method in a larger study may further demonstrate its value.

Data on cell proliferation in ileal mucosa in patients with FAP are limited. Previously, de Silva et al. [11] reported a labeling index of 19.8% in afferent ileal loop mucosa of patients with FAP or ulcerative colitis (UC), far lower than the 61.6% that was found here. Their labeling index for cell proliferation in ileal pouch mucosa (33.6%) was also much lower than in the present study. Only the labeling index of 51.7% as found by them in pouches with pouchitis nears the values we obtained. However, the majority of patients included in the study of de Silva et al. were patients with UC (23 of the 26 patients), so direct comparison with our results therefore seems inappropriate. In addition, Goldberg et al. [12] reported a median labeling index of 34.9% in the ileal pouch of patients with FAP, which is much lower than the 68.3% we found. However, their labeling indices were based on a minimum of three counted crypts, and if this number could not be reached, halves of crypts were included in the analyses. Moreover, their study group consisted of only 5 patients with FAP, whereas in the present study, 3 to 5 whole crypts of 20 patients were counted.

Several studies indicated that cell proliferation of normal-appearing colorectal mucosa of patients with adenomas or carcinomas was 19 to 86% higher compared to colorectal mucosa of healthy persons [1, 28, 30, 32]. These findings strongly suggest an association between the presence of adenomas or carcinomas and an increased mucosal cell proliferation. However, the difference in cell proliferation between pouch and afferent ileal mucosae of 6.7% as found in this study is less pronounced in comparison to the above referred findings. A possible explanation for this relatively small difference in cell proliferation between pouch and ileal mucosae in our study may be that intestinal epithelial cell proliferation is already very high in patients with FAP [19, 31], and these high cell proliferation rates make a further increase less pronounced.

The higher proliferation found in the pouch mucosa in comparison to mucosa of the afferent ileal loop can only be explained by intraluminal changes that occur after construction of the pouch. Whether changes in bacterial flora, bile acid composition, short-chain fatty acids, or other compounds are responsible for this finding remains unclear, but a better understanding of this process is necessary to find a possible treatment for this group of patients.

In conclusion, the increased cell proliferation in the ileal pouch mucosa compared to the mucosa of the afferent ileal loop may contribute to the enhanced risk for adenomas and carcinomas in the pouch of patients with FAP and emphasizes the need for regular endoscopical surveillance of the pouch in these patients. In addition, cell proliferation can be used as an early endpoint marker in chemopreventive studies in these patients.

The applied new method for semiquantitative evaluation of cell proliferation in immunohistochemically stained tissue sections seems promising, as it offers a relatively fast and easy means of assessment.

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ORIGINAL ARTICLE

Claudin 4 identifies a wide spectrum of epithelial neoplasms and represents a very useful marker for carcinoma versus mesothelioma diagnosis in pleural and peritoneal biopsies and effusions

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Abstract We evaluated the usefulness of the tight-junction associated protein Claudin 4 (CL-4) in the diagnosis of mesothelioma and mimickers, analyzing biopsies from 454 tumors, including 82 mesotheliomas, 336 carcinomas of different origin (278 primary, 58 metastatic to serosae), 36 nonepithelial spindle cell neoplasms, as well as 97 cytological samples from reactive effusions (12), mesothelioma (23) and metastatic carcinomas (62). CL-4 was consistently negative in normal and reactive mesothelium, as well as in all 82 mesotheliomas. In contrast, strong reactivity was found in 57/58 serosal metastasis, and in 245/278 primary carcinomas, with uppermost expression (150/153) in those most frequently involved in the differential with mesothelioma (lung, breast, gastrointestinal tract, pancreas, ovary, primary serous papillary carcinoma of peritoneum). On effusions, reactive and neoplastic mesothelial cells were regularly negative, while metastatic tumor cells stained positively in 60/62 (96.8%) cases. Among spindle cell neoplasms, only 2/9 biphasic synovial sarcomas and 4/4 follicular dendritic cell sarcomas stained positively. Results indicate that CL-4 reacts with the majority of epithelial neoplasms that often metastasize to serous membranes, representing a pancarcinoma marker with extremely high sensitivity and specificity. CL-4 may be considered a primary immunohistochemical reagent to rule out the diagnosis of mesothelioma.

Keywords Claudin 4 · Tight junctions · Mesothelioma · Carcinoma · Follicular dendritic cells · Immunohistochemistry

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Introduction

Mesothelioma is a seemingly rare tumor, and its incidence has increased steadily and progressively over the last 30 years on a worldwide basis [31, 55]. Its recognition is important not only for medical but also for public health reasons in consideration of its relationship to exposure to environmental asbestos and asbestiform minerals [31]. Because mesothelioma can exhibit many histological patterns, its diagnosis and distinction from cancers that most commonly metastasize to serosal surfaces represents a challenging diagnostic problem in surgical pathology, leaving to immunohistochemistry the role of diagnostic discriminator [46, 73]. Unfortunately, despite the large number of studies devoted to the immunohistochemistry of mesothelioma and mimickers [19, 43, 46–49, 76], to date none of the markers used in routine practice is diriment in all the cases. Results obtained in different studies are

conflicting, pointing out how relevant might be the quality of the primary antibodies, the sensitivity of the techniques used for antigen retrieval and signal amplification, and the interpretation of the results [42, 58]. On the other hand, it should be noted that absolute positive and negative markers for mesothelioma are still lacking [73], and even those characterized by very high sensitivity and specificity do not have universal diagnostic usefulness, because they largely depend on individual conditions related to the site involved by tumors (pleural vs peritoneal) and the neoplasm that is primarily considered in the differential [4, 19, 43, 44, 46–48, 73, 76]; furthermore, the diagnostic value of some markers can also depend on the sort of specimens analyzed (histological *versus* cytological samples).

Recently, claudins have been shown to represent new interesting targets for cancer diagnosis [36]. Claudins comprise a multigene family consisting of more than 20 members believed to be major functional constituents of tight junctions [38]. They are present at epithelial and endothelial cell interfaces and contribute to mechanical adhesion, control the paracellular ion flux, maintain cell polarity, and by their capacity to recruit signaling proteins [34], they may regulate cell proliferation and differentiation [36].

The expression of claudins mRNA and protein vary within normal epithelia [9, 15, 17, 22, 40, 60, 62, 63] and is altered in several cancers [68, 69]. However, whereas some of these molecules such as claudin 1 and claudin 7 are downregulated in carcinomas [36], in keeping with the generally accepted idea that tumorigenesis is associated by a disruption of tight junctions, others especially claudin 4, are generally overexpressed during neoplastic transformation [11, 13, 15, 17, 26, 35, 37, 56, 64, 67–69]. Claudin 4, a major modulator of tight junctions [23], identifies neoplasms potentially metastasizing to serosal surfaces, while it is usually not expressed in nonepithelial tumors [68], as claudin 4 is negative in normal mesothelium [17, 63, 71, 77], we investigated its possible diagnostic usefulness in the differential diagnosis between mesothelioma and carcinomas, analyzing claudin 4 protein expression in a large series of histological and cytological samples. The results indicate that claudin 4 is expressed by the majority of epithelial neoplasms with capacity to metastasize to pleura and peritoneum, while is consistently negative in mesothelioma. Claudin 4 is a candidate to be included as a primary reagent in the panel of antibodies to assist the diagnosis of tumors involving serosal cavities.

Materials and methods

Tissue and effusion specimens

The material used in this study was obtained from the files of the Department of Pathology at the University of Brescia and at the Hospital of Mestre, and included histological and cytological samples (Tables 1, 2 and 3) submitted for diagnostic purposes; the study was performed in accordance with Hospital Institutional Ethical Board protocols.

Diagnoses of tissue specimens and site of biopsy are detailed in Tables 1 and 2. They included normal tissues associated with the tumors or removed for different diagnostic purposes (Table 1), as well as 454 neoplastic samples (Table 2). All diagnoses of mesothelioma were based on WHO criteria [74], and confirmed in all instances by clinical, morphological and immunohistochemical data. They included 79 mesotheliomas from the pleura and 3 from peritoneum. Similarly, diagnoses of nonmesothelioma cancers were unequivocal and established on morphological and phenotypical data, as well as on clinical histories. The majority of tissue samples consisted in whole biopsy or

Table 1 Claudin 4 expression in normal tissues

Tissue	Positive cells
Lung	Bronchial, bronchiolar and alveolar epithelium (W)
Thymus	Medullary epithelium
Thyroid	Follicular epithelium; C cells (VW)
Breast	Ductal and acinar epithelium
Gastrointestinal tract	Fundal gastric glands (V); gut and colon mucosa
Liver	Biliary duct epithelium (W)
Pancreas	Acinar epithelium, ductal epithelium (VW) and endocrine cells (VW)
Kidney	Medullary collecting ducts; convoluted distal tubule; rare Bowman epithelial cells (V)
Bladder	Urothelium
Prostate	Acinar and duct epithelium
Testis	Epididymis e spermatic cord epithelium
Ovary, tuba, uterus	Tubal, cervical and endometrial mucosa
Others	Follicular dendritic cells

V, Variable; *W*, weak reactivity

Table 2 Tissue specimens, their distribution according to diagnoses, and reactivity for Claudin 4

Diagnoses	Number of cases	Reactivity with claudin 4	
		Positive	Negative
Serosal membrane neoplasms			
Malignant mesothelioma:	82	0	82
Epithelioid[a]	60		
Biphasic	11		
Sarcomatoid	9		
Desmoplastic	2		
Pleural involvement from:	50	43	7
Lung a denocarcinoma	18	18	0
Lung squamous cell carcinoma	10	10	0
Lung pseudosarcomatous carcinoma	2	2	0
Gastric adenocarcinoma	5	5	0
Pancreatic adenocarcinoma	3	3	0
Ovarian serous carcinoma	1	1	0
Breast carcinoma	1	1	0
Hepatocarcinoma	1	1	0
Bladder urothelial carcinoma	1	1	0
Renal clear cell carcinoma	1	0	1
Rhinopharyngeal undifferentiated carcinoma	1	1	0
Rhabdomyosarco ma	1	0	1
Leiomyosarcoma	1	0	1
Synovial sarcoma[b]	3	0	3
Epithelioid angiosarcoma[c]	1	0	1
Solitary fibrous tumor of the pleura	8	0	8
Peritoneal serous papillary carcinoma	8	8	0
Peritoneal metastases from:	16	14	2
Ovarian serous carcinoma	5	5	0
Hepatocarcinoma	3	3	0
Appendiceal mucinous tumor (pseudomyxoma)	1	1	0
Pancreatic adenocarcinoma	1	1	0
Renal clear cell carcinoma	1	1	0
Thyroid papillary carcinoma	1	1	0
Adenocarcinoma, primary undetermined	2	2	0
Rhabdomyosarcoma	1	0	1
Leiomyosarcoma	1	0	1
Primary non serosal neoplasms			
Lung:			
Adenocarcinoma	14	14	0
Squamous-cell carcinoma	11	11	0
Pseudosarcomatous carcinoma	3	3	0
Oat cell carcinoma	3	8	0
Thymus:			
Thymoma, type A	2	0	2
Thymoma, type AB	4	1	3
Thymoma, type B	3	0	3
Breast:			
Ductal carcinoma	19	19	0
Lobular carcinoma	11	11	0
Tubular carcinoma	5	5	0
Pseudosarcomatous carcinoma	1	0	1
Neuroendocrine carcinoma	1	1	0
Ovary:			
Serous carcinoma	10	10	0
Endometrioid carcinoma	10	10	0
Mucinous carcinoma	2	2	0
Clear cell carcinoma	4	4	0
Mixed-type carcinoma	2	2	0
Uterus:			
Endometrial adenocarcinoma	7	7	0
Adenosquamous endometrial carcinoma	3	3	0
Cervical adenocarcinoma	3	3	0
Stomach:			
Intestinal type adenocarcinoma	3	3	0
Diffuse type adenocarcinoma	15	13	2
Mixed-type adenocarcinoma	2	2	0
Small intestine:			
Adenocarcinoma	5	5	0
Neuroendocrine carcinoma (carcinoid)	2	2	0
Colon adenocarcinoma	10	10	0
Liver:			
Well differentiated hepatocarcinoma	5	2	3
Moderately differentiated hepatocarcinoma	5	2	3
Poorly differentiated hepatocarcinoma	2	0	2
Cholangiocarcinoma	10	10	0
Pancreas:			
Ductal adenocarcinoma	12	12	0
Endocrine tumor	5	5	0
Thyroid:			
Papillary carcinoma	5	5	0
Follicular carcinoma	9	9	0
Medullary carcinoma	5	5	0
Testis:			
Embrional carcinoma	3	2	1
Seminoma	3	0	3
Yalk-sac carcinoma	3	0	3
Choriocarcinoma	1	1	0
Prostate:			
Acinar adenocarcinoma	5	5	0
Ductal adenocarcinoma	2	2	0
Bladder:			
Low grade, invasive urothelial carcinoma	3	3	0
High grade, invasive urothelial carcinoma	6	4	2
Kidney:			
Clear cell carcinoma	8	7	1
Papillary, type I, carcinoma	2	2	0

Table 2 (continued)

Diagnoses	Number of cases	Reactivity with claudin 4	
		Positive	Negative
Papillary, type II, carcinoma	4	4	0
Cromophobe carcinoma	7	7	0
Collecting duct carcinoma	1	1	0
Adrenal cortical carcinoma	4	0	4
Other tumors:			
Synovial sarcoma	6	2	4
Gastrointestinal stromal tumor	7	0	7
Cutaneous melanoma	3	0	3
Follicular dendritic cell sarcoma	4	4	0

[a] 57 from the pleura, 3 from peritoneum
[b] 1 primitive of the pleura, 2 primitive of mediastinum, with pleura and lung involvement
[c] primary of the pleura.

surgical specimens, fixed in neutral formalin, and embedded in paraffin. In addition, tissue microarrays were constructed from representative areas of paraffin blocks obtained from different carcinomas, and assembled using a manual tissue-arraying instrument (Bio-Optica, Milan, Italy), sampling from each case two 2-mm-diameter tissue cores.

Effusions were submitted for routine diagnostic purposes, and samples were centrifuged, fixed in 95% ethanol, and stained with the Papanicolau or hematoxylin-eosin techniques. Diagnoses were established by morphological evaluation of smears, correlation with biopsy samples (if available), clinical information, and in appropriate cases, by the use of a panel of antibodies against carcinoma and mesothelioma antigens [52]. Diagnoses of cytological specimens are detailed in Table 3.

Immunostainings

On 30-μm-thick paraffin sections, endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol for 20 min, and epitope retrieval was performed using a slide steamer (DAKO, Glostrup, Denmark, EU) in 1.0 mM ethylenediamine tetraacetic acid (EDTA) buffer (pH 8.0), for 40 min at 98°C. Upon washing in Tris-HCl buffer (pH 7.4–7.6), sections were incubated for 5 min in Tris-HCl buffer containing 5% normal goat serum, followed by mouse monoclonal antibody anti-claudin 4 (clone 3E2C1, Zymed, San Francisco, CA, USA), diluted 1:100, for 60 min. Sections were then washed in Tris-HCl buffer before incubation for 30 min with the antimouse, biotin-free, peroxidase-conjugated polymer ChemMate (DAKO). The reaction was developed using diaminobenzydine as chromogen, and sections were counterstained with hematoxylin. On cytological slides, after coverslip removal by immersion in xylene for 48 h, cells were rehydrated through a scale of alcohols and endogenous peroxidase activity blocked with 0.3% H_2O_2 in methanol for 20 min; cytoplasmic staining was then cleared using a lithium sutured solution in 70% ethanol, and epitope retrieval was obtained by microwave heating in 1.0 mM EDTA buffer (pH 8.0), for 3×5 min, at 750 W. Subsequently, the immunostain followed the same procedure as for tissue sections.

The specificity of the antibody was evaluated using known positive (normal bronchial epithelium) and negative (lymphocytes) controls. Furthermore, omission of the primary antibody was also used as negative control.

In the evaluation, membrane-bound reactivity was considered significant, and positive cases were defined when at least more than 10% of tumor cells were distinctly labeled. On occasion, benign and neoplastic cells of different origin (such

Table 3 Effusion specimens, their distribution according to diagnoses, and reactivity with claudin 4

Diagnoses	Number of cases	Site		Reactivity with claudin 4	
		Pleura	Peritoneum	Positive	Negative
Malignant mesothelioma	23	16	7	0	23
Lung carcinoma[a]	12	12	0	12	0
Hepatocarcinoma	3	2	1	2	1
Ovarian carcinoma[b]	28	0	28	28	0
Ovarian small cell undifferentiated carcinoma, hypercalcemic-type	1	0	1	0	1
Peritoneal serous papillary carcinoma	1	0	1	1	0
Endometrial carcinoma	2	0	2	2	0
Other carcinomas[c]	12	7	5	12	0
Adenocarcinoma of unknown origin	3	0	3	3	0
Reactive mesothelium	12	4	8	0	12
Total	97	41	56	60	37

[a] 11 adenocarcinoma, 1 small cell carcinoma
[b] Ovarian carcinoma included serous, mucinous, endometrioid, clear cell, and mixed subtypes
[c] Gastrointestinal carcinoma (4 cases), breast (3 cases) and prostate, renal, pancreas, uterine cervical, papillary thyroid carcinoma (1 case each).

as endothelial cells, mesothelium, fibroblasts, smooth muscle cells) showed single or multiple positive intracytoplasmic dots: although we cannot rule out a binding to a cytosolic claudin 4-related epitope, this reactivity was considered nonspecific and excluded from the evaluation.

Statistic

Diagnostic sensitivity and specificity were calculated using the Bayesian statistical program at the Medical College of Wisconsin website http://www.intmed.mcw.edu/clincalc/bayes.html.

Results

Results on claudin 4 expression on tissue biopsies from normal tissues, pathological samples, and effusions are summarized in Tables 1, 2 and 3, respectively.

Normal tissues

Normal mesothelium was regularly negative for claudin 4. In the abdominal cavity, there is an abrupt transition from the intensely positive fallopian mucosa to the negative epithelium of ovarian surface and peritoneum (Fig. 1a,b). Among claudin 4-expressing cells, the vast majority were decorated along the entire cell membrane; however, in some epithelia such as lung alveoli (Fig. 1c), large pancreatic ducts, as well as endocrine cells of the pancreas and C cells in the thyroid, the reactivity was variable and frequently discontinuous; stomach mucosa showed only occasional positive cells at the base of fundal glands, but even minute foci of intestinal metaplasia were highlighted by strong reactivity for claudin 4. In the thymus, claudin 4 reactivity was restricted to medullary epithelial cells (not shown). Besides epithelia, claudin 4 was uniquely found in follicular dendritic cells of B follicles associated with some tumors. This finding was further supported by the demonstration of strong positivity of follicular dendritic cells occurring in reactive lymph nodes (Fig. 1d) and spleen, as well as in follicular B-cell lymphomas.

Reactive mesothelium and malignant mesothelioma

Similar to normal mesothelium, mesothelial cells, including those showing pronounced reactive features associated with metastases or overlying primary carcinomas, were also negative for claudin 4. No reactivity was also found in mesothelial cells occurring in inflammatory or metastatic effusions (Fig. 2a,e, and g).

All 82 tissue biopsies and 23 cytological samples from malignant mesothelioma resulted negative for claudin 4 (Fig. 2b,c, and d).

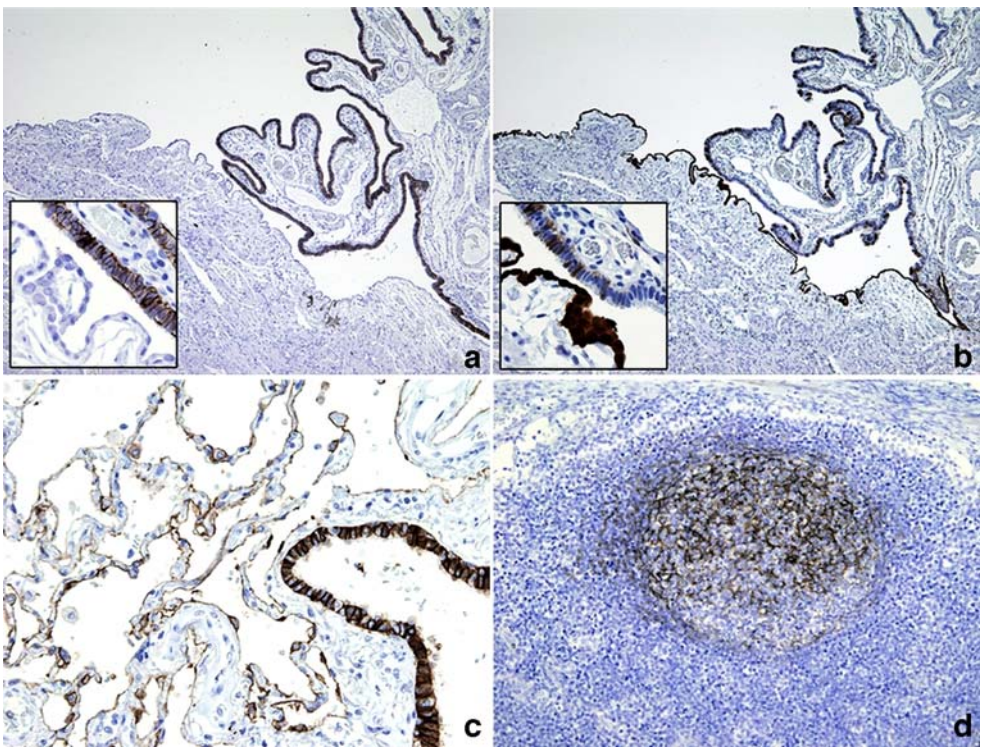

Fig. 1 Claudin 4 reactivity (**a**) compared to calretinin (**b**) in serial section form normal salpinx: note the abrupt transition from the tubal epithelium, strongly positive for claudin 4, to the negative mesothelial layer; in contrast, calretinin is expressed by mesothelium, as well as by scattered cells within the tubal epithelium. Details of claudin 4 and calretinin reactivity are illustrated in the insets. In lung parenchyma (**c**), reactivity for claudin 4 is shown by bronchial epithelium and, less intensely, by alveolar cells. Besides epithelia, claudin 4 is also expressed by follicular dendritic cells occurring in secondary B follicles (**d**). (Immunoperoxidase for claudin 4, counterstained with hematoxylin)

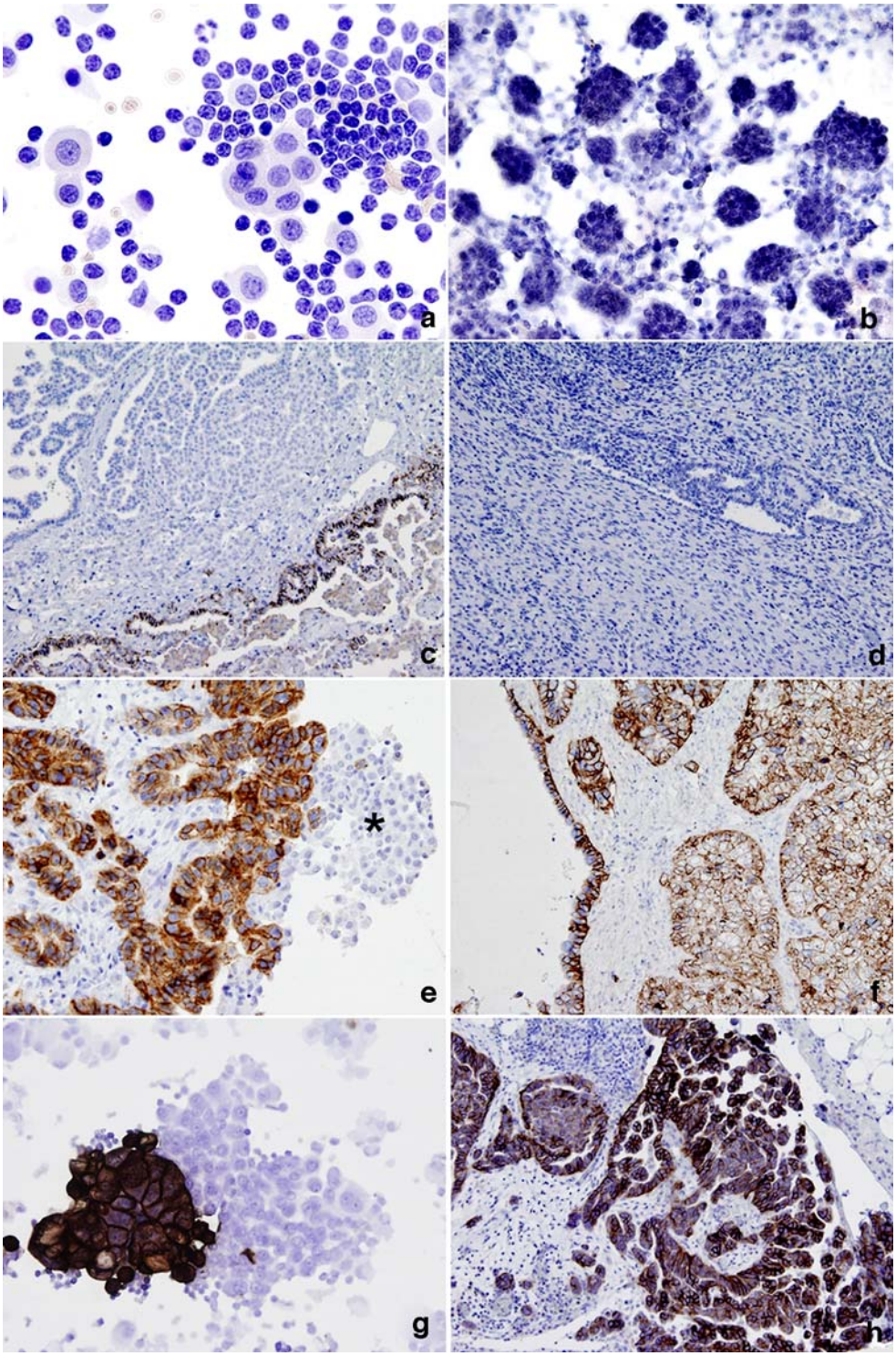

Fig. 2 Claudin 4 expression in mesothelial cells from inflammatory pleural effusion (**a**), mesothelioma (**b**–**d**), and neoplasms involving serous surfaces. Hyperplastic mesothelial cells occurring in a cell smear (**a**) do not express claudin 4. Representative examples of mesothelioma in a pleural effusion (**b**), and epithelioid (**c**) and biphasic (**d**) mesothelioma in pleural biopsies, all completely unreactive for claudin 4. Note the reactivity for claudin 4 on alveolar epithelium infiltrated by the tumor (**c**). Pleural metastases of adenocarcinoma originated from the stomach (**e**) and the lung (**f**) highlight the strong reactivity of tumor cells for claudin 4; positivity is also obvious in the malignant peritoneal effusion from an ovarian carcinoma (**g**). Note in **e** x(*asterisk*) and **g** aggregates of claudin 4-negative hyperplastic mesothelium. Strong and diffuse positivity is shown in a case of papillary serous carcinoma of peritoneum (**h**). (Immunoperoxidase for claudin 4, counterstained with hematoxylin)

Serosal metastases of carcinoma and primary serous papillary carcinoma of the peritoneum

Fifty-seven out of 58 (98.3%) biopsies from serosal metastases of different epithelial origin, as well as eight cases of primary serous papillary carcinoma of the peritoneum were found to express claudin 4 (Fig. 2e,f, and h). In all cases, positivity was strong and diffusely distributed along the cell membrane on most parts of the tumoral proliferation, including scattered neoplastic cells infiltrating the fibrous stroma. Pseudosarcomatous areas in two-pleural metastasis from lung carcinoma were also positive.

On effusions, 60 out of 62 (96.7%) cases originating from various epithelial neoplasms showed strong reactivity on all

tumor cells (Fig. 2g); the two negative samples were represented by a small-cell undifferentiated carcinoma of the ovary, hypercalcemic-type and by a case of hepatocarcinoma.

Primary extra-serosal tumors

Because antigenic properties may vary between primary and metastatic cancers, we extended the study to a series of epithelial neoplasms in their site of origin. Two hundred seventy primary carcinomas were analyzed; 237 (87.8%) resulted positive for claudin 4. It should be noted that positive cases included all epithelial neoplasms that are more frequently involved in the differential with mesothelioma, such as carcinomas of the lung (36 cases) and ovary (28 cases; Fig. 3a,b, and c). The positivity was membranous and occurred along the entire surface of the cells. In most of the samples the staining involved the whole tumor; however, in three cases of pulmonary squamous cell carcinoma, it was stronger at the outer part of large neoplastic aggregates, and in five carcinomas of the breast, positivity was variable throughout the tumor and occurred as both linear and multiple-dots reactivity along the plasma membrane. Because downregulation of claudin 4 expression has been previously recognized in diffuse-type of gastric carcinoma [57, 70], we studied 15 cases and found that the majority of them (12/15) were strongly positive in 50–100% of tumor cells; only a single case expressed claudin 4 in less than 50% of cells, and two cases were weakly positive in 5% of cells. Four cases of hepatocarcinoma and three of renal clear cell carcinoma contained between 20 and 50% of positive cells. We tabulated as negative those specimens with less than 10% of reactive cells, but only seminomas, adrenal carcinomas, and eight hepatocarcinomas completely lacked claudin 4 reactivity. Except for a single-type AB thymoma, which contained numerous positive cells within areas of glandular differen-

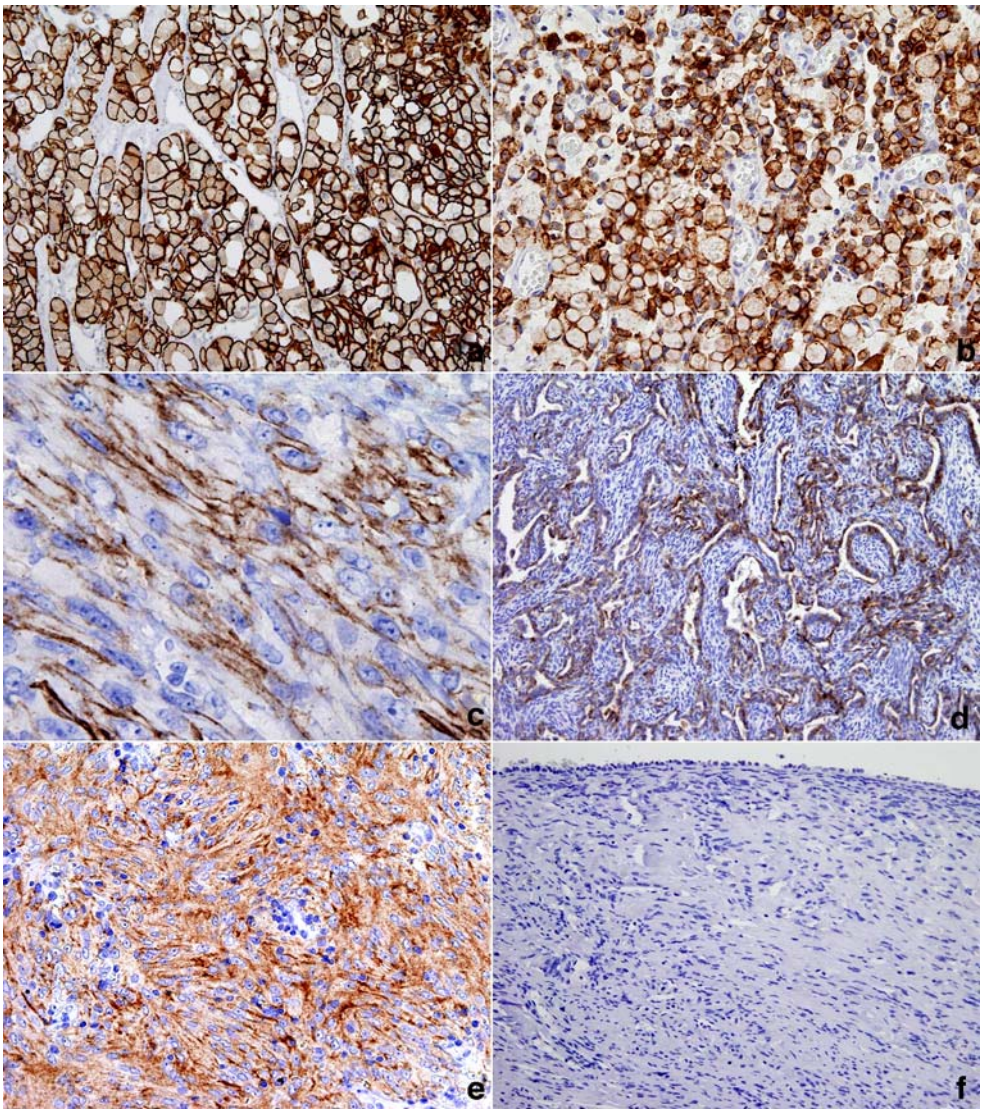

Fig. 3 Examples of primary epithelial (**a**–**c**) and mesenchymal neoplasms (**d**–**f**) stained for claudin 4 Homogeneously strong positivity is shown in a case of renal chromophobe carcinoma (**a**), and gastric carcinoma diffuse-type (**b**), while reactivity is more variable in a case of pseudosarcomatous carcinoma of the lung (**c**). Only the epithelial cell component in a biphasic synovial sarcoma (**d**) reacts for claudin 4, while the spindle cells are completely negative. In keeping with the reactivity of normal follicular dendritic cells, also neoplastic cells from a case of follicular dendritic cells sarcoma are strongly positive for claudin 4 (**e**). No reactivity is evident in a solitary fibrous tumor of the pleura (**f**). (Immunoperoxidase for claudin 4, counterstained with hematoxylin)

tiation, the other eight cases showed only focal scattered reactivity (data not shown). Six synovial sarcomas arising in the soft tissues were also analyzed, two of which revealed reactive cells, that were regularly restricted to areas of epithelial differentiation (Fig. 3d). Melanomas and gastrointestinal stromal tumors were regularly negative, while, in keeping with the reactivity on the normal cell counterpart, all four cases of follicular dendritic cell sarcoma (one spindle and three epithelioid cell-type) showed strong reactivity for claudin 4 (Fig. 3e).

Solitary fibrous tumor of the pleura, and other nonepithelial tumors involving the serous surfaces

Mesothelioma can be simulated histologically by spindle cell tumors, either arising in or metastatic to serosal surfaces, such as solitary fibrous tumor, synovial sarcoma and epithelioid angiosarcoma [4, 24, 29]. We therefore investigated eight cases of solitary fibrous tumor of the pleura (Fig. 3f), three cases of monophasic synovial sarcoma involving the pleura, four metastatic myogenic sarcomas, and a single epitheliod angiosarcoma, all of which lacked any reactivity for claudin 4.

Discussion

In the present study, we analyzed by immunohistochemistry the expression of the tight-junction-associated protein claudin 4 in a large series of normal and neoplastic tissues. Results show that all eighty-two cases of mesothelioma were negative, while strong reactivity was found in 57/58 serosal metastasis of carcinomas, and in 245/278 primary carcinomas of different origin, with uppermost expression (150/153) in those most frequently metastasizing to serosal surfaces or simulating mesothelioma (lung, breast, gastrointestinal tract, pancreas, ovary, and primary serous papillary carcinoma of the peritoneum). Taken together, these data indicate that claudin 4 represents a very robust pancarcinoma marker, with an extremely high sensitivity (90.0%) and specificity (100%) for distinction from mesothelioma.

These data are in keeping with the study by Davidson et al. [12], who demonstrated that claudin 4 gene expression is significantly lower in mesotheliomas compared with ovarian carcinomas; in contrast, Soini et al. [71] found claudin 4 positivity in 8 out of 35 (23%) mesotheliomas; because we used the same anti-claudin 4 primary antibody and similar antigen retrieval techniques, we cannot exclude that the discrepancy with the results of Soini et al. might depend on variability in the quality and preservation of the tissues.

Claudin 4 exhibits several advantages compared with other pancarcinoma markers qualified for optimal specificity and sensitivity (Table 4). Firstly, it was regularly negative in mesotheliomas, while cumulative data from several studies indicate that MOC-31 and carcinoembryonic antigen (CEA) label approximately 5–10% of mesotheliomas [19, 44, 46, 49], and even higher expression rates are reported for BG-8, B72.3, and Ber-EP4 [4, 46, 47, 73]. In addition, claudin 4 demonstrated to be of similar diagnostic usefulness in pleural and peritoneal pathology, because tumors most frequently implicated in the differential with mesothelioma in these locations (respectively, pulmonary carcinomas, and ovarian and peritoneal papillary serous carcinomas) [6, 7, 19, 43, 45, 47, 48, 76] were regularly positive. In contrast, sensitivity of other markers is known to greatly vary according to specific tumor subtypes: CEA is frequently expressed in adenocarcinoma derived from the lung, gastrointestinal tract, and breast but is mostly negative in the ovary and peritoneal serous papillary carcinoma [4, 47, 49]. Similarly, squamous cell carcinoma of the lung frequently lacks CD15 and B72.3 [48], and Ber-EP4 has been reported to be poorly expressed in peritoneal serous papillary carcinoma [4]. The strong and constant reactivity of claudin 4 on a wide range of epithelial neoplasms simplifies the choice of immunohistochemical

Table 4 Data from the literature [1, 5, 14, 19, 25, 27, 30, 39, 41–44, 46–50, 54, 68, 69, 71, 75, 76] on the percentage of reactivity of the most used pancarcinoma markers with mesothelioma and with carcinomas frequently involved in the differential diagnosis, compared with claudin 4 expression as found in the present and previous studies (between brackets)

	Ber-EP4	BG-8	B72.3	CEA[a]	CD15	MOC-31	Claudin 4
Epithelioid mesothelioma	0–88%	3–23%	0–48%	0–5%	0–32%	0–10%	0% (23%)
Lung adenocarcinoma	100%	89–100%	35–85%	25–100%	50–85%	90–100%	100% (100%)[b]
Lung squamous cell carcinoma	87%	80–83%	40–84%	77%	30%	97%	100% (100%)[b]
Ovary/peritoneum serous carcinoma	50–100%	73%	65–100%	0–8%	30–80%	98%	100% (100%)[c]
Kidney carcinoma	35–50%	0%	0%	0%	75–100%	50%	95.5% (50%)
Hepatocarcinoma	8–36%	n.a.	46%	0–11%	n.a.	87–93%	33.3% (0–10%)
Cholangiocarcinoma	100%	n.a.	50–100%	n.a.	75%	83–100%	100% (100%)

n.a., not available.
[a] CEA monoclonal antibody
[b] Results were not distinguished according to the histological subtype of lung carcinoma
[c] Data are related only to ovarian serous carcinoma.

reagents and avoids tailoring it to the specific circumstances of each case.

Renal carcinomas less commonly metastasize to serosal surfaces, but represent potential mimickers of mesothelioma [44, 46]. The expression of classical negative mesothelioma markers such as CD15, MOC-31 and Ber-EP4 is quite variable in these tumors, and largely depends on the histological subtype [44, 51]. Furthermore, Ordonez [44] found that epithelioid mesotheliomas can express renal carcinoma associated antigens, such as CD10 (38% of cases) and RCC-Ma (8%). In the present study, claudin 4 was positive in 22 out of 24 primary and metastatic renal cell carcinomas; the reactivity was independent from the histological subtype, indicating that this marker has a high negative (0.98) and positive (1.0) predictive value in the differential with mesothelioma.

The choice of immunohistochemical reagents for the diagnosis of sarcomatoid and desmoplastic mesothelioma is challenging, because these tumors frequently loose expression of some of the more common positive markers [73]. Moreover, several spindle cell neoplasms involving the serosal surfaces may mimic mesothelioma, both clinically and histologically [73]. Claudin 4 showed to be useful in the distinction of sarcomatoid mesothelioma from sarcomatoid carcinoma, whereas it was of no value for its differentiation from mesenchymal spindle cell neoplasms that more frequently occur in the pleura [4, 29, 73], as well as thymomas [73], thus confirming the limited role of immunohistochemistry in the differential diagnosis of sarcomatoid compared with epithelioid tumors occurring in serosal surfaces [29, 73]. Interestingly, among mesenchymal cells, claudin 4 showed selective expression on follicular dendritic cells and strongly stained four cases of follicular dendritic cell sarcoma. This observation is not unexpected, considering the presence of ultrastructural (such as desmosomes) and antigenic (epithelial membrane antigen, epidermal growth factor receptor, desmosome-associated proteins) features of epithelial cells in follicular dendritic cells [10, 72].

More than 80% of all mesothelioma patients develop a serous effusion at an early stage of the disease and cytological examination frequently represents the first diagnostic procedure. However, metastasis involving the serosal cavities are by far more frequent than mesothelioma, and the differential diagnosis between metastatic carcinoma and mesothelioma represents a common challenge on cytological specimens from body cavity fluids [53]. Similar to histological diagnosis, this process can be assisted by immunohistochemistry, but individual positive mesothelial markers have been found to be of lower diagnostic value compared to tissue biopsies, and the sensitivity of adenocarcinoma markers (such as CEA, Ber-EP4, MOC-31, and CD15) is extremely variable (43–96.6%) [2, 8, 28, 59, 65, 66]. In the present study, anti-claudin 4 antibody was successfully applied on Papanicolau or hematoxylin-eosin stained cytospins, and strongly labeled 60 out of 62 (96.8%) metastatic adenocarcinomas, while all 23 mesotheliomas and reactive mesothelium were regularly negative (specificity, 100%; sensitivity, 96.7%). The regular negativity for claudin 4 on reactive mesothelium is of particular relevance, considering the common quandary in cytopathology of distinguishing reactive mesothelial cells from metastasis [20, 52, 53].

In keeping with previous observations [11, 13, 15, 17, 26, 35, 37, 56, 64, 67–69], this study shows that claudin 4 is widely expressed in carcinomas from different origin, including those in which the normal epithelial counterpart lacks any significant reactivity, such as stomach, kidney, ovary, as well as endocrine tumors of pancreas and thyroid medullary carcinomas. Mechanisms responsible for the increase in claudin 4 expression in malignant epithelial neoplasms are unknown: epigenetic modifications of claudin 4 promoter occur in ovarian cancer cells [16] and lead to increase survival, motility, and invasion [3]; in contrast, claudin 4 reduces invasiveness of pancreatic tumor cells [33]. In the present study, claudin-4-negative epithelial neoplasms were related either to poor differentiation (such as bladder urothelial carcinoma), or to specific histological types (such as adrenal carcinoma, most germ cell tumors of the testis, thymoma and, as previously reported [25], hepatocarcinoma). These data support the hypothesis that functions of claudin 4 may be highly tissue specific and may depend on the exact molecular circuitry of the cells.

Finally, we cannot disregard the implication of claudin 4 reactivity in the treatment of neoplasms involving the serosal surfaces. Claudin 4 identifies the functional receptor of *Clostridium perfringens* enterotoxin (CPE) [18], and recent studies demonstrated that treatment of xenograft cancer cells with CPE induces necrosis in tumors expressing claudin 4 [21, 32, 61]. Despite the fact that claudin 4 expression in normal epithelia is a cause of concern towards the use of CPE for systemic cancer therapy [36], its absence on normal and hyperplastic mesothelium opens the possibility of intracavitary treatment with CPE or monoclonal antibodies of claudin 4-positive tumors.

In conclusion, data provided by this study indicate that claudin 4 is a pancarcinoma marker with a broad spectrum of reactivity, while it is regularly negative in normal and neoplastic mesothelium; due to its high specificity and sensitivity for carcinomas on both tissue biopsies and cell smears, claudin 4 appears to be an ideal single-shot marker to be applied in a wide range of circumstances to assist the immunohistochemical diagnosis of mesothelioma and mimickers. If claudin 4-targeting drugs will be delivered for humans, the immunohistochemical evaluation of protein expression will be also relevant for selection of patients eligible for treatment.

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ORIGINAL ARTICLE

Expression of LAT1 predicts risk of progression of transitional cell carcinoma of the upper urinary tract

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Abstract L-type amino acid transporter 1 (LAT1), a neutral amino acid transporter, requires covalent association with the heavy chain of 4F2 cell surface antigen (4F2hc) for its functional form. We investigated the importance of LAT1 and 4F2hc expressions to progression in upper urinary tract cancer. We examined their expressions and their relationships to clinicopathologic parameters and clinical outcome in 124 cases. Positive expressions of LAT1 (protein and messenger ribonucleic acid) and 4F2hc (protein) were recognized in 79.8, 89.5, and 87.9% of tumor samples, respectively. In tumor cells, LAT1 protein was detected either as nodular granules within the cytoplasm or diffusely within the cytoplasm and/or on plasma membrane. In the normal urothelium, its expression was detected as nodular granules within the cytoplasm. A correlation with stage was shown for LAT1 protein expression and for a cooperative expression of LAT1 protein with 4F2hc protein (active form of LAT1 protein). Further, in all tumors, a cooperative expression of LAT1 protein and 4F2hc protein was significantly correlated with both overall and disease-free survival rates in the univariate analysis but not in the multivariate analysis. In conclusion, the detection of the active form of LAT1 protein would appear to be of value in informing the risk of progression in transitional cell carcinoma of the upper urinary tract.

Keywords Transitional cell carcinoma · Upper urinary tract · LAT1 · 4F2hc · Prognosis

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Introduction

Facilitated diffusion and active transport are responsible for the transport of substances such as glucose and amino acids, respectively. System L amino acid transport mediates the sodium-independent uptake of nonpolar branched-chain or aromatic neutral amino acids and is the major route by which mammalian cells take up nutritionally essential amino acids from extracellular fluids [2, 3,7, 8]. Indeed, system L amino acid transport plays a critical role in the absorption of amino acids by the intestine, kidney, and placenta. The genes encoding the proteins responsible for this type of transport have now been cloned, and the L-type amino acid transporter 1 (LAT1) was the first member of the system L amino acid transporter family to be identified [9, 15]. LAT1 requires covalent association with the heavy chain of 4F2 cell surface antigen (4F2hc) for its functional expression in the basolateral plasma membrane of epithelial

cells [29]. Previous studies have shown LAT1 to be highly expressed in proliferating tissues, many tumor cell lines, and primary human tumors but to be at barely detectable levels in adult tissues (except brain, ovary, and placenta), while 4F2hc is ubiquitous [2, 3, 7, 8, 10–13, 16, 23, 25–27, 30, 31, 34–36]. Furthermore, overexpression of LAT1 in hepatocytes has been found to enhance their growth [1], leading the authors of that paper to suggest that LAT1 may play an important role in cell growth. However, little is known about how the expression of LAT1 is related to clinicopathological findings or the prognosis of tumors. In transitional cell carcinoma of the upper urinary tract (TCC-UUT), no study has yet been made of the relationship between LAT1 protein expression and either tumor progression or prognosis.

In the present study, we examined the expressions of LAT1 protein and messenger ribonucleic acid (mRNA), 4F2hc protein, and proliferating cell nuclear antigen (PCNA) in 124 cases of TCC-UUT. Our goal was to evaluate the importance of LAT1 expression as a prognostic tool in TCC-UUT.

Materials and methods

The material used comprised 124 surgically resected specimens from patients with primary TCC-UUT. These specimens had been obtained at the Mutual Aid Associations' Hospital, Tachikawa, and National Defense Medical College Hospital, Tokorozawa, between 1970 and 1995. Histopathologic stage was determined according to the criteria proposed by the International Union Against Cancer [32]. Tumor cells were divided histopathologically into two grades using the criteria for urinary bladder tumors laid down by the Armed Forces Institute of Pathology [17].

Immunohistochemistry

We used the polymer-peroxidase method (EnVision+/HRP; Dako Cytomation, Denmark) on deparaffinized sections, employing mouse monoclonal antibodies against LAT1 (1:20; clone no. 4D9; Trans Genic, Kumamoto, Japan) and 4F2hc (1:200; polyclonal antibody; Trans Genic). For LAT1 expression, the sections received autoclave pretreatment with 0.05 M citrate buffer, pH 6.0, for 20 min before immunohistochemistry against the antibody. For the negative control, the incubation step with the primary antibody was omitted.

In situ hybridization

In situ hybridization was performed essentially as previously described [18]. Briefly, sections were treated with 0.2 N HCl for 20 min, then incubated in 2× sodium chloride–sodium citrate (SSC) for 10 min at 37°C, then incubated in 5 μg/ml proteinase K for 10 min at 37°C. Sections were subsequently postfixed in 4% paraformaldehyde for 5 min, then incubated in 0.1 mol/l triethanolamine buffer, pH 8.0, containing 0.25% (vol/vol) acetic anhydride for 10 min to prevent nonspecific binding because of oxidation of the tissue. Hybridization was carried out overnight at 42°C in 50% (vol/vol) deionized formamide, 5× Denhardt's solution, 5% (wt/vol) dextran sulfate, 2× SSC, 0.3 mg/ml salmon sperm deoxyribonucleic acid (DNA), 5 mM ethylenediamine tetraacetic acid (EDTA), and 10 ng/ml biotin-labeled probes. After performing a final stringent wash at 37°C for 20 min, hybridization was detected immunologically. The LAT1 complementary (cDNA) probe used was a 251-bp fragment (obtained from positions 918 to 1,168 in the cDNA of human LAT1) subcloned into the *Eco*RI site of a pGEM-T Easy Vector (Promega, Madison, WI). The antisense probe and the corresponding sense probe were labeled with biotin using SP6 and T7 polymerases, respectively, by means of an RNA-labeling kit (Roche Diagnostics GmbH, Mannheim, Germany).

Evaluation of immunohistochemistry and in situ hibridization

For the analysis of immunoreactivity and in situ hybridization, the extent of staining was scored as "–," indicating negative reaction of tumor cells, "±," less than or equal to 10% of tumor area stained, "+," 11 to 25% stained, "2+," 26 to 50% stained, or "3+," greater than or equal to 51% stained. The tumors in which stained tumor cells made up more than 25% of the tumor were graded as positive. The evaluation was performed twice by one investigator (Nakanishi) who was blind to both tumor stage and grade. PCNA was evaluated immunohistochemically; the technique used and the results obtained in these same patients have been reported elsewhere [24]. For the analysis of PCNA, the percentage of nuclei exhibiting a positive immunoreaction (PCNA index) was determined (on the basis of the immunoreaction in at least 1,000 tumor cells) as previously described [24]. The PCNA index was classified as high if it was greater than or equal to 70.0%, a figure representing the median value for the carcinomas.

Western blotting analysis

For Western blotting analysis, tissue samples were homogenized using a polytron homogenizer (Kinematica AG, Lucerne, Switzerland) for 1 min, then sonicated (UD200; Tomy Seiko, Tokyo, Japan) for 1 min in 500 μl of an ice-

cold homogenizing buffer of the following composition: 20 mM Tris/HCl, pH 7.4, containing 250 mM sucrose, 2 mM EDTA, and protease inhibitor cocktail (Complete Mini, Roche Diagnostics GmbH, Roche Applied Science, Penzberg, Germany). An 800-g crude particulate fraction was discarded, and the supernatant was centrifuged at 100,000×*g* for 60 min at 4°C. The resulting pellet constituted the membrane–particulate fraction, while the supernatant was the cytosolic fraction. The particulate fraction was resuspended in homogenizing buffer containing 0.5% Triton X-100, then centrifuged at 100,000×*g* for 60 min, and the resulting detergent-treated supernatant was the membrane fraction. The protein concentration of each sample was determined by means of a BC protein assay system (Bio-Rad Laboratories, CA). Then, 25 μg amounts of protein extracts, in the absence (nonreducing condition) of 2-mercaptoethanol, were separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluroride membranes (Hybond-N+; Amersham Pharmacia Biotech, Buckinghamshire, England). Membranes were blocked with 5% nonfat dry milk for 60 min at room temperature, then incubated overnight at 4°C with LAT1 antibody (clone no. 4D9; Trans Genic) diluted at 1:20. Finally, after blots had been incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies, they were visualized by enhanced chemiluminescence (Amersham Life Science, Arlington Heights, IL).

Statistical analysis

For statistical analysis, disease-free and overall survival rates were the two main dependent variables tested in this study. "Disease-free survival" was defined as the period between the initial radical operation and the subsequent appearance of recurrence or metastasis. Recurrence was defined as TCC occurring anywhere in the genitourinary tract. The end-point was either recurrence/metastasis of TCC or the closing date of the study, whichever came first. "Overall survival" was defined as the interval between surgery and death; the end point for this variable was either death or the closing date of the study.

Disease-free and overall survival curves for all of the univariate analyses were assessed using the Kaplan–Meier method. Comparisons between two or more survival curves were assessed using Wilcoxon and log-rank tests. Multivariate analysis of the clinicopathologic parameters was performed using the Cox stepwise-regression model. The above analyses were performed using the SAS statistical software package (SAS Institute, Carey, NC) [28]. The comparison in respect of stage in all tumors was performed using the Kruskal–Wallis test. Comparisons in respect of age, sex, grade, pattern of growth, and PCNA index were performed using the Chi-squared analysis.

Results

Clinicopathological findings

The patients' age at diagnosis was within the range of 34 to 84 years, with a median age of 67 years. Twenty-nine of the 124 patients died as a result of their tumors 2 to 132 months after surgery (mean, 24 months; median, 15 months). The remainder survived 0 to 257 months after surgery (mean, 69 months; median 63 months). Among the 124 cases, 53 tumors (42.7%) were in the renal pelvis or calyces, 47 (37.9%) were in the ureter, and 24 (19.4%) were multicentric. Ten patients had simultaneous bladder tumors at the time of diagnosis, 27 had subsequent bladder tumors, and six had an antecedent bladder tumor. In all, 41 (33.0%) patients had an associated bladder neoplasm; of these, six had such a neoplasm at more than one of the above times and thus appear in more than one group. The initial management of the 118 patients who were not treated as having bladder cancer included complete nephroureterectomy with a bladder cuff (88 patients), nephroureterectomy without a bladder cuff (eight patients), nephroureterectomy with total cystectomy (ten patients), and nephrectomy (12 patients). Thirty-two patients received adjacent chemotherapy (21 patients), radiotherapy (seven patients), or both (four patients), in addition to surgery.

The tumors were divided into three groups (A, B, and C) on the basis of tumor stage. There were 47 cases (37.9%) in group A (papillary, noninvasive tumors, pTa), 20 cases (16.1%) in group B (tumors invading the submucosa or muscularis, pT1 and pT2), and 57 cases (46.0%) in group C (tumors invading beyond the muscularis or renal parenchyma or metastasizing the regional lymph node or a distant site, pT3 and pT4). Ten of the 47 patients in group A showed recurrence (seven patients), metastasis (one patient), or both (two patients), but 37 developed neither recurrence nor metastasis within the follow-up period. The mean follow-up for patients with a noninvasive tumor who had a recurrence and/or metastasis was 67 months (median, 48; range, 14 to 124), whereas the mean follow-up for those without recurrence or metastasis was 81 months (median, 86; range, 4 to 203). Of the 20 patients in group B, seven showed either recurrence (four patients) or recurrence with metastasis (three patients), but the remaining 13 developed neither recurrence nor metastasis within the follow-up period. The mean follow-up for group B patients who showed recurrence and/or metastasis was 69 months (median, 66; range, 9 to 175), whereas the mean follow-up for those without recurrence or metastasis was 86 months (median, 33; range, 0 to 257). Of the 57 patients in group C, five either had metastasis at surgery, or the tumor could not be excised totally by surgery. In this group, a total of 33 showed recurrence, metastasis, or both (5, 18, and 10

patients, respectively), but the remaining 24 developed neither recurrence nor metastasis within the follow-up period. The mean follow-up for group C patients who showed recurrence and/or metastasis was 28 months (median, 15; range, 2 to 167), whereas the mean follow-up for those without recurrence or metastasis was 44 months (median, 41; range, 4 to 119).

At the time of diagnosis, tumors were in low grade in 76 of the patients (61.3%) and high grade in 48 (38.7%). Inspection of the pattern of growth of the tumors revealed that 88 cases (71.0%) showed a papillary pattern and 36 cases (29.0%) a nonpapillary pattern (solid tumor). Of the 29 patients who died of their tumors, 11 (37.9%) had a tumor in the renal pelvis or calyces, 13 (44.9%) in the ureter, and five (17.2%) had multicentric tumors. Twelve of the 29 (41.3%) who died had an associated bladder neoplasm. Of these 29, two were in group A, three in group B, and 24 in group C.

Immunohistochemistry and Western blot analysis for LAT1 protein

A positive expression of LAT1 protein was recognized in 99 (79.8%) of the samples. Cells either expressed nodular granules within the cytoplasm (Fig. 1a), or exhibited a diffuse expression within the cytoplasm (Fig. 1b and/or on the plasma membrane, Fig. 1c). Expression of LAT1 protein diffusely within the cytoplasm and/or on the plasma membrane was recognized in 59 of the 99 LAT1 protein-positive samples. In the normal urothelium, its expression was detected as nodular granules within the cytoplasm (Fig. 1d). In our Western blotting analysis, conducted in the absence (nonreducing condition) of 2-mercaptoethanol, LAT1 protein was detected as one band of 125 kDa in the cytoplasm or membrane of both normal pelvic mucosal tissue and tumor tissues (Fig. 2). In normal pelvic mucosal tissues and in the illustrated TCC case 1—in which LAT1 protein was detected as nodular granules within the cytoplasm using immunohistochemistry—LAT1 protein was revealed more strongly in the cytoplasmic extraction than in the membrane extraction. In TCC case 2—in which diffuse cytoplasmic staining and plasma membrane staining was revealed by immunohistochemistry—LAT1 protein was seen as a distinct band for both the membrane extraction and the cytoplasmic extraction.

A positive expression of PCNA was recognized in all tumors, the immunoreactivity being confined to tumor cell nuclei. The PCNA index was within the range of 7.4 to 93.0% (mean and median values, 66.7 and 69.9%, respectively).

In our assessment of whether LAT1 protein expression was related to PCNA index or clinicopathologic findings, a LAT1 protein-positive expression was found to be associated with sex (p=0.018) and stage (p=0.014; Table 1). However, a LAT1 protein-positive expression was not associated with the PCNA index. When LAT1 protein expression was assessed separately for noninvasive and invasive tumors, a significant correlation with a LAT1 protein-positive expression was found for sex (p=0.029) in

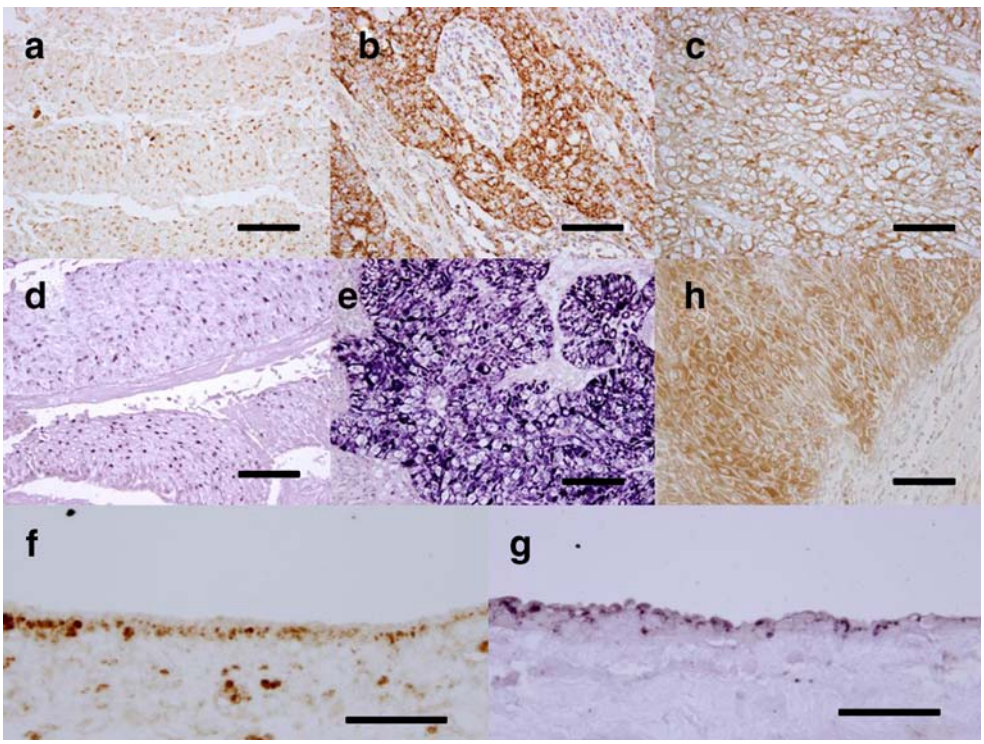

Fig. 1 Expressions of LAT1 (**a**, **b**, **c**, **d**, protein; **e**, mRNA) and 4F2hc (**h**, protein) in normal pelvic mucosa (**d**) and transitional cell carcinoma of the upper urinary tract (**a**, **b**, **c**, **e**, **f**). In the case of LAT1 protein, tumor cells displayed nodular granules within the cytoplasm (**a**) or a diffuse expression within the cytoplasm (**b**) and/or on the plasma membrane (**c**). LAT1 mRNA was displayed by tumor cells within the cytoplasm (**e**). In the normal pelvic mucosa, LAT1 protein was detected as nodular granules within the cytoplasm (**d**). 4F2hc protein was displayed by tumor cells within the cytoplasm (**f**). *Scale bar*=100 μm

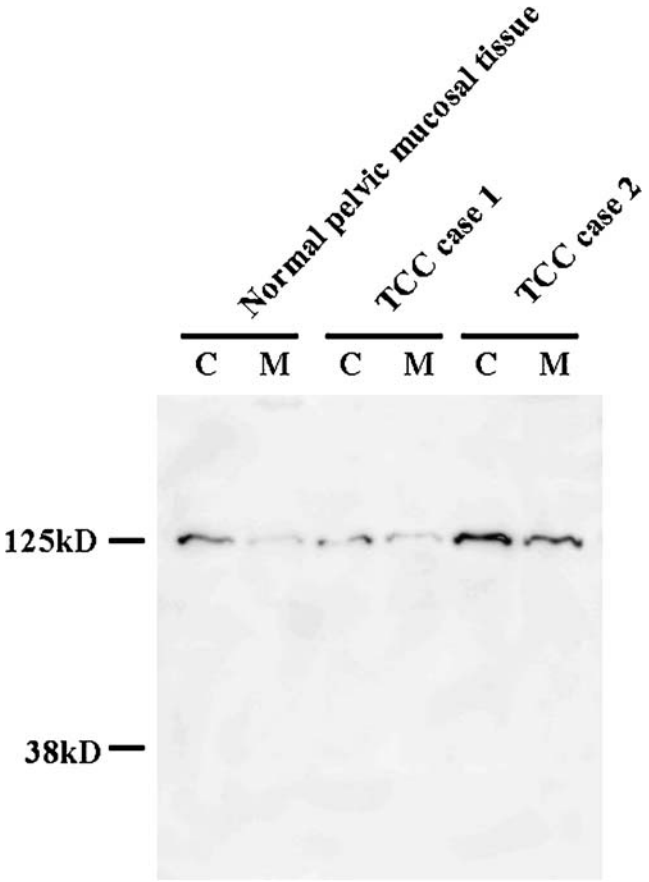

Fig. 2 Western blot analysis performed in the absence (nonreducing condition) of 2-mercaptoethanol for LAT1 in normal pelvic tissue and transitional cell carcinomas (*TCC*). LAT1 protein was detected as one band of 125 kDa in the cytoplasmic extraction (*C*) or in the membrane extraction (*M*) of all three tissues. TCC case 1 displayed nodular granules within the cytoplasm, whereas TCC case 2 displayed diffuse cytoplasmic staining and membrane staining (using immunohistochemistry)

the case of noninvasive tumors and for stage (p=0.019) in invasive tumors (Tables 2, 3).

In situ hybridization for LAT1 mRNA

By in situ hybridization, LAT1 mRNA was detected within the cytoplasm of tumor cells (Fig. 1d). A positive expression of LAT1 mRNA was recognized in 111 (89.5%) of all samples. In the normal urothelium, a moderate expression was detected within the cytoplasm. In our assessment of whether LAT1 mRNA expression was related to PCNA index or clinicopathologic findings in all cases, noninvasive cases, and invasive cases, no significant correlations were found with a LAT1 mRNA-positive expression (Tables 1–3).

Immunohistochemistry for 4F2hc protein

A positive expression of 4F2hc protein was recognized in 109 (87.9%) of samples. The expression of this protein was confined to the cytoplasm of both tumor cells and normal urothelial cells (Fig. 1f). In our assessments of whether 4F2hc protein expression was related to PCNA index or clinicopathologic findings in all cases, noninvasive cases, and invasive cases, no associations were found with 4F2hc protein-positive expression except that it was associated with age (p=0.039) in all cases and with sex (p=0.029) in noninvasive cases (Tables 1–3).

Relationship between LAT1 (protein and mRNA) expression and 4F2hc protein expression

This relationship was examined because it is well known that LAT1 requires 4F2hc for its functional expression. A cooperative expression of LAT1 protein with 4F2hc protein ("LAT1 protein with 4F2hc") was recognized in 89 (71.7%) of all samples. In our assessment of whether simultaneous expression of LAT1 and 4F2hc proteins was related to the PCNA index or clinicopathologic findings, "LAT1 protein with 4F2hc" was found to be associated with sex (p=0.016) and stage (p=0.0013; Table 1). When a cooperative expression of LAT1 and 4F2hc proteins was assessed separately for noninvasive and invasive tumors, a significant correlation with "LAT1 protein with 4F2hc" was found for sex (p=0.033) in noninvasive tumors and for stage (p=0.0071) in invasive tumors (Tables 2, 3).

Cooperative expressions of LAT1 mRNA with 4F2hc protein ("LAT1 mRNA with 4F2hc") was recognized in 99 (79.8%) of the samples. In our assessment of whether cooperative expressions of LAT1 mRNA and 4F2hc protein were related to the PCNA index or clinicopathologic findings, "LAT1 mRNA with 4F2hc" was found to be associated with age (p=0.028; Table 1). When a cooperative expression of LAT1 mRNA and 4F2hc protein was assessed separately for noninvasive and invasive tumors, a significant correlation with "LAT1 mRNA with 4F2hc" was found for age (p=0.016) in noninvasive tumors (Tables 2, 3).

Distant-metastasis-free interval and overall survival rates

Our assessments of distant-metastasis-free interval and overall survival revealed rates for a 5-year disease-free survival and a 5-year overall survival of 60.2 and 73.3%, respectively. In the assessment of disease-free survival, 119 patients who had no metastasis at surgery and in whom the malignant tumor was excised totally by surgery were included in the analysis.In the assessment of overall survival, all 124 patients were included in the analysis. Univariate analyses of disease-free and overall survivals in all patients revealed that "LAT1 protein with 4F2hc," PCNA index, stage, grade, and pattern of growth each had a significant effect on both of the two survival rates in all tumors (Table 4, Fig. 3). In patients with invasive

Table 1 Relationship[a] between protein and mRNA expressions (LAT1 protein, LAT1 mRNA, and 4F2hc protein) and other tumor characteristics (clinicopathologic findings and proliferating cell nuclear antigen [PCNA] index) in all 124 cases

	Number of cases	LAT1 protein expression		LAT1 mRNA expression		4F2hc protein expression		LAT1 protein expression in 4F2hc protein-positive cases		LAT1 mRNA expression in 4F2hc protein-positive cases	
		Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value
Age (year)											
≤67	64	52	0.16	59	0.31	60	0.039	50	0.10	56	0.028
>67	60	47		52		49		39		43	
Sex											
Male	91	68	0.018	80	0.33	78	0.21	60	0.016	70	0.17
Female	33	31		31		31		29		29	
Stage											
Group A	47	33	0.014	46	0.059	40	0.57	27	0.0013	39	0.46
Group B	20	14		17		17		12		14	
Group C	57	52		48		52		50		46	
Grade											
Low	76	59	0.44	70	0.23	64	0.11	50	0.062	60	0.75
High	48	40		41		45		39		39	
Pattern of growth											
Papillary	88	69	0.39	79	0.88	78	0.69	61	0.34	70	0.89
Nonpapillary (solid tumor)	36	30		32		31		28		29	
PCNA index											
<70%	62	46	0.72	54	0.86	54	0.48	44	0.84	47	0.26
≥70%	62	53		57		55		45		52	

The tumors were divided into three groups on the basis of tumor stage (A, papillary, noninvasive tumors, pTa; B, tumors invading the submucosa or muscularis, pT1 and pT2; and C, tumors invading beyond the muscularis or renal parenchyma or metastasizing the regional lymph node or a distant site, pT3 and pT4).

[a] Comparisons (for all except stage) were performed using the Chi-squared analysis. The comparison in respect of stage in all tumors was performed using the Kruskal–Wallis test.

tumors, stage had a significant effect on overall survival rate.

In the final models of the multivariate analysis for all tumors, stage was shown to be a prognostic factor for disease-free and overall survivals (for disease-free survival, the value obtained for risk ratio was 8.11 [$p<0.0001$], while for overall survival, it was 7.60 [$p<0.0001$]).

Discussion

In urothelial carcinoma, differences in the anatomy of tumors are associated with differences in survival. Although prognostic significance has been established for both stage and grade [4, 6], it is important to identify prognostic markers that will predict which patients are likely to experience disease progression. Several markers, including epithelial growth factor receptor, p53, E-cadherin, and HIF-1α, are known to be associated with progression and prognosis in TCC-UUT [18–22]. The purpose of our investigation was to look for possible relations between LAT1 (protein and mRNA) expression and PCNA index, clinicopathologic findings, or clinical outcome in TCC-UUT. Our analysis revealed that a LAT1 protein-positive expression was significantly associated with stage in all TCC-UUT cases. When the relationship between LAT1 and 4F2hc was examined (because LAT1 requires 4F2hc for its functional expression), a cooperative expression of LAT1 protein with 4F2hc protein ("LAT1 protein with 4F2hc") was found to be more strongly associated with stage than suggested by a simpler analysis. Further, in all tumors, significant correlations were found between "LAT1 protein with 4F2hc" and both overall and disease-free survival rates in the univariate analysis. Although the expression of the active form of LAT1 protein (cooperative expression of LAT1 protein with 4F2hc protein) was not found to be associated with prognosis in the multivariate analysis, the detection of the active form of LAT1 protein would appear to be of value as a prognostic tool in TCC-UUT.

It has previously been reported that LAT1 is expressed in some normal tissues, including the blood–brain barrier, activated lymphocytes, the basal layer of the skin, the

Table 2 Relationship[a] between protein and mRNA expressions (LAT1 protein, LAT1 mRNA, and 4F2hc protein) and other tumor characteristics (clinicopathologic findings and proliferating cell nuclear antigen [PCNA] index) in 47 noninvasive cases

	Number of cases	LAT1 protein expression		LAT1 mRNA expression		4F2hc protein expression		LAT1 protein expression in 4F2hc protein-positive cases		LAT1 mRNA expression in 4F2hc protein-positive cases	
		Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value
Age (year)											
≤67	24	17	0.92	24	0.30	23	0.034	17	0.057	23	0.016
>67	23	16		22		17		10		16	
Sex											
Male	38	24	0.029	37	0.62	32	0.72	19	0.033	31	0.59
Female	9	9		9		8		8		8	
Stage											
Group A	47	33		46		40		27		39	
Grade											
Low	42	30	0.59	41	0.72	35	0.32	24	0.90	34	0.28
High	5	3		5		5		3		5	
Pattern of growth											
Papillary	47	33		46		40		27		39	
Nonpapillary (solid tumors)	0	0		0		0		0		0	
PCNA index											
<70%	30	21	0.96	29	0.44	27	0.21	18	0.63	26	0.37
≥70%	17	12		17		13		9		13	

The tumors were divided into three groups on the basis of tumor stage (A, papillary, noninvasive tumors, pTa; B, tumors invading the submucosa or muscularis, pT1 and pT2; and C, tumors invading beyond the muscularis or renal parenchyma or metastasizing the regional lymph node or a distant site, pT3 and pT4).

[a] Comparisons (for all except stage) were performed using the Chi-squared analysis. The comparison in respect of stage in all tumors was performed using the Kruskal–Wallis test.

proximal tubules of the kidney, the placenta, and testis, and also in a variety of tumor cell lines such as hepatoma, leukemia, breast cancer, oral squamous cell carcinoma, bladder carcinoma, and glioma [10–13, 16, 23, 25–27, 29–31, 34–36]. In normal urothelial cells, we found, using immunohistochemistry, that LAT1 protein was expressed within the cytoplasm as nodular granules, an observation indicating nonfunctionality because of their absence from the plasma membrane. In contrast, LAT1 protein was detected in the plasma membrane (which contains the sites of action for LAT1 protein) in about half of the tumor cells. Furthermore, our Western blot analysis revealed that (a) in the normal pelvic mucosal tissues and in a TCC case in which nodular granules were observed by immunohistochemistry, the LAT1 protein expression was stronger in the cytoplasmic extraction than in the membrane extraction, while (b) in a TCC case in which diffuse LAT1 expression was detected in both the cytoplasm and the plasma membrane by immunohistochemistry, a distinct band for this protein was seen in the membrane extraction as well as in the cytoplasmic extraction. Thus, LAT1 protein localized to its sites of action in cells may be present at significantly higher levels in tumors than in normal urothelial tissues.

In an in vitro study, Campbell and Thompson [1]—who transiently overexpressed LAT1 and 4F2hc (either alone or together) in nontransformed mouse hepatocytes—demonstrated (a) that overexpression of LAT1 alone was sufficient to increase system L transport activity significantly in these hepatocytes and (b) that hepatic cells overexpressing LAT1 displayed a growth advantage relative to control cells under conditions involving limited arginine. They therefore proposed that cells in which LAT1 is not normally expressed, such as hepatocytes, may acquire a growth advantage if LAT1 becomes constitutively expressed at some stage. After an examination of human materials, Kim et al. [10]—who looked for expressions of LAT1 and 4F2hc proteins in oral normal mucosa, oral precancerous lesions, and oral squamous cell carcinomas using immunohistochemistry—reported that LAT1 and 4F2hc protein expressions increased progressively from normal mucosa to dysplasia and carcinoma. More recently, we demonstrated (a) that the incidence of positive expressions for LAT1

Table 3 Relationship[a] between protein and mRNA expressions (LAT1 protein, LAT1 mRNA, and 4F2hc protein) and other tumor characteristics (clinicopathologic findings and proliferating cell nuclear antigen [PCNA] index) in 77 invasive cases

	Number of cases	LAT1 protein expression		LAT1 mRNA expression		4F2hc protein expression		LAT1 protein expression in 4F2hc protein-positive cases		LAT1 mRNA expression in 4F2hc protein-positive cases	
		Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value	Positive cases	*p* value
Age (year)											
≤67	40	35	0.64	35	0.43	37	0.38	33	0.10	33	0.31
>67	37	31		30		32		29		27	
Sex											
Male	53	44	0.31	43	0.23	46	0.22	41	0.29	39	0.17
Female	24	22		22		23		21		21	
Stage											
Group A			0.019		0.93		0.61		0.0071		0.32
Group B	20	14		17		17		12		14	
Group C	57	52		48		52		50		46	
Grade											
Low	34	29	0.92	29	0.85	29	0.26	26	0.42	26	0.78
High	43	37		36		4/		36		34	
Pattern of growth											
Papillary	41	36	0.57	33	0.31	38	0.88	34	0.56	31	0.60
Nonpapillary (soild tumor)	36	30		32		31		28		29	
PCNA index											
<70%	32	27	0.77	27	0.99	27	0.99	26	0.89	26	0.55
≥70%	45	39		38		38		36		34	

The tumors were divided into three groups on the basis of tumor stage (A, papillary, noninvasive tumors, pTa; B, tumors invading the submucosa or muscularis, pT1 and pT2; and C, tumors invading beyond the muscularis or renal parenchyma or metastasizing the regional lymph node or a distant site, pT3 and pT4).

[a] Comparisons (for all except stage) were performed using the Chi-squared analysis. The comparison in respect of stage in all tumors was performed using the Kruskal–Wallis test.

Table 4 Univariate analysis of overall and disease-free survival rates

Prognostic indicator	All tumors				Noninvasive tumors				Invasive tumors			
	Overall survival (*n*=124)		Disease-free survival (*n*=119)		Overall survival (*n*=47)		Disease-free survival (*n*=47)		Overall survival (*n*=77)		Disease-free survival (*n*=72)	
	Wilcoxon	Log rank	Wilcoxon	Log rank	Wilcoxon	Log rank	Wilcoxon	Log rank	Wilcoxon	Log rank	Wilcoxon	Log rank
LAT1 protein expression	0.22	0.23	0.18	0.26	0.23	0.51	0.18	0.55	0.51	0.61	0.24	0.49
LAT1 mRNA expression	0.79	0.80	0.40	0.42	0.76	0.72	0.78	0.71	0.36	0.42	0.18	0.20
4F2hc protein expression	0.11	0.13	0.10	0.14	0.64	0.61	0.62	0.58	0.16	0.19	0.14	0.21
LAT1 protein expression with positive 4F2hc	0.045	0.046	0.037	0.054	0.74	0.39	0.35	0.82	0.23	0.28	0.11	0.25
LAT1 mRNA expression with positive 4F2hc	0.19	0.18	0.081	0.10	0.57	0.52	0.57	0.49	0.12	0.15	0.059	0.097
Stage	<0.0001	<0.0001	<0.0001	<0.0001	*	*	*	*	0.023	0.018	0.11	0.051
Grade	0.0010	0.0023	0.0023	0.0015	0.72	0.72	0.72	0.72	0.15	0.24	0.29	0.23
Pattern of growth	0.0071	0.033	0.0094	0.021	*	*	*	*	0.62	0.93	0.96	0.65
PCNA[a] index	0.0052	0.012	0.045	0.024	0.40	0.38	0.39	0.35	0.041	0.056	0.26	0.11

*p value was not determined because of one factor or because that particular analysis was not done.

[a] Proliferating cell nuclear antigen

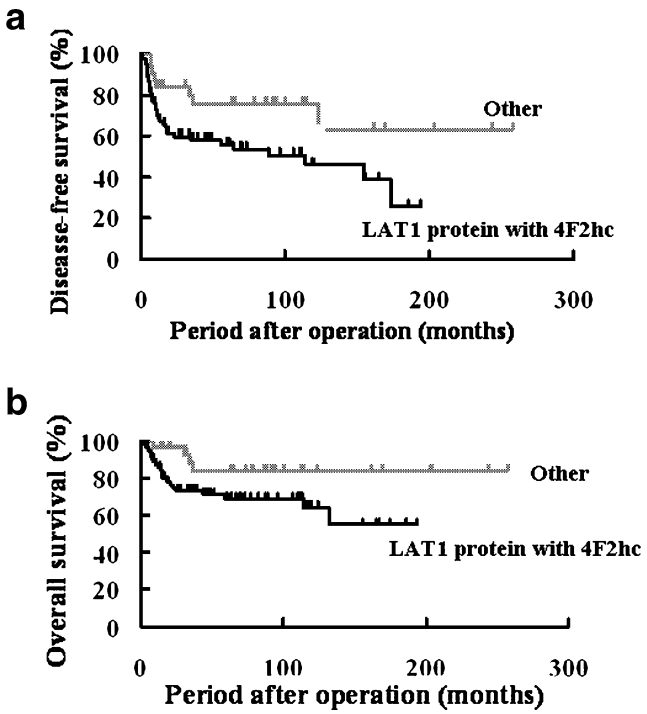

Fig. 3 Disease-free and overall survival curves for all patients with transitional cell carcinoma (subdivided according to presence or absence of cooperative expression of LAT1 protein with 4F2hc protein ["LAT1 protein with 4F2hc"]). **a** Disease-free survival curve for patients displaying LAT1 protein with 4F2hc (n=85) or other expressions (n=34). **b** Overall survival curve for patients displaying LAT1 protein with 4F2hc (n=89) or other expressions (n=35)

protein and LAT1 mRNA increased or tended to increase from low-grade atypical adenomatous hyperplasia (AAH, a precancerous lesion of the lung) through high-grade AAH to early adenocarcinoma of the lung (using immunohistochemistry and in situ hybridization) and also (b) that the labeling index calculated using Ki-67 antibody (which was detected in all stages of the cell cycle except for the G0 period) was significantly higher in LAT1 protein-positive AAH and early adenocarcinoma than in their LAT1 protein-negative counterparts [23]. Furthermore, Kobayashi et al. [13], who examined esophageal carcinoma using immunohistochemistry, reported that LAT1 expression increased as the depth of invasion and the tumor size increased. On the basis of the above evidence, LAT1 has been concluded to be associated with tumor growth, proliferation, and aggressiveness [10, 13, 23]. In TCC cases, to our knowledge, no previous report dealing with LAT1 and 4F2hc has been published. In our immunohistochemical and in situ hybridization examinations, LAT1 protein-positive expression was significantly associated with stage in all cases, indicating an association with the aggressiveness of the tumors. Thus, our findings support the above idea of a relationship between LAT1 and tumor growth and aggressiveness. In our study, however, we failed to find an association between LAT1 expression and PCNA index (cell proliferation index). A similar lack of association (between LAT1 expression and tumor mitosis) was noted in a previous study of colon carcinoma [10]. We are not entirely surprised at this lack of a direct correlation between LAT1 expression and PCNA index because of the diversity of the other biological factors involved in the promotion of cell proliferation.

Concerning tumor prognosis, to our knowledge, no report dealing with LAT1 and 4F2hc has been published. When a tumor exhibits enhanced uptake and transport of amino acids into select metabolic pathways, including those involving LAT1, it would be expected to benefit from the metabolic and regulatory effects of those amino acids and to experience an accelerated rate of growth. Although in our multivariate analysis for all tumors, we failed to find a relationship between LAT1 expression and either disease-free or overall survival rates, significant correlations were found between "LAT1 protein with 4F2hc" and both overall and disease-free survival rates for all tumors in the univariate analysis. On that basis, the expression of LAT1 protein with 4F2hc (that is, the active form of LAT1 protein) may be expected to be one of the tools that can be used to evaluate the prognosis in TCC-UUT. However, this may not result in a direct association being found between LAT1 expression, for example, and prognosis. Therefore, future studies should attempt to elucidate the relationships between such expression(s) and clinicopathologic findings or clinical outcome in several tumors.

In conclusion, the detection of the active form of LAT1 protein (a cooperative expression of LAT1 protein with 4F2hc protein) would appear to be of value as a prognostic tool in TCC-UUT. In the last few years, several investigators have demonstrated that the expressions of ASCT2 and other neutral amino acid transporters are coordinately elevated in a wide spectrum of primary cancers [5, 14, 33]. The suggestion is that these transporters support the "tumor metabolome." Therefore, future studies should examine other neutral amino acid transporters and seek to elucidate the relationship between their expressions and clinicopathologic findings or clinical outcome in TCC-UUT.

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ORIGINAL ARTICLE

Establishment of novel human dedifferentiated chondrosarcoma cell line with osteoblastic differentiation

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Abstract Dedifferentiated chondrosarcoma is a rare, highly malignant variant of chondrosarcoma in which a high-grade sarcoma coexists with a low-grade chondroid tumor. We herein review a case of dedifferentiated chondrosarcoma with an osteosarcoma omit component that occurred in the distal femur of a 38-year-old man. We established the cell line (NDCS-1) from a pleural effusion of the metastatic lung tumor. The cell line was characterized by a the G-banded karyotype, polymerase chain reaction (PCR) single-strand conformation polymorphism analysis, spectral karyotyping, and reverse transcriptase PCR (RT-PCR). The tumor exhibited complex karyotypes and a high frequency of chromosomal amplication with p53 mutation. This tumor revealed an osteoblastic and chondroblastic character in vitro and in severe combined immunodeficiency mice. The expression and phosphorylation of platelet-derived growth factor receptor-β, which seemed to play a major role in the malignant phenotype of chondrosarcoma, was confirmed by RT-PCR and Western blotting. To our knowledge, this is the first report of the establishment of a human dedifferentiated chondrosarcoma.

Keywords Dedifferentiated chondrosarcoma · Chondrosarcoma · Osteoblastic differentiation · Characterization · New cell line

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Introduction

Dedifferentiated chondrosarcoma was first proposed in 1971 by Dahlin and Beabout. They described a high-grade nonhchondroid sarcoma, such as osteosarcoma, rhabdomyosarcoma, fibrosarcoma, and malignant fibrous histiocytoma (MFH), arising within a pre-existing low-grade chondrosarcoma. Dedifferentiated chondrosarcoma is rare variant, accounting for about 10% of all chondrosarcomas. The importance of recognizing this dedifferentiated neoplasm relates its locally aggressive behavior, frequency of recurrence, and ultimately its poor prognosis [10, 14, 15]. Many analyses of dedifferentiated chondrosarcoma are available at the clinico-pathological level, but only few molecular studies have so far been published, and the cell biology of this neoplasm is still not clearly understood. To our knowledge, there has not been any report describing the establishment of cell lines of dedifferentiated chondrosarcoma. We have established this cell line, NDCS-1, derived from a pleural effusion of a metastatic lung tumor that may serve as a useful in vitro and in vivo model for study of the molecular pathogenesis of dedifferentiated chondrosarcoma.

Materials and methods

Source of tumor cells

A 38-year-old man was founded to have a bone tumor on the left distal femur (Fig. 1a, b). The patient underwent wide excision. Histopathological diagnosis was grade I chondrosarcoma. Five years after the surgery, the tumor recurred, and open biopsy revealed it to be dedifferentiated to osteosarcoma (Fig. 1c, d). He was operated on with an above-knee amputation.

The tissue specimen was examined histopathologically. The patient did not react to chemotherapy with cisplatinum, ifosmfamide, and methotrexate and died of lung metastasis after 7 months after the above-knee amputation.

Establishment of cell line

A sample from the pleural effusion of the lung metastasis was seeded in dishes, containing Roswell Park Memorial Institute Tissue Culture Medium (RPMI) 1640 supplemented with 10% heat-inactive fetal bovine serum and 300 mg kanamycin sulfate (Wako Tokyo, Japan). These were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C.

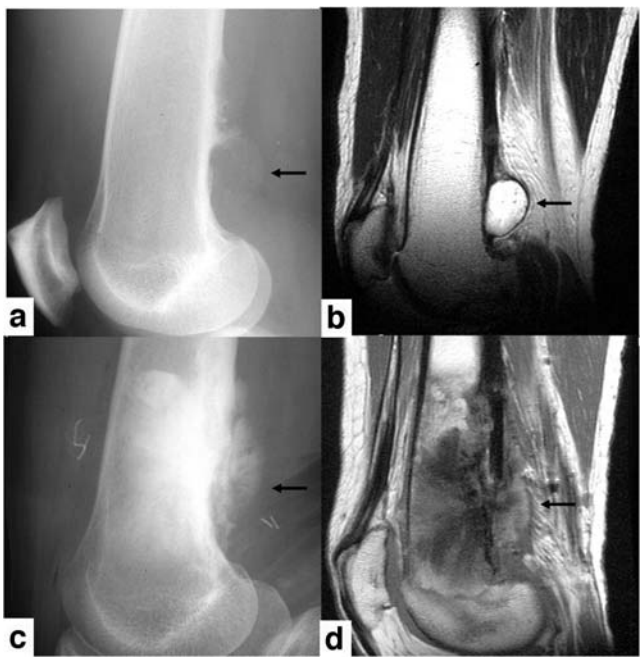

Fig. 1 **a** Plain radiograph of the distal femur at the first operation. The lateral rentogenogram showing a periosteum elevation. **b** MR imaging showing a bone periosteal tumor of the distal femur. **c**, **d** The *arrows* pointed at the recurrent tumor. The tumor invaded the intramedullary space. A sagittal MRI demonstrated the tumor to have spread to the soft tissue and the borders of the tumor were indistinct

Histological examination of the xenografted tumor

Seven-week-old mice with severe combined immunodeficiency (SCID; Icr/SCID purchased from Nihon Clea, Tokyo, Japan) were used. The tumor cells (1×10^{7} cells suspended in 0.2 ml RPIM; the passage number was 20) were subcutaneously implanted into the mice. Three months after the tumor cell inoculation, the mice were killed, and a specimen was obtained. We stained 4-μm-thick sections of the specimen with routine hematoxylin–eosin staining and analyzed with p53 (DO7; DAKO, Japan), platelet-derived growth factor receptor (PDGFR; Lab Vision, CA, USA), and Ki-67(MIB-1; IMMUNOTECH, France) immunohistochemical staining.

Cytogenetic analysis

The Standard trypsin–Giemsa banding technique [51] was performed for cells obtained after 20 passages, and additional slides were made for spectral karyotyping analysis, according to the manufacturer's protocol (Applied Spectral Imaging, Migdal Ha'Emek, Israel) [25, 50, 58]. Clonal chromosomal abnormalities were described according to the International System for Human Cytogenetics (ISCN 1995) [42].

PCR-SSCP analysis

A polymerase chain reaction (PCR) single-strand conformation polymorphism (SSCP) analysis was performed on exons 4–8 of p53. High-molecular-weight deoxyribonucleic acid (DNA) was isolated from the original tumor samples using standard methods [52]. PCR-SSCP was performed [51] and analyzed using the Allele Link software package (Amersham Pharmacia Biotech). Abnormal bands during the SSCP analysis were amplified and sequenced using an autosequencer [52].

Reverse transcriptase polymerase chain reaction

We used Reverse transcriptase PCR (RT-PCR) to detect the expression of the gene-triggering lineage differentiation in mesenchymal cells. The total cellular ribonucleic acid (RNA) was prepared from the following: cultured cell and human osteosarcoma cell lines, NOS-1 [24] and human chondrosarcoma cell line, and OUMS [30]. These were harvested for RNA extraction with guanidine thiocynate/secium chloride gradient centrifugation. The RNA was then reverse transcribed to complementary DNA using 100 U of Moloney murine leukemia virus reverse transcriptase per reaction with an oligo-dT primer (Promega, Madison, USA). The oligonucleotide primers have been described in previous reports (Table 1). Aggrecan was designed in our laboratory, and the specific amplification was confirmed by

Table 1 PCR primers

Primer	nucleotide	ANT	Cycle	Size
RUNX2 [16]	5′-CTC ACT ACC ACA CCT ACC TG-3′ 5′-TCA ATA TGG TCG CCA AAC AGA TTC-3′	55	35	320
ALP	5′-GGG CTT CTT GTC CGT GTC GCT C-3′ 5′-CTT GCT GGT GGA AGG AGG CAG G-3′	55	30	244
COL I [59]	5′-AAG AAC CCC AAG GAC AAG AGG-3′ 5′-GGA GGG AGT TTA CAG GAA GCA-3′	55	35	461
OST	5′-TGA AAG CCG ATG TGG TCA GC-3′ 5′-TCA CAC TCC TCG CCC TAT TG-3′	60	30	884
Sox9 [59]	5′-AGA ACC CCA AGA TGC ACA AC-3′ 5′-TTT GCT TCG TCA ATG AAT GG-3′	58	45	277
COL II [29]	5′-AAC TGG CAAGCA AGG AGA CA-3′ 5′-AGT TTC AGG TCT CTG CAG GT-3′	60	30	420
COL X [29]	5′-CTG GGA CCC CTC TTG TTA GT-3′ 5′-GTT TTT ACG TTG CTG CTC AC-3′	55	35	622
ChM-I [22]	5′-CAT CGG GGC CTT CTA CTT CT-3′ 5′-GGC ATG ATC TTG CCY YCC AG-3′	60	35	312
Aggrecan	5′-TTT GAG GAG GCA CAG CAG G-3′ 5′-GGG CTC ACA ATG GGG GTA TC-3′	60	30	146
PPAR-γ [48]	5′-ATG GCA ATT GAA TGT CGTGTC TG-3′ 5′-GCA TTA TGA GAC ATC CCC AC-3′	58	45	213
MyoD [18]	5′-AGC ACTACA GCG GCG ACT-3′ 5′-GCG ACT CAG AAG GCA CGT C-3′	60	35	263
Myogenin [18]	5′-TAA GGTGTGTAA GAGGAA GTC-3′ 5′-TAC ATG GAT GAG GAA GGG GAT-3′	64	35	622
BMP2 [61]	5′-GGA AGA ACT ACC AGA AAC GAG-3′ 5′-AGA TGA TCA GCC AGA GGA AAA-3′	55	35	657
BMP4 [61]	5′-ACC TGA GAC GGG GAA GAA A-3′ 5′-TTA AAG AGG AAA CGA AAA GCA-3′	55	34	358
PDGFR-α [3]	5′-ATCAATCAGCCCAGATGGAC-3′ 5′-TTCACGGGCAGAAAGGTACT-3′	60	30	891
PDGFR-β [3]	5′-AATGTCTCCAGCACCTTCGT-3′ 5′-AGCGGATGTGGTAAGGCATA-3′	58	30	668

PCR base sequence of the sense primer and antisense primers. PCR condition of each primers and product size
OST is osteocalcin, Type2, Type10 is type II, Xcollagen. ChM-I is chondromodulin-I.
ANT is annealing time.

the direct sequencing analysis with dideoxy-chain termination methods employed using an ABI Prism 310 genetic analyzer and Big Dye Terminator cycle sequencing ready reaction kit (Perkin Elmer, Foster City, CA). The PCRs were analyzed for these primer sets [3, 16, 18, 22, 29, 48, 59, 61].

Western blotting

A whole-cell lysate was prepared by scraping the cells into Laemmli buffer (62.5 mM Tris, pH 6.8, 2% [w/v] sodium dodecyl sulfate [SDS], 5% [v/v] glycerol, 6 M urea [Fisher: BP169–212] with proteinase inhibitors [Complete: Roche]). The protein concentration was determined by the bicinchoninic acid method (PIERCE) with bovine serum albumin as the standard. The proteins were separated by SDS–polyacrylamide gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane. The membranes were blocked with 5% nonfat dry milk with 0.2% Tris-buffered saline–Tween for 1 h and incubated overnight at 4°C with PDGFR-β antibody (Santa Cruz Biotechnology, dilution 1:200) and phosphorylated PDGFR-β (p-PDGFR-β, Santa Cruz Biotechnology, dilution 1:500). After washing, the membranes were incubated for 1 h with biotin-conjugated second antibodies (anti-rabbit, dilution 1:3000) at room temperature. After washing, the immunoreactivity was visualized with electrochemiluminescence (Amersham Biosciences).

Result

Establishment of NDCS-1 of cell line and doubling time

Spindle-shaped, polygonal tumor cells reached confluence with some piled-up foci of cells (Fig. 2a). This new cell line was designated NDCS-1 and has been maintained in vitro for more than 50 passages (a period of more than 6 months).

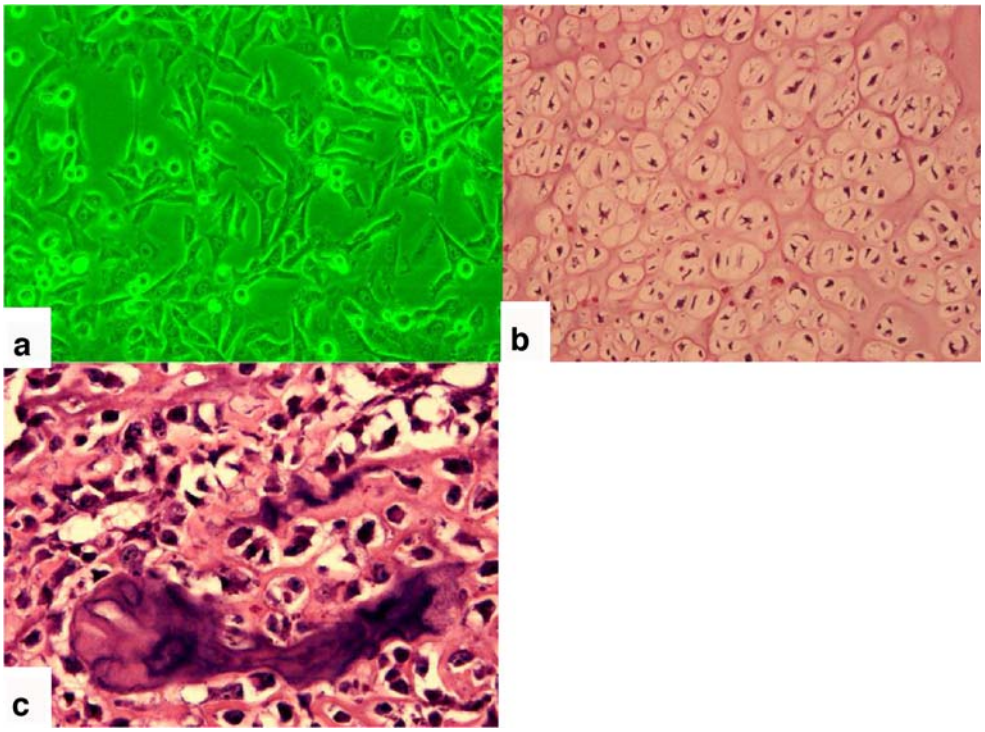

Fig. 2 **a** Light microscopic findings of the NDCS-1 cells in vitro. The NDCS-1 cells were spindle to polygonal in shape, with oval nuclei and extended elongated the cytoplasmic processes (original magnification, 200×). **b** The primary tumor showed low-grade chondrosarcoma (original magnification, 100×). **c** The recurrent tumor had an osteosarcomatous component with abundant bone formation (original magnification, 200×)

The population-doubling time of NDCS-1 cells in the logarithmic growth phase was 30 h. The maximum cell density was approximately 5.0×10^6 cells/cm^2.

Histological examination

The primary tumor showed grade I chondrosarcoma (Fig. 2b). The recurrent tumor showed dedifferentiation component of high-grade osteosarcoma with massive bone formation, and chondroblastic areas were also found (Fig. 2c). The p53 immunoactivities were positive in a recurrent tumor but were hardly seen in the original one. The Ki-67 immunoreactivity rose to 30% at the time of recurrence from 2% of the primary tumor (Fig. 3a, b, c, d). The small elastic hard nodules were first palpable in the mice 2 weeks after implantation. Three months later, the

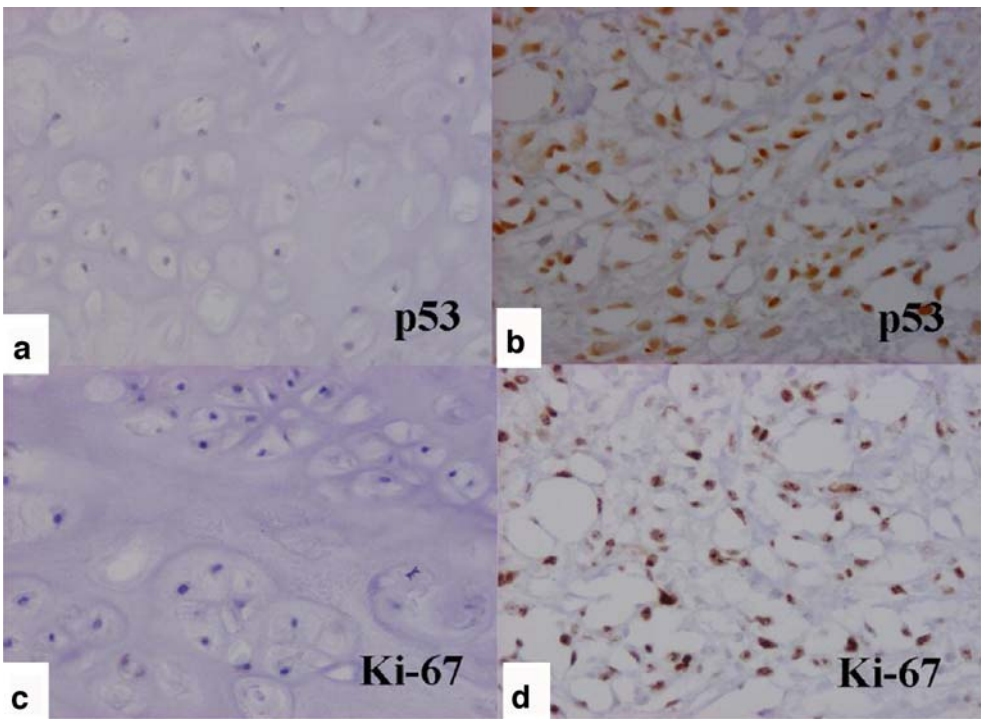

Fig. 3 **a** p53-positive cells were not seen in the primary tumor. **b** There were numerous p53-positive cells in the recurrent tumor. **c** Ki67-positive cells were hardly detected in the primary tumor. The rate of the Ki67-positive cells was only 2%. **d** Ki67-positive cells were 30% in the recurrent tumor, (**a**–**d**, original magnification, 200×)

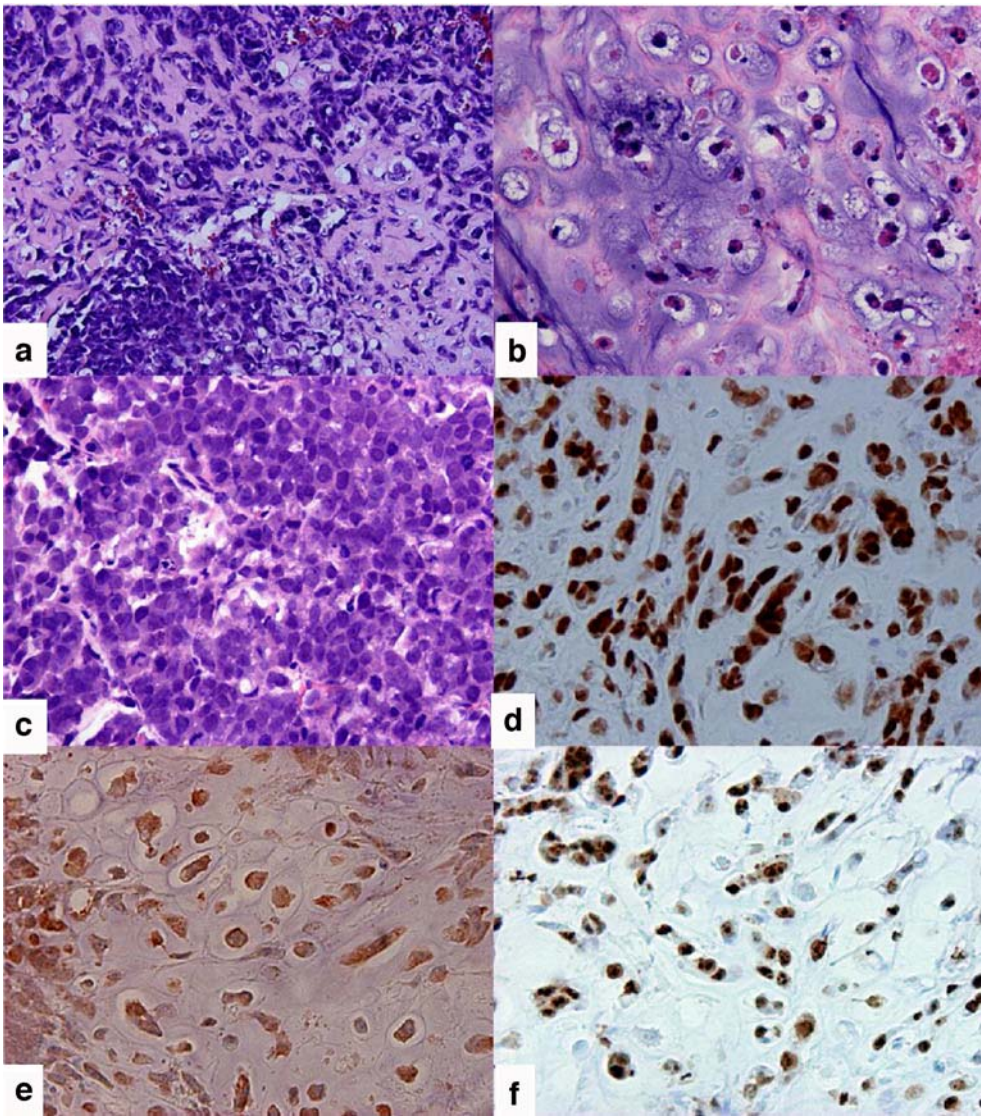

Fig. 4 Light microscopic findings of NDCS-1 cells in vivo. The specimens were from the tumor implanted into the subcutaneous tissue of severe combined immunodeficiency (SCID) mice. **a** Osteoblastic area in the tumor. A lace-like osteoid was observed (original magnification, 200×). **b** Chondroblastic area in this tumor (original magnification, 400×). **c** Anaplastic area in this tumor (original magnification, 400×). Immunoactivities of the p53, PDGFR, and Ki67 proteins. There were strongly p53- (**d**) and PDGFR-positive cells (**e**) in the tumor. The positive rate of the Ki67 protein was 60% in the tumor (**f**; original magnification, 4000)

tumor had grown up to 3.0 cm in diameter. The cut surface of these tumor were solid and white including mineralized areas.The transplanted NDCS-1 cell tumors were composed mainly of three parts: the osteoblastic (immature bone) lesion (Fig. 4a), the chondroid lesion (Fig. 4b), and the anaplastic lesion (Fig. 4c). Based on the immunohistochemistry, the tumor cells were diffusely positive for p53 and the PDGFR, and the positive rate of Ki-67 was 60% (Fig. 4d, e, f).

Cytogenetic examination

The results of the cytogenetic analyses were very complex (Fig. 5). Cytogenetic analyses of ten metaphases were

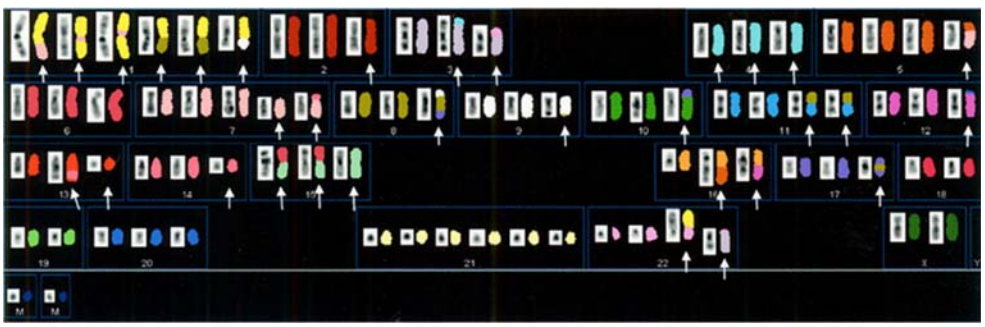

Fig. 5 Representative SKY of the cell line. *Many arrows* point to the abnormal chromosomes

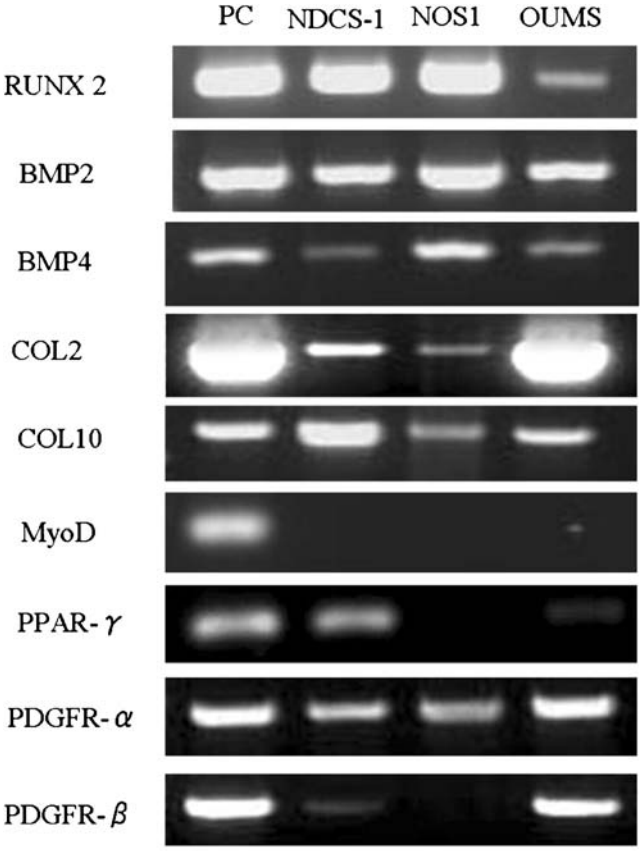

Fig. 6 RT-PCR revealed the expression of RUNX2, Type II collagen, Type X collagen, BMP2, BMP4, PPAR-γ, MyoD, PDGFR-α, and PDGFR-β

analyzed from this cell line. The G-banding was analyzed based on the results of the spectral karyotyping.

All ten cells had numerical and structural abnormalities, as follows: 76~78<3*n*>,XX,-Y[10], +der (1) t (1;7) (q25;q11) [10], +der (1;9) (p10;p10) [10], +der (1) (1pter→1q11::22q?::1q21→1qter) [10], der (1) (1qter→1q21::22q?::1p11→1qter) [10], der (1;8) (p10;?) ×2 [10], del (2) (q11q21) [10], der (3) t (3;4) (p25;?) [10], der (3;22) (q10;q10) [10], del (4) (p14) [10], del(4) (q21q22) [10], der (4) ?inv (4) (q12q21) del (4) (q?) [10], +der (5;7) (p10;q10) [10], +7[8], del (7) (p12) [9], der (7) t (7;14) (p11;q?) [9], +8[10], der (8) (17qter→17q21::8?→cen→8?::9q32→9qter) [8], der (9) t (8;9) (?;q32) [4], der (9) (9pter→9q32::8?::17?) [6], der (10) t (10;17) (p15;q11) [10], +der (11) t (8;11) (?;p11) [9], der (11) t (8;11) [8], add (12) (p13) [3], der (12) t (12;20) (p13;?) [9], ?del (13) (q12) [8], der (13) [2], der (13;19) (q10;p10), ?idic (14) (q11) [10], der (13) (13pter→13q32::14q21→14q32::13q?) [9],-15[3], der(15) (15qter→15p11::6p11→6p23::6?) [9], I (15) (q10) [8], der (15) [7], der (16) t (5;16) (q13;q24) [4], der (16) t (12;16) (q13;q22) [10],-17[3], der (17) (17pter→17q11::8?::17?) [3]-18[10],-19[10], +21[10], +21[8], +der (22) t (3;22) (p12;q11) [10], der (22) t (1;22) (q21;p13) [8], +0~4 mar [cp10].

These alterations were all clonal abnormality.

Polymerase chain reaction single-strand conformation polymorphism

The PCR-SSCP sequence analyses of the original tumor and cultured cells revealed a functionally relevant p53 missense mutation from TGC to AGC in codon 242 of exon 7 (T-to-A transvesion).

RT-PCR and Western blotting

As shown in Fig. 6, we performed an RT-PCR analysis to confirm the expression of various mRNAs in NDCS-1, NOS1, and OUMS cells (Table 2). Although NDCS-1 had osteoblastic characterization (Runx2, ALP, Type I collagen, and osteocalcin were positive), chondroblastic characterization had been also present (Type II collagen, Type X collagen, aggrecan, Sox9 were positive). The myogenic master genes, which were MyoD1 and myogenin, were negative. Peroxisome proliferator-activated receptor (PPAR) γ, bone morphogenetic protein (BMP) 2, BMP4, and the BMP receptor were positive. PDGFR-α and PDGFR-β were positive in the RT-PCR. PDGFR-β and

Table 2 Result of RT-PCR

	NDCS-1	NOS-1	OUMS
RUNX2	+	+	+
ALP	+	+	+
COL I	+	+	+
OSC	+	+	+
SOX9	+	+	+
COL II	+	+	+
COL X	+	+	+
ChM-1	−	−	−
Aggrecan	+	−	+
PPARγ	+	−	+
MyoD	−	−	−
Myogenin	−	−	−
BMP2	+	+	+
BMP4	+	+	+
BMPR-II	+	+	+
PDGFR-α	+	+	+
PDGFR-β	+	−	+

Result of the RT-PCR conducted to detect the expression of a gene triggering lineage differentiation in mesenchymal cells.
ALP Alkalin phosphatase, *COL I* type I collagen, *OSC* osteocalcin, *COL II* type II collagen, *ChM-1* chondromodulin-1, *NDCS-1* this cell line, *NOS-1* a cell line of osteosarcoma, *OUMS* a cell line of chondrosarcoma

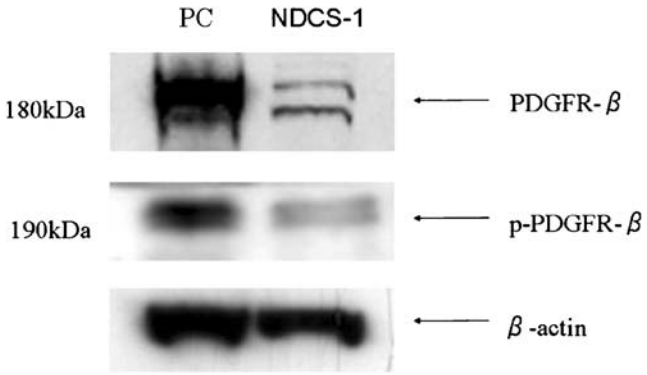

Fig. 7 Western blotting revealed the expression of PDGFR-β and p-PDFGR-β at the protein level

p-PDFGR-β expression were determined by Western blotting (Fig. 7).

Discussion

Dedifferentiated chondrosarcoma has a very poor prognosis, with a poor 5-year survival rate ranging from 0 to 18% [10, 11, 17, 26, 27, 35, 36, 40, 41, 47]. In addition, the dedifferentiated component usually is a high-grade spindle sarcoma that may respond to chemotherapy, but the response of this tumor to chemotherapy is very poor. In particular, the prognosis of the osteosarcomatous component in the dedifferentiated chondrosarcoma is extremely bad in comparison to other tumors such as MFH and fibrosarcoma [14]. The mechanism of resistance to chemotherapy has not yet been fully understood. Although intensive chemotherapy has been administrated in our case, no significant effect was observed.

This tumor has a strong tumorigenicity and shows chondroblastic and osteoblastic characteristics in vitro and in vivo. The expression of PPAR-γ might have contributed to the multifunctional property of the cell line. This cell line showed extremely complicated karyotype based on cytogenetic analysis. To our knowledge, 15 cases of cytogenetic analysis of dedifferentiated chondrosarcoma have been reported previously. Including that in this case, the cytogenetic findings of dedifferentiated chondrosarcoma were very complex. Although no specific chromosomal abnormality has yet been identified; a part of the chromosome commonly demonstrated a repeated numerical aberration of +7, +8, +20, and +21 [9, 12, 34, 46, 49, 55]. In addition, this cell line shared a part of cytogenetic findings with chondrosarcoma and osteosarcoma. In conventional chondrosarcoma, the most frequent chromosomes having structural aberrations was 1 and 9 [44], and numerical aberration was 7 and 19 [12, 44, 47]. In the loss of heterozygosity studies, the loss of chromosomes or chromosomal regions 1p36, 1p13p~22, 4, 5q13~q31, 6p22~qter, 9p22~pter, 10p, 10q24~qter, 11p13~pter, 11p25, 13q21~qter, 14q24~qter, 18p, 18q22~qter, and 22q13 and the gain of 7p13~pter,12q15~qter,19,20pter~q11, and 21q have been reported [8, 46]. In comparative genomic hybridization studies, the most frequently gained regions were 20q 12~qter (37%), 20q whole arm (32%), 8q24~qter (27%), 20p (24%), and 14q24~qter, while the most common losses were Xcen~q21, 6cen~q22, and 18cen~q11.2 (11%) [28, 30, 33, 35]. The cell line reported herein exhibited a partly shared structural and numerical aberration with the abovementioned cell lines namely, 1p13 and ~22, 10p, 20q.

In osteosarcoma, frequent structural alterations were found in 1p11–13, 1q11–12, 1q21q22, 11p15, 12p13, 17p11–13, 19q13, and 22q11–13 and common numerical abnormalities were at +1 -9, -10,-13, and -17 [4, 19, 32, 54, 56, 63]. This cell line also partly shared a part of structural and numerical aberration in the abovementioned, which were namely, 1q11, 12p13, and 17p11–13.

There is increasing evidence that tumor suppressor gene mutations play a major role in the development, progression, and /or differentiation of tumors, with p53 mutations occurring frequently in the oncogenesis of various mesenchymal neoplasms [1, 2, 6, 7, 13, 20, 57, 60, 62]. This cell line has a p53 point mutation (exon 7,codon 242 [Cys→Ser] [TGC→AGC]) and results in an immunnohistochemistry with strong positivity in high-grade cells with p53 staining. Grade I chondrosarcoma, in this case, did not show immunoreactivity for p53. Simms et al. reported that p53 overexpression was consistently present in dedifferentiated components with accompanied increased proliferative activity and an absence in low-grade cartilaginous component. It has been documented that the overexpression of p53 measured immunohistochemically is usually due to the accumulation of the mutated gene product, which exhibits a longer half-life than the wild-type protein. Recent genetic analyses revealed that p53 mutations were detected exclusively in dedifferentiated components. These evidences of a p53 mutation in the dedifferentiated component may be regarded as at least a cofactor that switched the pre-existing low-grade chondrosarcoma to a highly malignant dedifferentiated tumor [20, 43, 45, 53].

Recent advances in understanding chondrosarcoma development have suggested several molecular targets for the future development of new adjuvant therapy for this chemoresistant neoplasm. Many studies of receptor tyrosine kinase signaling for possible candidates for molecular therapies have been performed [5, 21, 23, 31, 37–39]. Receptor tyrosine kinase inhibitors have recently been developed to block deregulated protein kinase activity and thus slow tumor progression. The expressions of PDGFR-α and PDGFR-β have been detected in conventional chondrosarcoma [31]. Langonigro et al. reported that PDGFR-β seemed to play a major role in the malignant phenotype of chondrosarcoma.

In this cell line, we showed the expression of PDGFR-β in immunnohistochemistry, Western blotting, and RT-PCR. The phosphorylation of PDGFR-β in the cell line offers a rationale for the use of receptor tyrosine kinase inhibitors, alone or in combination with chemotherapy, for dedifferentiated chondrosarcoma. The NDCS-1 cell line appears to maintain the morphological and genetic characteristics of dedifferentiated chondrosarcoma in vitro and in vivo. This cell line may therefore be a useful tool for investigating the biology and molecular target of treatment in dedifferentiated chondrosarcoma.

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ORIGINAL ARTICLE

Inter-laboratory validation of PCR-based HPV detection in pathology specimens

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Abstract The detection and typing of human papilloma virus (HPV) in pathology specimens is gaining increasingly in importance. In the context of the initiative for quality assurance in pathology (QuIP) of the German Society of Pathology and the Professional Association of German Pathologists, four panel laboratories with experience and expertise in polymerase chain reaction (PCR)-based HPV detection were selected to establish an inter-laboratory trial. In a first step, these laboratories performed an internal testing of their own methodologies, which comprised DNA sequencing, multiplex nested PCR and hybridization techniques. Material from 39 samples including paraffin sections and DNA preparations of tissues and plasmids were evaluated by each panel institute according to their own protocols. Despite the different methodologies, a high degree of inter-laboratory reliability was achieved. In this report, we summarise the results. Pretested specimens are available for the external trail and can be ordered from the steering institute via provitro GmbH Berlin (http://www.provitro.de). Supplementary data are online available at http://pathologie-ccm.charite.de (rubric "Forschung"), which includes a web-based photo gallery of HPV-associated lesions and their potential association with specific virus types. The initiative is intended to foster the quality assurance of molecular HPV analysis in pathology and its correlation with morphological changes.

Keywords PCR · HPV · Human papilloma virus · Multicentre study · Quality assurance · Reference standards · Histology · Morphology · HPV gallery

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Introduction

Infection with certain types of the human papilloma virus (HPV) is the primary risk factor for the development of cervical cancer and its precursor lesions [53]. In addition, HPV may be associated with other anogenital neoplasias, cancer of the respiratory tract, and in particular, tonsillar carcinomas [50]. More than 100 different HPV types have been identified with about 40 of these infecting the anogenital epithelium. These have been classified as either low-risk (LR) or high-risk (HR) for the development of cervical cancer based upon their identification in cervical tumour samples. A recent analysis of 11 studies has designated 15 anogenital HPV types as HR (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), with a further three types designated as probably HR (26, 53 and 66) [3, 9].

Molecular biological detection of HPV has become an important method in gynecology [2, 33]. The relevance is based on the fact that screening for HR HPV types together with cytology can increase considerably the sensitivity for identifying individuals with the propensity to develop high-grade intraepithelial lesions and cervical cancer [4, 7, 10, 15, 31].

Persistent HPV infection is a necessary, although not a sufficient, cause of cervical cancer [3, 32]. It is the only cancer that is almost completely preventable through regular screening. Incorporation of HPV tests into screening programs might identify women who are at risk of developing invasive cervical cancer. Therefore, it is logical that HPV testing could be a useful cervical cancer-screening tool, and its use has been proposed for primary screening, for the triage of equivocal Pap smears and for the follow-up of patients after treatment for cervical intraepithelial neoplasia (CIN). Large-scale controlled trials are currently conducted in Europe using the Hybrid Capture 2 HPV test (HC2, Digene) or HPV-polymerase chain reaction (PCR) [9] examining the performance of HPV testing as a primary screening test. The techniques are widely applied on cytological specimens assisting and/or replacing conventional Pap smear cytology.

Detection and identification of cancer-related genes in surgical pathology specimens, i.e. in biopsies and resections, have gained increasing importance in recent years and are the domain of molecular pathology laboratories. There are certain peculiarities that are related to the use of formalin-fixed and paraffin-embedded (FFPE) tissue imposing several methodological obstacles [8, 18, 19, 36, 49].

As part of a joint initiative of the German Society of Pathology (Deutsche Gesellschaft für Pathologie http://www.mh-hannover.de/institute/pathologie/dgp) and the Professional Association of German Pathologists (Berufsverband Deutscher Pathologen, http://www.bv-pathologie.de) for quality control in pathology (QuIP), we performed an internal comparative inter-laboratory trial in the field of PCR-based HPV detection. Four institutes were selected by both societies because of their expertise and experience in this area [20, 37, 40, 44–46, 50]. Our aim was to compare and to validate methodological variants employed by the four panel laboratories. This study followed the general guidelines in molecular pathology [22] and is similar to another multicentre trial on tuberculosis PCR detection that was recently performed [39, 41].

Despite a number of methodological variations, PCR-based diagnostics of HPV proved to be reliable and reproducible. The specimens tested constitute the first stock of a sample collection that is available for external molecular pathology laboratories to test their methodology and to increase quality assurance.

Materials and methods

Design of study and sample collection

Each panel laboratory contributed between 8 and 13 cases (paraffin sections and/or DNA solutions) that had been previously analyzed in their own laboratory for the presence and type of HPV. The material of each set of cases was sent by the respective laboratory to each of the three other panel laboratories. In total, 39 cases comprising DNA solutions as well as paraffin sections were analyzed by all panel laboratories. The collection consisted of four negative controls (two fetal lungs, two bone marrow aspirates of breast cancer patients), two positive controls (plasmid solutions with defined HPV types) and 33 clinical samples including 18 portio conisations, five uterus hysterectomy specimens without dysplasia, three portio/cervix biopsies, two cervical (neck) lymph node metastases, two portio smears, one lung metastasis, one introitus vaginae biopsy and one penile amputation specimen. The DNA solutions that were sent by the respective panel laboratories had a volume of 10–12 µl and a DNA concentration of about 50 ng/µl. The number of sections of the paraffin samples is listed in Table 1.

Preparation of DNA from paraffin-embedded tissue and cytological swab/smear samples or cervical scrape specimens

A survey on the methodological steps is shown in Fig. 1, and details are listed in Table 1. For the FFPE specimens, all panel laboratories transferred a variable number of sections into reaction tubes for deparaffinization. The panel laboratories A–C de-waxed the paraffin sections according to standard protocols using xylene and ethanol; the number of treatments varied between the laboratories from one to three times. Laboratory D removed the paraffin mechanically after heating and cooling down of the sections in buffer solution. For disruption of the tissue and cell lysis, all laboratories performed proteinase K digestion using standard protocols. Some details in brief:

Laboratory A Extraction of DNA was performed by the use of QIAamp DNA Mini Kit (Qiagen, http://www.qiagen.com) according to the instruction of the manufacturer with the following modifications: The de-waxed tissue or an aliquot of 100 µl of cervical scrape specimens in liquid cytology medium were pre-treated 10 min at 95°C prior the proteinase K incubation step at 55°C. The DNA was eluted in a final volume of 50–100 µl. After determination of the DNA concentration, about 100 ng DNA was used for PCR reaction [41].

Table 1 Comparison of the methodological steps of laboratories A–D

Methodological steps	Laboratory A (Berlin)	Laboratory B (Bonn)	Laboratory C (Tübingen)	Laboratory D (Zürich)
FFPE specimen cutting	3–5×3 μm sections	3×10 μm sections	5×8 μm sections	3–5×10 μm sections
Cytological specimen handling	100 μl cervical swab in ViraPap™ transport solution (Digene) or *PreserveCyt*™ Solution (Cytyc)	200 μl concentrated swab sample in *PreserveCyt*™[46]	Cervical scrape in 400 μl PBS buffer	Gynecological cell suspension
Pre-treatment of FFPE specimens	De-wax with xylene (1×), wash with ethanol (1×), air dried [40]	De-wax with xylene (2×), wash with ethanol (2×), air dried	De-wax with xylene (3×), wash with ethanol (2×), vacuum dried [44, 45]	Boiling 10 min with buffer, centrifugation at 4°C, removing the paraffin lid
Pre-treatment of cytological samples (fresh material)	Nothing	10 ml cervical samples were concentrated 10 min 2,000×*g*	Snap-frozen (1×)	Centrifugation (20 min at 14,000 rpm each) and washing (1×) of the pellet
DNA preparation	Heating of the sample in lysis buffer (10 min), QIAamp DNA Mini Kit	QIAamp DNA Mini Kit	QIAamp DNA Mini Kit, extraction with phenol/$CHCl_3$/Isoamyl alcohol, ethanol precipitation	Proteinase K lysis (15–18 h, 37°C), heat denaturation (10 min), centrifugation [29]
Final extraction volume	50–100 μl	100 μl	100–200 μl	Variable
DNA quality	Pure	Pure	Pure	Crude cell or tissue extract
DNA quantity per PCR	1–5 μl (50–200 ng)	3 μl	100 ng DNA	1–10 μl (~50 ng)
PCR volume	25 μl	20 μl	50 μl	50 μl
DNA quality control	$OD_{260/280\ nm}$	Without	$OD_{260/280\ nm}$	1% agarose gel, comparison with standard DNA solution
DNA integrity control	β globin PCR (268 bp)	β globin PCR (268 bp)	β globin PCR (268 bp)	Duplex versican PCR (268 bp, 589 bp)
PCR and HPV detection method	Electrophoresis (3.3% agarose gel), DIG-PCR/ELISA (Roche) HPV PCR Array (Chipron)	Fluorescence capillary electrophoresis, DNA sequencing, INNO-LiPA HPV assay	Electrophoresis (2% agarose gel), sequencing by dye-deoxy terminator method (377 ABI Prism Sequencer)	Electrophoresis (3% MetaPhor-agarose gel), sequencing

Laboratory B For DNA preparation, 3×10 μm thick slices of the paraffin blocks were used. Isolation of DNA was performed with the QIAamp DNA Mini Kit (QIAGEN, Hilden) according to the manufacturer's protocol. Cytological samples (10 ml) in PreservCyt™ (Cytyc) solution were centrifuged 10 min at 2,000×*g*. After removing the supernatant, 200 μl of the concentrated sample was used for DNA isolation (QIAamp DNA Mini Kit). DNA was eluted from the columns in a volume of 100 μl 10 mM Tris–HCl pH 8.0. [46].

Laboratory C For extraction of total genomic DNA from biopsy specimens, five 8-μm thick sections were cut from each paraffin block for microdissection. The FFPE material was transferred into reaction tubes and de-waxed according to standard protocols. In brief, tissue was vortexed in 1 ml xylene (100%) for 1 min and centrifuged at 13,000 rpm for 5 min. The procedure was repeated three times. Then, samples were washed twice in pure ethanol and vacuum-dried. Proteinase K digestion was carried out in a final volume of 200 μl buffer (50 mM Tris, 1 mM ethylenediamine tetraacetic acid (EDTA), 0.5% Tween-20, pH 8.5) containing 0.2 mM proteinase K at 55°C for 2 h. Total DNA was extracted with phenol/chloroform/isoamyl-alcohol (*v*/*v*/*v*, 25:24:1) and precipitated with ethanol (100%)/LiCl (8 M) as described [44, 45]. Cervical scrapes were transferred in 400 μl phosphate buffer, rigorously vortexed for 1 min and the swab removed. An amount of 20 μl of Qiagen protease stock solution and 400 μl AL buffer were added. The extraction of total DNA was performed using the QIAmp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of extracted DNA was determined by spectrophotometry at 280 nm. One hundred nanograms of total extracted DNA was used for PCR amplifications.

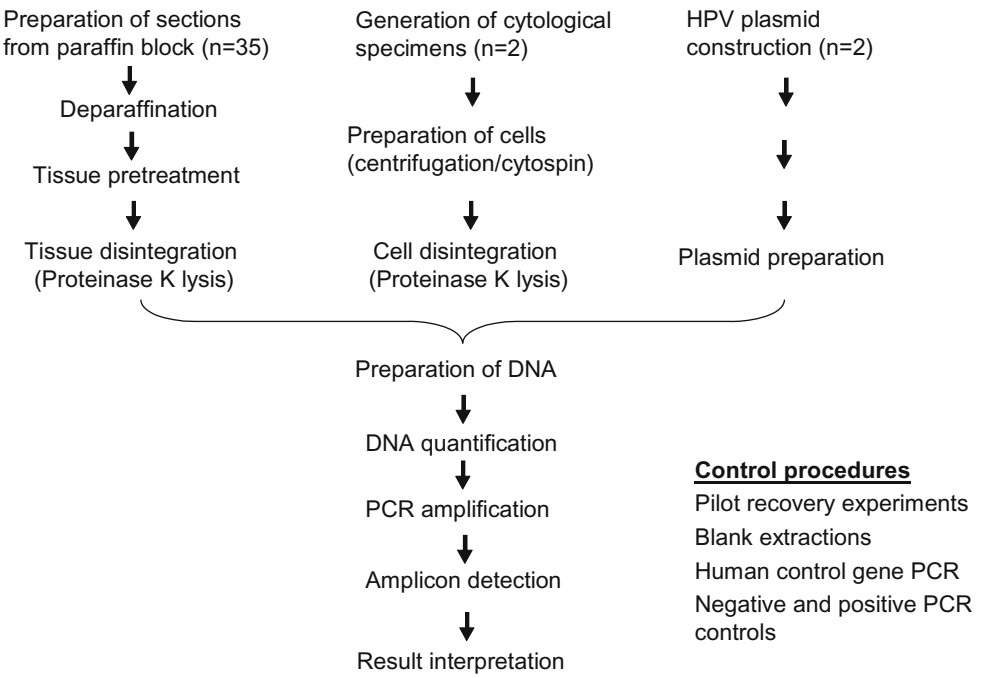

Fig. 1 Methodological steps in PCR-based HPV detection

Laboratory D The paraffin sections (three to five, 10 μm thick) were boiled in 200 μl buffer (50 mM Tris pH 8.5/1 mM EDTA/0.5% Tween 20) with vigorous mixing for 10 min, then centrifuged 30 min full speed in an Eppendorf centrifuge and cooled down to 4°C. The solid paraffin lid was removed immediately using a sterile pipette tip. After addition of 2 μl proteinase K (19 mg/ml stock solution) the incubation was carried out with shaking at 55°C. Cell suspensions of cytological specimens were centrifuged 20 min at 14,000 rpm, the supernatant discarded and the cells were carefully washed with phosphate buffered saline (PBS) and centrifuged again 14 min at 14,000 rpm. The cells were also dissolved in the lysis buffer and treated as mentioned above. After complete lysis of the tissue or the cells, the proteinase K was inactivated for 10 min at 95°C.

DNA quantification control procedures, quality assurance

To standardise the conditions and to estimate the amount of extracted DNA, the concentration of DNA was measured spectrophotometrically and the optical density ratio (260 nm/280 nm) was calculated to obtain information about the purity of the template DNA (laboratories A and C). The integrity and the quantity of the DNA was assessed semi-quantitatively via gel electrophoresis and comparison of the sample with other standard DNA samples by laboratory D. DNA amounts and PCR reaction volumes of each laboratory are specified in Table 1.

To examine whether amplifiable DNA was present in the samples, all panel laboratories assessed the quality of the prepared DNA by control PCR to amplify an appropriate segment of a human gene (Table 2), laboratories A–C used the β globin sequence as target. Laboratory D selected a multiplex PCR to amplify a small and a large amplicon of the CSPG2 (versican) gene, the larger fragment being greater as the largest HPV PCR product [35, 52]. Details of the control PCR are described in the next section.

Other control procedures were blank extractions and the selection of negative controls consisted of tissue samples that could not have had contact with HPV, similar as previously described [41]. Additionally, sterilised water or buffer without DNA were used as standard PCR negative controls, which were included in each run to check the reagents. Positive PCR controls for all amplifications included well-characterized HPV-infected samples, i.e. HPV11 (laboratory A), purified DNA from the HPV16-positive cell line Caski (laboratory B) and HPV16-infected clinical sample (laboratory D). The specificity of the nested PCR used for HPV typing was verified by including plasmid solutions of either HPV16, 33, 35 or 68 as positive controls in the PCR runs (laboratory C).

PCR amplification and HPV detection

Primer sets as well as detection methods for HPV are provided in Tables 2 and 3. All panel laboratories

Table 2 PCR targets and primers

Lab	Aims/target	Name sequences[a] 5′→ 3′	A	Size (bp)	Reference
A–C	Control/β globin PCR	PC04 (Glob-F) CAA CTT CAT CCA CGT TCA CC GH20 (Glob-R) GAA GAG CCA AGG ACA GGT AC	55°C	268	[38]
D	Control duplex PCR/ versican Exon 3	VExon3up: CAACGATGCCTACTTTGCCACCC VExon3low: ATCCCGTACATGACGTCACAGCG	55°C	268	[29, 52]
	versican Exon 8	VC589-up: TGAGCCTCCCTCTTTATCTCCT VC589-low: CGTGCCTTCCACTGAC		589	
A, B	HPV consensus/L1 gene	MY 09 CGT CCM ARR GGA WAC TGA TC MY 11 GCM CAG GGW CAT AAY AAT GG	55°C	~450	[1, 21, 47]
A	HPV consensus/L1 gene	125.for Pool of 18 oligonucleotide primer 125.rev Pool of 16 oligonucleotide primer, biotinylated	45°C	~170	Chipron GmbH, Germany HPV 3.1 LCD-Array Kit
D	HPV consensus/L1 gene	PGMY09 F-R pool of a total of 13 oligonucleotide primers PGMY11 A-E pool of five oligonucleotide primers	55°C	~450	[1, 16, 26]
B	HPV consensus/L1 gene	GP5+ TTT GTT ACT GTG GTA GAT ACT AC GP6+ GAA AAA TAA ACT GTA AAT CAT ATT C	40°C	125–165	[11, 26, 43]
C	HPV consensus MY/GP, L1 gene nested PCR	Outer Primers MY09/11 plus HMBB01 (5′-GCG ACC CAA TGC AAA TTG GT-3′)	55°C	~450	[44]
		Inner primers GP5+/6+	40°C	125–165	
D	HPV/ L1C1/2	L1C1 CGT AAA CGT TTT CCC TAT TTT TTT L1C2 TAC CCT AAA TAC YCT RTA TTG	48°C	253/256	[34, 51]
C	HPV consensus/GP-E6/E7 gene	GP-E6-3F GGG WGK KAC TGA AAT CGG T GP-E7-5B CTG AGC TGT CAR NTA ATT GCT CA GP-E7-6B TCC TCT GAG TYG YCT AAT TGC TC	40°C	602–666	[44]
C	HPV type-specific nested multiplex PCR (NMPCR)	Four cocktails of 4–5 primer pairs amplify products of markedly different sizes	56°C	151–457	[44]
B	HPV consensus/L1 gene	Mixture of defined primers for the SPF1 and SPF2 target regions SPF1 A-D, SPF2B-bio and SPF2D-bio	45°C	65	[28], INNO-LiPA HPV Genotyping v2

A Annealing temperature

[a] The degenerate base code is as follows: K=G or T, M=A or C, W=A or T, Y=C or T, R=A or G, N=A;C;G;T

performed consensus PCR for the amplification of variable regions differing between the HPV genotypes. The consensus or general PCR primers were selected to amplify a broad spectrum of HPV genotypes and were mostly located in the L1 open reading frame region of the HPV genome. It is a highly conserved region, and several universal PCR primer sets were described in the literature, most of which were applied by the panel laboratories (Table 2).

Specifically, the MY09/11 primers were used by laboratories A and B, the PGMY09/PGMY11 primers by laboratory D, the GP5+/6+ primers by laboratory B, and a nested PCR which combines the MY09/11 and GP5+/6+ primers was applied by laboratory C (Fig. 2). Commercially available primer mixtures consisted of 125.for/125.rev (LCD-Array HPV 3.1, Chipron, Berlin), which combined a number of distinct forward and reverse primers, aimed at the same position of the L1 gene (laboratory A), and the short PCR fragment (SPF) primers of INNO-LiPA HPV detection/genotyping assay, which yield a short PCR fragment of the L1 region of a broad spectrum of HPV types (laboratory B).

Another consensus PCR targeted the E6 and E7 region by a nested multiplex PCR approach (NMPCR) assay (laboratory C). Using a single consensus forward primer (GP-E6-3F) and two consensus reverse primers (GP-E7-5B and GP-E7-6B), genomic DNA of all known mucosal HPV genotypes were amplified. The PCR products of the first round were the target of four second-round reactions with primer cocktails each containing four to five different primer pairs for the amplification of specific HPV types (Table 3), which were identifiable by the size of the nested PCR amplification product in the gel electrophoreses [44].

The main procedures of HPV amplification and typing used by each laboratory are specified in the following and in Table 3.

Laboratory A All amplifications were performed with the commercially available ready mixture (Reddy Mix™, *Ab*gene,

Table 3 HPV PCR product identification/typing

Lab	Method	Potentialities	Detection of HPV types
A	DIG-PCR/ELISA (Roche)	Screening method to detect the subgroups:	
		"Low risk"	{6, 11, 42, 43, 44}
		"High risk"	{16, 18, 31, 33, 35, 45, 51, 52, 56, 58}
A	LCD chip HPV Array 3.1 (Chipron)	Screening to detect the single type	5, 6, 11, 16, 18, 31, 33, 35, 39, 42, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 67, 68, 70, 74, 82, 90, 91
		Identification of HPV types, potential to identify individual HPV types in multiple infected material	
B	Consensus PCR (GP5+/6+; MY09/11)	Screening method to detect HPV: Fluorescence capillary electrophoresis	One or more peaks Mono or mixed HPV infection
B–D	DNA sequencing	Sequence analysis and comparison with documented virus sequences, detection of mono HPV infection (Mixed infection after purification/separation of the peaks)	Available virus sequences in the GenBank database
B	INNO-LiPA HPV detection/genotyping assay	Screening to detect the single type	6, 11, 16, 18, 31/40/58, 31/33/54, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 56/74, 56/58, 58, 59, 66, 68/45/70, 68/18/39, 70
		Identification of HPV types, potential to identify individual HPV types in multiple infected material	
C	HPV type-specific nested multiplex PCR (NMPR)	First-round PCR (GP-E6/7)	Large consensus PCR product
		Nested PCR to identify HPV types with four primer cocktails in the second round PCR:	
		1. Cocktail	16, 18, 31, 59, 45
		2. Cocktail	33, 6/11, 58, 52, 56
		3. Cocktail	35, 42, 43, 44
		4. Cocktail	68, 39, 51, 66

http://www.abgene.com). The final reaction volume of 25 µl contained: 75 mM Tris–HCl (pH 8.8 at 25°C), 20 mM ammonium sulphate, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide trisphosphate, 0.01% (*v*/*v*) Tween 20, 1.25 units Thermoprime DNA polymerase, as well as loading buffer and dyes for electrophoresis. For the Digoxygenin (DIG)-PCR, the twofold concentrated kit reagents were complemented by a PCR DIG labelling mix (DIG detection, Roche, http://www.roche.com), final DIG-dUTP concentration of 120 µM and primer concentration of 0.1 µM. The cycle conditions were: 5 min 95°C, 40 cycles with 1 min 94°C, 2 min annealing temperature (see Table 2), 2 min 72°C and a final 7-min extension step at 72°C. PCR products were detected by standard electrophoresis using a 10-µl PCR aliquot and a 3.3% agarose gel containing ethidium bromide.

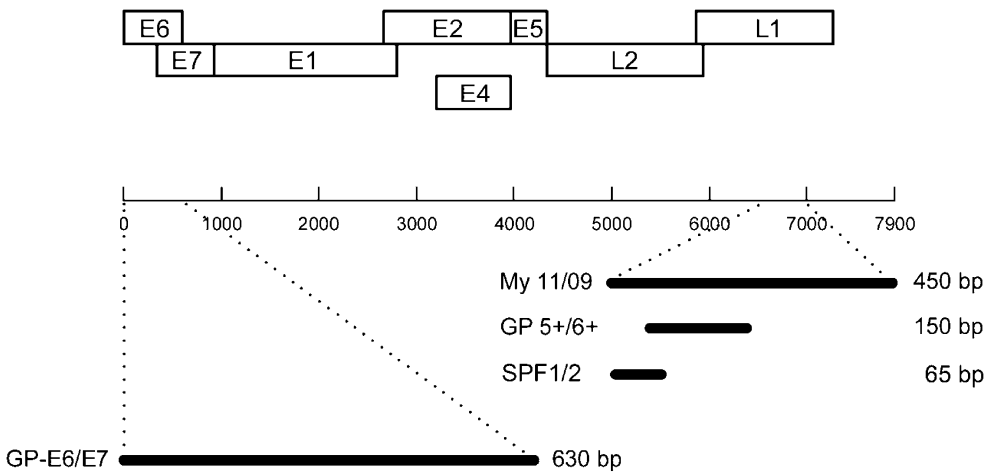

Fig. 2 Diagram of the positions of PCR amplicons used by the panel laboratories. The positions are indicated relative to the HPV-16 genome (modified from Sotlar et al. [44, 45])

In the following enzyme-linked immunosorbent assay (ELISA) detection, two 2.5-µl aliquots containing the digoxygenin-labelled PCR products were hybridized with mixtures of biotinylated probes of HR and LR HPV types (TIB MOLBIOL, Berlin, probe sequences from Bauer et al. [1], Hildesheim et al. [21] and Gravitt et al. [16]), respectively. The hybridization solutions containing DIG-labelled PCR product and biotinylated probe are then subjected onto streptavidin-coated microtiter plates; the unattached DNA strand is removed by washing. The DIG-containing hybrid can be detected and photometrically measured by binding of anti-DIG/enzyme conjugate and the substrate colour reaction as described by the instruction manual (Roche, Penzberg, Germany). This DIG-PCR/ELISA allows the differentiation of HR and LR HPV types and corresponds to the Hybrid Capture 2 analysis (Digene) used for cytological screening.

For HPV typing, a chip-based detection system ("LCD-array HPV 3.1", Chipron GmbH, Berlin) was used according to manufacturer's instructions. In brief, the biotinylated primer mixtures MY09/11 and 125 for/rev were used to generate labelled PCR products from the L1 region of the HPV genome. An amount of 10 µl of PCR products was then hybridized to HPV type-specific capture probes immobilized as two-dimensional arrays on the surface of a transparent polymer support, i.e. the chip/LCD array. Eight identical arrays of capture probes are present on one chip in separated reaction wells. After hybridization (30 min, 35°C) and high stringency washes (5 min, 24°C), the hybridization events were visualized by binding of streptavidin-horseradish peroxidase (HRP) conjugate and the precipitation of a chromogenic substrate (5 min, 24°C). Data analysis was mediated by high-resolution transmission light scanning and software-supported analysis. Specific Probes of 29 HPV types are spotted in duplicate onto the surface of the reaction well (Table 3).

Laboratory B All reactions were performed in a final volume of 20 µl consisting of 3 µl extracted DNA as a template, 2 µl 10× PCR buffer, 1 U Platinum Taq Polymerase (Invitrogen) with 20 pmol of each primer and 0.2 mM deoxyribonucleotide triphosphates (dNTPs). A $MgCl_2$ concentration of 1.5 mM was used for all reactions. The forward primers used in the PCR were labelled with fluorescence dyes: GP5+, MY11, Glob-F with HEX, fluorescein amidite derivate (FAM) and NED, respectively. The conditions were as follows: preheating for 5 min at 94°C was followed by 40 cycles of 20 s at 94°C, 20 s at the annealing temperatures (see Table 2), 40 s at 72°C and a final extension of 5 min at 72°C.

The PCR products were analyzed by fluorescence capillary electrophoresis using an automated ABI 310 Genetic Analyzer (Applied Biosystems). Therefore, 0.8 µl of each of the three PCR products were mixed with 10 µl formamide and 0.5 µl internal standard (Genescan 500-ROX, Applied Biosystems). After denaturation at 90°C for 2 min and cooling, the size of the PCR products were determined using the GeneScan software 2.1.1 (Applied Biosystems, Weiter-stadt, Germany).

The products of positive reactions were purified using the High Pure PCR product purification kit (Roche Diagnostics) according to the manufacturer's instructions and subsequently sequenced with the Big-Dye Terminator sequencing kit (Applied Biosystems) using one of the PCR primers as sequencing primer. The sequencing reactions were also analyzed on the ABI 310 Genetic Analyzer (Applied Biosystems). The obtained sequences were compared with documented virus sequences available in the GenBank databank using the BLAST program. HPV type-specific PCR was performed as described by van den Brule et al. [5] with modifications. All forward primers used in the reactions were labelled with FAM. This allowed the analysis of the PCR fragments by fluorescence capillary electrophoresis as described for the consensus PCR.

In case of negative PCR with the MY09/11 and GP5+/6+ primers or when the capillary electrophoresis showed more than one peak, a third PCR approach with SPF1/2 primers and a line probe assay (INNO-LiPA Genotyping v2 Amplification and Detection, Innogenetics, Heiden, Germany) was applied.

The line probe assay contained multiple probes immobilized as parallel lines (INNO-LiPA HPV Genotyping v2) on membrane strips. The SPF primers [28] amplify a short PCR fragment (65 bp) of a broad spectrum of HPV types in the L1 open reading frame region and allows detection of at least 24 different single HPV types and HPV patterns. The PCR was performed in a final reaction volume of 50 µl, containing 10 µl of the isolated DNA, PCR buffer II (Applied Biosystems), 2.0 mmol/l $MgCl_2$, 200 µmol/l each deoxynucleotide triphosphate, 20 pmol of forward and reverse biotinylated primer and 1.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR conditions were as follows: preheating for 9 min at 94°C was followed by 40 cycles of 30 s at 94°C, 45 s at 52°C, 45 s at 72°C and a final extension of 5 min at 72°C.

After denaturation of the PCR product, the single biotinylated strand in the liquid phase is hybridized with the multiple oligonucleotide probes of the line probe assay according the manufacturer's instructions. After hybridization and stringent washing, the hybrids can be detected by addition of a streptavidin conjugate and a substrate generating colour as lines which can be visually interpreted. This method permits detection of multiple HPV types in a single step.

Laboratory C HPV detection with primers MY09-MY11 was performed in a final volume of 50 µl. Each PCR mixture contained 50 mM KCl, 10 mM Tris–HCl (pH 8.5), 6 mM $MgCl_2$, a 200 µM concentration of each deoxy-

nucleoside triphosphate, 5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Weiterstadt, Germany), 50 pmol of primers MY09 and MY11, 5 pmol of primer HMBB01 (5′-GCG ACC CAA TGC AAA TTG GT-3′) and 5 pmol of primers PC04 and GH20 for the simultaneous amplification of a 268-bp product of the human β globin housekeeping gene [38]. Amplifications were performed with the following cycling profile: activation of AmpliTaq Gold at 94°C for 10 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 1 min elongation at 72°C at final extension step of 7 min at 72°C. To increase the sensitivity of HPV detection, nested PCRs were performed using MY09-MY11 as outer and GP5+-GP6+ as inner primers. Two microliters of the MY09–MY11 PCR product was used as template for the nested PCR amplification with GP5+-GP6+ primers [44].

To achieve exact typing of the HPV infections, a nested multiplex amplification of the GP-E6/E7 PCR with type-specific primers was chosen (NMPCR). Nested primers were arranged in multiplex PCR which discriminate among a broad spectrum of different HPV genotypes (Table 3). The primers were used in four cocktails, each containing four to five different primer pairs [44]. The identification of each HPV type present was achieved by determining the size of the nested PCR amplification product by gel electrophoresis. For HPV-6 and -11, only one specific primer pair was designed because of their high-sequence homologies and their biological similarity.

The conditions for the reactions with E6 consensus primers were 4-min denaturation step at 94°C followed by 40 amplification cycles at 94°C for 1 min, 40°C for 1 min and 72°C for 2 min for a total. The last step was an additional 10-min elongation at 72°C. NMPCRs with type-specific primers were performed under the following conditions: The first cycle was preceded by a 4-min denaturation followed by 35 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 45 s step, and the last cycle was a 4-min elongation step. All PCRs were performed in a final volume of 50 μl containing 50 mM KCl, 10 mM Tris–HCl pH 8.3, a 200-μM concentration of each dNTP, 1.5 mM $MgCl_2$, 1 U of thermostable DNA polymerase (AmpliTaq DNA polymerase; Applied Biosystems) and 15 pmol of each primer. Two microliters of the PCR product served as template for the nested PCR. Ten microliters of the amplification products were analyzed by electrophoresis on 2% agarose gels and ethidium bromide staining. With clinical samples, 100 fg of HPV-16, -33, -35 and -68 DNA-containing plasmids were used as positive controls.

For sequencing of PCR products, PCR products were excised from 2% Tris–acetate–EDTA (TAE) agarose gels and purified with a gel-extraction kit (QIAGEN) according to the manufacturer's instructions. PCR products were sequenced by the dye-deoxy terminator method on a 377 ABI Prism Sequencer (PE Applied Biosystems) using 5 pmol of either forward or back primers.

Laboratory D Essentially, PCR protocols of Gravitt et al. [17] and Yoshikawa et al. [51] were followed. The total reaction volume was 50 μl. All PCR mixtures included 1× reaction buffer II (Applied Biosystems), 0.2 mM primers, 0.2 mM dNTPs (Roche) and 2.5 U Amplitaq Gold (Applied Biosystems). In the L1C1/2 reaction, the final magnesium chloride concentration was adjusted to 2.5 mM, while 4 mM $MgCl_2$ was used in the PGMY 09/11 and the versican reactions. Cycling was performed in an Applied Biosystems 9700 thermocycler starting with a enzyme activation step at 96°C for 3 min followed by 40 cycles consisting of 1 min at 95°C, 1 min at 55°C (MY 09/11/Versican-PCR) or 48°C (L1C1/2 PCR), respectively, and 1 min 72°C. After final extension at 72°C at 5 min, samples were cooled to 4°C.

The PCR products were subsequently analyzed by gel electrophoresis on 3% Metaphor agarose (Cambrex Bio Science) and visualized on a UV screen after staining with ethidium bromide. PCR products were excised, purified with a Qiagen MinElute gel extraction kit and directly subjected to sequencing on an ABI 3100 genetic analyzer using Big Dye terminator chemistry (Applied Biosystems). All products were sequenced from both strands. Sequences were analyzed with Sequencher 3.1.1. software (Gene Codes). In some instances, mixed infections were identified by manually calling the secondary sequence.

Statistical analysis

For the nominal attribute infection type and dichotomous attribute HPV, HR HPV and LR HPV, we measured agreement across laboratories by using Fleiss' kappa statistic [14], which is a variant of Cohen's kappa for more than two raters. Kappa was determined by a custom-made program that calculated values in a range of 0 to 1. In accordance with the method of Landis and Koch [30], estimated kappa values below 0.40 were considered poor to fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial and 0.81 to 1.00 almost perfect.

The concordance for the attributes and main result were measured pair-wise for all laboratories. For each pair, the ratio of matched cases to the total number of cases was determined, which leads to a range 0 (total disagreement) to 1 (full agreement). Finally, the mean agreement was averaged over all pairs. This alternative concordance measure was used to take the strong imbalance of attribute values (yes/no) into account.

The HPV detection methods were also evaluated for their sensitivity and specificity being defined as follows.

Sensitivity: proportion of true positives among all positive cases, i.e. number of true positive results/(number of true positives+number of false negatives), specificity: proportion of true negatives among all negative cases, i.e. number of true negative results/(number true negatives+false positives). The consensus result among the laboratories was used as "gold standard".

Results

The detailed results of all PCR analyses which were performed in the different laboratories are provided in Table 4. The interpretation of the HPV results and the histopathological diagnosis are presented in Table 5. Scores were established for each positive result corresponding to the number of laboratories, which identified a specific virus type. A consensus HPV type was defined by a positive finding in the majority of the laboratories (score 4 or score 3 cases).

Mono infections

HPV DNA was consistently found in 28 cases. In 22, a single-consensus HR HPV type was found by the majority of the laboratories. These cases were interpreted as mono infections. In two of these (A1, D28), one laboratory using PCR after DNA sequencing failed to find the consensus virus type (score 3). In the remaining 20 cases, the consensus type was consistently found (score 4). However, in three of these (A8, C23, D34), one laboratory using PCR after hybridization found a weak additional signal for HPV16 being distinct from the consensus HR type. All the 22 cases consisted of clinical samples, i.e. 5 invasive carcinomas, 13 cases with severe dysplasia or carcinomas in situ (CIN 3 or VAIN 3), 3 CIN 2 lesions and 1 Pap IIID smear.

Mixed infections

In six cases, the majority of the laboratories detected more than one HPV type. These cases were formally interpreted as mixed infections. Two of these (C24, C26) consisted of a mixture of DNA plasmids representing two distinct HPV types that were included into the collection as positive controls. In these two controls, only one laboratory did not succeed to determine one of the two HPV types correctly. For the four clinical samples, three revealed one HPV consensus type (A2, C21, D35), and one carried two (D29). The fact that the consensus types belonged to the HR group was determined by all laboratories. Additionally, the consensus types were always correctly determined except for two cases (A2, D29) in which one specific HPV type (HPV53) was not covered by detection method of the respective laboratory. Up to four additional types were observed in these clinical samples, which consisted of two CIN 3 lesions, one Pap IIID smear and one cervix biopsy with inflammation and very minor epithelial polymorphism compatible with a CIN 1 lesion.

Negative samples

A negative result in HPV detection and subtyping analysis was only scored if the amplification of control genes for testing the DNA integrity of the tissue gave a positive result. Two fetal lung tissues and two breast cancer biopsies were selected as true negative controls. In one of these four cases, a positive result for HPV6 (LR) and HPV45 (HR) was found by one laboratory using a hybridization detection method, while the others were consistently negative. Additionally, seven clinical samples (two cervix/portio biopsies and five hysterectomy specimens) were analyzed, which were consistently negative (score 4) in two cases, while single positive results (score 3) were detectable in five cases. All these aberrant positive findings were identified by hybridization methods, i.e. one ELISA test indicated a LR infection that could not be further sub-typed (D33), two (A6, D37) LR types (HPV6, HPV11), and in two cases (D30, D39), HR viruses (HPV51, HPV16) were found. In two of these five aberrant positive cases, the finding was only evident in the paraffin sample, while the corresponding DNA solutions were negative. Notably, there were pathological findings on the respective histological evaluations that consisted of squamous metaplasia in two cases (one with CIN 1) and microglandular hyperplasia in one additional case.

Statistical analysis

Sensitivity and specificity of the different methods for detecting HPV DNA and the group of HR types were calculated. In addition, the concordance of detecting specific HPV types was calculated (Table 6). Interestingly, specific methods applied in individual laboratories showed higher values than the most widespread method using the MY09/11 primers.

The statistical analysis of the test results between the four laboratories is represented in Table 7, indicating substantial concordance for the detection of HPV and the discrimination of the infection type. Almost perfect concordance was reached for the detection of HPV HR, while only moderate matching was achieved for HPV LR. The congruence for the detection of the consensus HPV types including only the positive cases was 95%. Overall, the concordance in the detection of the consensus HPV types or a consensus negative result was 89%. In Fig. 3,

Table 4 Results of PCR-based HPV testing of the panel laboratories

Code	Laboratory A		Laboratory B			Laboratory C		Laboratory D				
	MY09/11 (PCR/ ELISA)	MY09/11+125 (Chipron)	MY09/11 (FluCapElpho)	MY09/11 (Sequence)	SPF1/2 (INNO LiPA)	MY/GP (nested PCR)	Cocktail 1-4 (NMPCR)	PGMY09/ 11 (PCR)	PGMY09/11 (Sequence)	L1C1/2 (PCR)	L1C1/2 (Sequence)	
A1	HR	18	pos.	18	n.d.	pos.	18 (1)	neg.	n.d.	neg.	n.d.	Mono infections
A3	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16 (16W12E)	pos.	16	
A5	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16	pos.	16	
A8	HR	56, 16[a]	pos.	56	n.d.	pos.	56 (2)	pos.	56	pos.	56	
A9	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16	pos.	16	
A10	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16	pos.	16	
B11	HR	33	pos.	33	n.d.	pos.	33 (2)	pos.	33	pos.	33	
B12	HR	58	pos.	58	n.d.	pos.	58 (2)	pos.	58 (99%)	pos.	58	
B13	HR	33	pos.	33	n.d.	pos.	33 (2)	pos.	33 (99%)	pos.	33	
B14	HR	18	pos.	18	n.d.	pos.	18 (1)	pos.	18 (99%)	pos.	18	
B15	neg.	66	pos.	66	n.d.	pos.	66 (4)	pos.	66 (SDL106)	pos.	66	
B16	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16	pos.	16	
B17	neg.	58	pos.	58	n.d.	pos.	58 (2)	pos.	58 (ISO68)	pos.	58	
B18	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16	pos.	16	
C20	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16 (As.-Am.V.)	pos.	16	
C23	HR	16[a], 18	pos.	18	n.d.	pos.	18 (1)	neg.	n.d.	pos.	18 (L2574)	
C25	HR	33	pos.	33	n.d.	pos.	33 (2)	pos.	33	pos.	33	
D27	neg.	56	pos.	56	n.d.	pos.	56 (2)	pos.	56 (X01)	pos.	56 (X01)	
D28	neg.	58	pos.	58	n.d.	pos.	58 (2)	neg.	n.d.	neg.	n.d.	
D32	HR	16	pos.	16	n.d.	pos.[a]	16 (1)	pos.	16 (16W12E)	pos.	16	
D34	HR	51 (P)/16[a](D)	pos.	51	n.d.	pos.	51 (4)	pos.	51	pos.	51	
D38	HR	16	pos.	16	n.d.	pos.	16 (1)	pos.	16	pos.	16	
A2	HR	61, 53	pos.	n.d.	11 + 53 (+54?)	pos.	52 (2), 66 (4)	pos.	61, 53 (Bsb-1)	pos.	52	Mixed
C21	HR	18, 31	pos.	18	n.d.	pos.	18 (1), 31 (1)	pos.	18 (L2574)	pos.	18	
D29	HR	33, 35, 53	pos.	n.d.	35, 53, 68	pos.	31 (1), 33 (2), 35(3), 66(4)	pos.	35, 53	pos.	35, 53	
D35	HR	16, 33	pos.	16	n.d.	pos.	16 (1), 33 (2), 68(4)	pos.	16	pos.	16	
C24	HR	45, 58	pos.	n.d.	45, 58	pos.	45 (1), 58 (2)	pos.	45	pos.	58	
C26	LR/HR	6, 52	pos.	6	n.d.	pos.	6 (2), 52 (2)	pos.	52	pos.	52, 6	
A4	neg.	neg.	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	Negative
A7	neg.	neg.	pos.	n.d.	6, 45	neg.	n.d.	neg.	n.d.	neg.	n.d.	
C19	neg.	neg.	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	
C22	neg.	neg.	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	
A6	LR	6	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	

D30	neg.	neg.	pos.	n.d.	51	neg.	n.d.	neg.	n.d.	neg.	n.d.	
D31	neg.	neg.	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	
D33	LR (P)/ neg.(D)	neg.	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	
D36	neg.	neg.	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	
D37	neg.	neg.	neg.	neg.	11	neg.	n.d.	neg.	n.d.	neg.	n.d.	
D39	neg.(D)/ HR[a](P)	neg.(D)/16[a](P)	neg.	neg.	neg.	neg.	n.d.	neg.	n.d.	neg.	n.d.	

pos. Positive, *neg.* negative, *n.d.* not determined, *P* paraffin tissue, *D* DNA solution
[a] Weak signal

all consensus HPV types together their respective detection rates are represented.

Representative histological images of the clinical samples are available as supplementary data at http://pathologie-ccm.charite.de under the rubric "Forschung".

Discussion

In this study, we performed for the first time an inter-laboratory trial on PCR based HPV detection in surgical pathology specimens. Previously, only a few studies were published. They were all performed on cytological specimens, some at a much larger scale, mostly using hybridization detection methods and in particular hybrid capture (Digene) [42]. The scope and intention of these trials were manifold; besides validating the sensitivity and specificity of the methodology, an important focus was the applicability of testing in the prevention of HPV-associated disease and the triage of women with increased risk. The hybrid capture technology is a non-PCR based methodology using signal amplification, which is widely used in the analysis of cytology specimens. Importantly, it can detect an HPV infection but can only differentiate between a HR and LR infection [25]. Furthermore, due to the limited number of probes represented by the system and potential cross-hybridizations, it harbours the risk of wrong classifications in a substantial fraction of cases. One study comparing sequencing with hybrid capture identified revealed that about 15% of HPV infections were not found, of which 50% represented HR types [27]. In this study, the hybrid capture technique was not applied.

Our objective was the evaluation of techniques for HPV typing being in daily use at four experienced molecular pathology laboratories and the establishment of a sample collection for an external inter-laboratory trial. In general, we achieved a substantial concordance for the detection of HPV with almost perfect match in detecting a HPV HR infection. Concordance for HPV LR was moderate, which was related to the fact that only two cases were analyzed both harbouring a second HR virus type. The identification of the main HPV type was also achieved with very good concordance (Table 7). However, there was greater variability in the detection of specific virus types (Fig. 3) being related to methodology (detection technique etc.) on the one hand but also to HPV biology (infection type, genome variability etc.) on the other hand.

Methodologically, all laboratories analyzed multiple regions of the HPV genomes, most frequently in the L1 gene. This precaution was taken to minimize false negative results in the consensus PCR. The positive PCR is the

Table 5 Histopathological characteristics and compiled HPV results of the sample collective

Code	Specimen	Intervention	Type	P/D	Pathological diagnosis	Consensus HPV types	Score	Risk types	Other HPV types	Scores	Infection type
A1	Cervical lymph node	Resection	Clinical	Paraffin	Squamous cell carcinoma metastasis	18	3	HR			Mono
A3	Lung	Resection	Clinical	Paraffin	Squamous cell carcinoma metastasis	16	4	HR			Mono
A5	Cervical lymph node	Biopsy	Clinical	Paraffin	Squamous cell carcinoma metastasis	16	4	HR			Mono
A8	Portio	Biopsy	Clinical	Paraffin	CIN 3	56	4	HR	16*	1	Mono
A9	Portio conisation	Biopsy	Clinical	Paraffin	CIN 3 (Carcinoma in situ)	16	4	HR			Mono
A10	Portio conisation	Biopsy	Clinical	Paraffin	CIN 3 (Carcinoma in situ)	16	4	HR			Mono
B11	Portio conisation	Biopsy	Clinical	DNA	CIN 2	33	4	HR			Mono
B12	Portio conisation	Biopsy	Clinical	DNA	CIN 3 (carcinoma in situ)	58	4	HR			Mono
B13	Portio conisation	Biopsy	Clinical	DNA	Squamous transitional cell carcinoma	33	4	HR			Mono
B14	Vagina	Biopsy	Clinical	DNA	VAIN 3	18	4	HR			Mono
B15	Portio conisation	Biopsy	Clinical	DNA	CIN 2 (previously Pap IVb)	66	4	HR			Mono
B16	Portio conisation	Biopsy	Clinical	DNA	CIN 3 / Carcinoma in situ	16	4	HR			Mono
B17	Portio conisation	Biopsy	Clinical	DNA	CIN 3 (carcinoma in situ)	58	4	HR			Mono
B18	Introitus vaginae	Biopsy	Clinical	DNA	Condyloma acuminata with VAIN 2-3	16	4	HR			Mono
C20	Portio conisation	Biopsy	Clinical	DNA	CIN 2-3	16	4	HR			Mono
C23	Portio conisation	Biopsy	Clinical	DNA	CIN 2-3	18	4	HR	16*	1	Mono
C25	Portio smear	Cytology	Clinical	DNA	Pap IIID	33	4	HR			Mono
D27	Portio conisation	Biopsy	Clinical	D+P	CIN 1-2, cervicitis	56	4	HR			Mono
D28	Portio conisation	Biopsy	Clinical	D+P	CIN 3	58	3	HR			Mono
D32	Penis amputation	Resection	Clinical	D+P	Squamous cell carcinoma	16	4	HR			Mono
D34	Portio conisation	Biopsy	Clinical	D+P	CIN 3, CIN 1	51	4	HR	16*	1	Mono
D38	Portio conisation	Biopsy	Clinical	D+P	CIN 3	16	4	HR			Mono
A2	Cervix	Biopsy	Clinical	Paraffin	CIN 1 (previously Pap IIID)	53	3	HR, LR	52,61, 66,54	2/2/1/1	Mixed
C21	Portio smear	Cytology	Clinical	DNA	Pap IIID	18	4	HR	31	2	Mixed
D29	Portio conisation	Biopsy	Clinical	D+P	CIN 3	35, 53	4/3	HR	31,33, 66,68	2/2/1/1	Mixed
D35	Portio conisation	Biopsy	Clinical	D+P	CIN 2-3	16	4	HR	33, 68	2/1	Mixed
C24	Plasmid solution	Lab control	P.c.	DNA		45, 58	4/4	HR			Mixed
C26	Plasmid solution	Lab control	P.c.	DNA		6, 52	4/3	LR, HR			Mixed
A4	Lung	Autopsy	N.c.	Paraffin	Fetal lung	Negative	4	no			Negative
A7	Lung	Autopsy	N.c.	Paraffin	Fetal lung	Negative	3	no	6, 45	1/1	Negative
C19	Bone marrow aspiration	Biopsy	N.c.	DNA	Breast carcinoma	Negative	4	no			Negative
C22	Bone marrow aspiration	Biopsy	N.c.	DNA	Breast carcinoma	Negative	4	no			Negative
A6	Cervix	Biopsy	Clinical	Paraffin	Squamous cell metaplasia (CIN1)	Negative	3	no	6	1	Negative
D30	Portio conisation	Biopsy	Clinical	D+P	No dysplasia, cervicitis, metaplasia, hyperplasia	Negative	3	no	51	1	Negative
D31	Uterus	Resection	Clinical	D+P	No dysplasia	Negative	4	no			Negative

D33	Uterus	Resection	Clinical	D+P	No dysplasia	Negative	3	no	LR (P)		Negative
D36	Uterus	Resection	Clinical	D+P	No dysplasia	Negative	4	no			Negative
D37	Uterus	Resection	Clinical	D+P	No dysplasia, cervicitis, hyperplasia	Negative	3	no	11	1	Negative
D39	Uterus	Resection	Clinical	D+P	No dysplasia	Negative	3	no	16* (P)	1	Negative

* Weak signal; other legends see Table 4
Pc. Positive control, *N.c.* Negative control

prerequisite for HPV typing, which was performed by all laboratories and was a major endpoint of the analysis.

In general, the sensitivity of DNA detection by PCR is inversely related to the size of the amplimer, as the kinetics of the PCR reaction favour smaller amplimers. Moreover, as the efficacy of PCR also depends on the quality of the DNA extracted from the clinical specimen, detection with small amplimers will be advantageous, especially when using formalin-fixed, paraffin-embedded materials. The PCR products varied between 65 bp (SPF1/2, L1 region), 150 bp (GP5+/6+, L1 region), 450 bp (MY09/11, L1 region) and 630 bp (GP-E6/E7 region). The two negative results of positive samples were indeed achieved with the MY09/11 primers using conventional PCR while these cases were still detectable using nested PCR of the even longer fragment GP-E6/E7. In contrast, the smallest fragment detected additional samples, however, once also resulting in a false positive result of a negative control. Subjecting clinical samples to pre-treatments such as formalin fixation and paraffin-embedding degrades DNA. Consequently, the efficiency of PCR primers generating a small product is considerably higher than primer sets yielding larger amplimers [24, 28].

Typing was performed by DNA sequencing, hybridization techniques and nested multiplex PCR. All methods have certain advantages and disadvantages. While DNA sequencing may be most specific by the capability to detect all virus types, it seems to be less sensitive in the detection of multiple infections. Hybridization and multiplex PCR are more sensitive but they can identify only those virus types that are covered by the assay and may show false positive results by mismatches of primers or probes.

The biology of HPV infection and its potential progression to cancer is complex [13]. One prerequisite seems to be the integration of HPV into the host genome which, being generally found in invasive carcinomas, is uncommon or absent in CIN 1 lesions [23, 48]. Similarly, persistence of HPV HR infection was observed in low-grade dysplasia which showed progression to severe dysplasia (CIN 3) while clearance of up to five different HPV types was seen in 17% of cases with low-grade histologies [6]. The presence of multiple HPV genotypes is a common phenomenon in many patient groups. Up to 35% of HPV-positive samples from patients with advanced cytological disorders contain multiple HPV genotypes, whereas multiple genotypes are less prevalent in carcinoma patients [31].

In view of these observations, it is not surprising that we observed an excellent concordance in cases with HPV HR mono infections while a higher variability was found in cases with mixed infections. Although for the latter there was also a good concordance for the detection of the predominant type, the detection of the additional HPV

Table 6 Statistical analysis of the different PCR-based HPV detection methods

Method	Used by laboratory	Number of analyses	HPV detection		HR detection		Type detection
			Sensitivity	Specificity	Sensitivity	Specificity	Comparison with consensus results (%)
(PG)MY 09/11	A, B, D	117	0.93	0.85	0.90	0.97	87
Chipron	A	39	1.00	0.91	1.00	1.00	95
NMPCR	C	39	1.00	1.00	1.00	1.00	95
L1C1/2	D	39	0.93	1.00	0.93	1.00	90.5

genomes frequently differed between the laboratories. In this context, it is also important to mention material heterogeneity as a potential factor with influence on the results. Copy number differences and the ratios of the various HPV types being present in the sample can obviously influence the sensitivity of detection. This is particularly true for PCR using consensus primers. Single HPV types may show a better primer affinity than others, and the less frequent genomes may be overlooked by the exponential amplification of the predominant type.

Furthermore, tissue heterogeneity is an inherent problem of our study because we analyzed tissue sections in addition to aliquots of DNA solutions. Particularly, the use of distinct tissue sections may have caused some positive results in our 11 samples of presumably negative cases. These consisted of four most probably HPV-unaffected cases (two bone marrow aspirates and two fetal lungs) as negative controls and seven uterus samples (cervix/portio biopsies and uterus resections) with potential contact to HPV. In addition, these cases carried histological changes (cervicitis, squamous metaplasia, microglandular hyperplasia, CIN 1) indicative for an external irritation which might have been caused by virus infection.

One by-product of our study was the observation of the morphological changes being associated with different HPV types. In our experience, there is some variability in the morphology. This is not surprising, given the limited number of histological patterns (CIN 1, CIN 2, CIN 3, koilocytosis, parakeratosis) linked to HPV so far on the one hand and the existence of more than 100 viral subtypes on the other hand. To enhance our perception and to better correlate morphology with viral subtypes, we initiated a photo gallery of HPV positive cases (see http://pathologie-ccm.charite.de, rubric "Forschung"). We feel that HPV analysis and typing will become an integral part of the molecular pathology diagnostics. There is a demand for the identification of women at risk of developing cervical cancer and an increased public interest in HPV typing due to the introduction of the recently approved vaccines. Therefore, surgical pathologists should also become more aware about potential HPV-related lesions in their daily morphological triage of histological and cytological specimens. Furthermore, specific HPV types may not only carry a different morphology but also distinct risk profiles.

Conclusions

HPV analysis is complex and should be carefully evaluated for its capacity to detect HPV DNA, HR infections and finally the correct virus type. Within our inter-laboratory trial, we tested several methods that showed reliable and consistent results in daily practice. Pretested specimens will be made available for the quality testing of other laboratories and the implementation of an external inter-laboratory trial. The initiative will hopefully increase the quality of the HPV identification and typing in surgical pathology specimens. Together with the web-based photo gallery, it may enhance the perception of the diversity of HPV-induced pathology and its putative association with specific virus type.

Table 7 Statistical analysis of test results of the panel laboratories

Parameter	HPV (yes/no)	Infection type (neg/mono/mixed)	HPV high risk (yes/no)	HPV LR (yes/no)	Main HPV type (yes/no)	Main result (first type/neg)
Concordance	0.731[a]	0.723[a]	0.810[a]	0.511[a]	0.946[b]	0.885[b]

[a] Fleiss' Kappa value (interpretation of the degree of concordance by Landis & Koch 1977: moderate, 0.4–0.6; substantial, 0.61–0.8; almost perfect, 0.81–1.0)
[b] Mean concordance

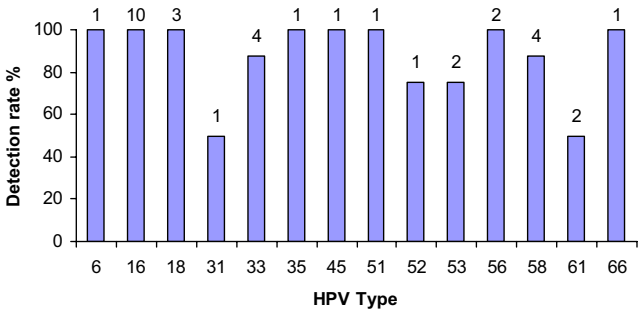

Fig. 3 Consensus HPV types (*x-coordinate*) and the rate of detection by the four laboratories (*y-coordinate*) together with the number of samples of each virus type (*numbers on top of each column*) are represented

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CASE REPORT

Psammomatous melanotic schwannoma presenting as colonic polyps

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Abstract Psammomatous melanotic schwannoma is an uncommon neoplasm that usually occurs in the setting of Carney's complex. They can occur in the gastrointestinal tract with preferential location in the stomach. A 43-year-old female presented with two colonic polyps at routine endoscopy. The patient was asymptomatic and did not have features of Carney's complex. Both polyps were composed of melanin-containing epithelioid and spindle cells with several psammoma bodies. There was no evidence of cytological atypia or necrosis. The tumor was diffusely positive for S-100, and focally for HMB-45 and melan-A. The differential diagnosis includes melanoma, GIST, pigmented neuroendocrine tumor, and epithelioid leiomyoma. The lack of malignant features separates this lesion from melanoma while the immunophenotype of the other lesions is characteristic.

Keywords Psammomatous melanotic schwannoma · Carney's complex · Colon polyp

Introduction

Schwannomas are encountered in the gastrointestinal tract with the most commonly affected site being the stomach [6, 9, 10]. However, cases have also been documented within the colon [8, 11, 13]. The histological spectrum that may be encountered in schwannomas can vary widely but psammomatous melanotic schwannoma is a characteristic lesion that is often seen in association with Carney's complex [2]. Carney first described this unique variant of schwannoma in 1990 which he noted in peripheral and posterior spine nerve roots near the midline, bone, soft tissue, heart, liver, skin, bronchus, retroperitoneum, and the gastrointestinal tract [2].

We wish to highlight an unusual case of psammomatous melanotic schwannoma presenting as an incidental colonic polyp in an asymptomatic patient without stigmata of Carney's complex.

Clinical features

A 43-year-old female presented for routine colonoscopy and was found to have two separate polyps, measuring 0.8 and 0.5 cm in greatest diameter, respectively, at 25 cm. They were pedunculated and thus easily snared and removed in toto. No other lesions were noted endoscopically. The patient did not have any of the clinical manifestations of Carney's complex despite relevant investigations. The patient made an uneventful recovery. The patient has not yet been investigated for germline mutations of the *PRKAR1A* gene.

Materials and methods

The specimens were fixed in 10% buffered formalin and routinely processed, cut, and stained with H&E. In addition, stains for melanin and iron were performed as well as

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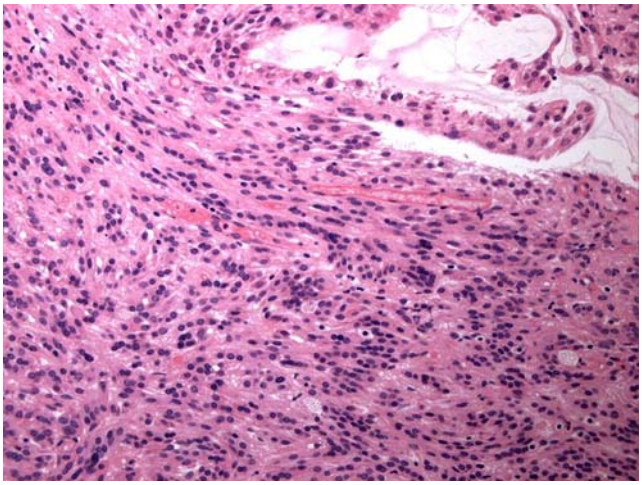

Fig. 1 The lesion composed of epithelioid cells and occasional spindle cells arranged in sheets (H&E×100)

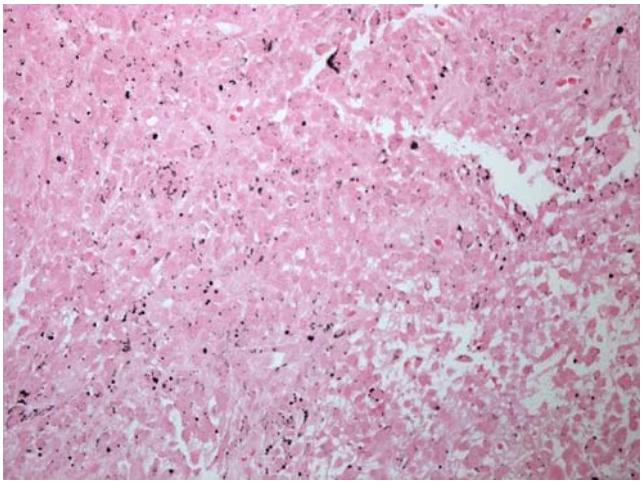

Fig. 3 The pigment was positive for the Masson-Fontana stain for melanin (Masson Fontana×400)

immunohistochemistry for: S100 protein, HMB-45, melan-A, synaptophysin, chromogranin, somatostatin, desmin, actin, CK7, CK20, CD68, CD34, and CD117.

Pathology

Sections from both polyps showed identical histological features. There was focal surface ulceration with fibrin and acute inflammatory exudate. Glands subjacent to the ulceration showed rupture with extravasation of mucin in to the lamina propria and regenerative activity. A cellular proliferation occupied the entire lamina propria and focally extended beyond the muscularis mucosae, creating a polypoid lesion. The tumor was composed of epithelioid cells (mainly) and a component of spindle cells arranged in sheets with focal areas of suggesting palisading and pseudorosette formation around vessels (Fig. 1). Several of the spindle cells had dendritic cytoplasmic processes. The epithelioid cells had abundant eosinophilic cytoplasm and several contained brown nonrefractile pigment (Fig. 2). The brown pigment was positive with the Masson Fontana melanin stain (Fig. 3) and negative for iron. Occasional lipid-laden macrophages were interspersed but obvious fat was not detected within the tumor. The nuclei were uniform with minimal pleomorphism, some contained small nucleoli and many contained intranuclear pseudoinclusions. The mitotic count was extremely low and only one mitotic figure per 10 high power fields was counted. Several multinucleated cells with clustering of nuclei were scattered throughout the lesion (Fig. 4). The stroma varied from fibrillar to oedematous to microcystic. Several psammoma bodies were liberally distributed throughout the lesion (Fig. 5). Foci of hypercellularity and necrosis were not evident.

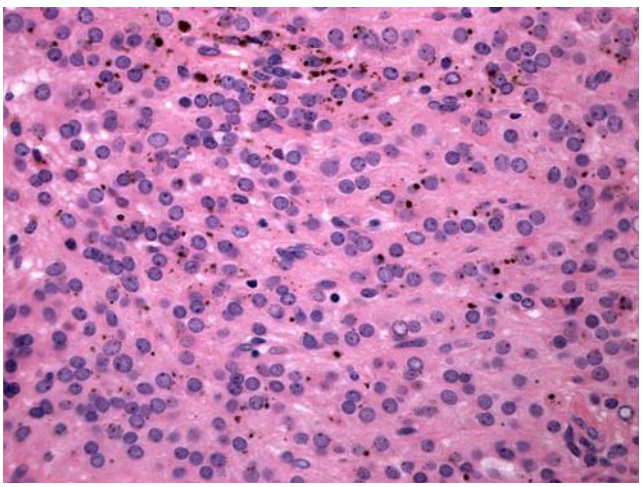

Fig. 2 Several of the tumor cells contained brown, nonrefractile pigment within the cytoplasm (H&E×400)

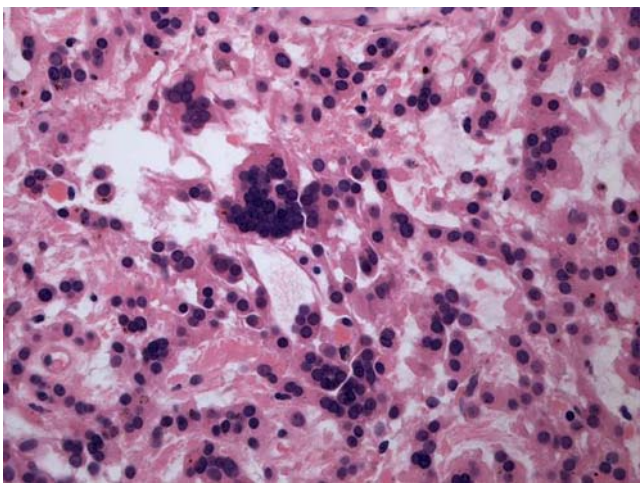

Fig. 4 A prominent finding was the presence of multinucleated giant cells scattered throughput the lesion. The number of nuclei varied from two to in excess of 25 (H&E×400)

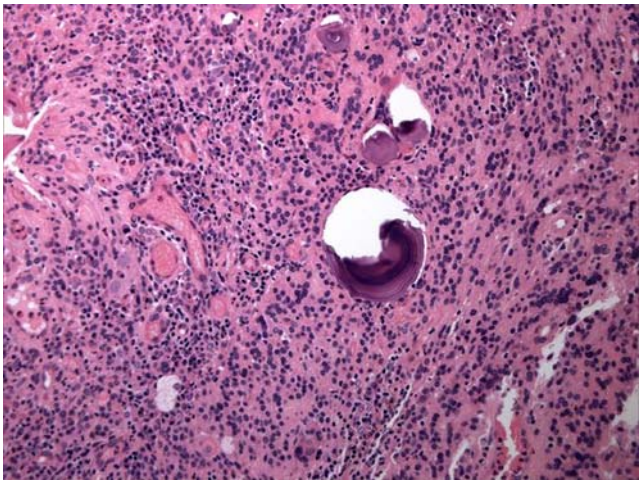

Fig. 5 The other typical and characteristic feature of the tumor was the presence of psammomatous calcification (H&E×400)

The tumor cells were strongly and diffusely positive for S-100 (Fig. 6), and focally for HMB-45 and melan-A. All other markers were negative.

Discussion

In a series of 20 schwannomas of the colorectum, Miettinen et al. noted that there was an equal gender distribution, wide age range (18–87 years; mean 65 years), size ranging from 0.5 to 5.5 cm, most frequently seen in the cecum followed by sigmoid and rectosigmoid, then transverse colon, descending colon, and rectum only [8]. The schwannomas appear as polypoid lesions causing bleeding, obstruction, and abdominal pain. The commonest pattern seen consists of spindle cells with Verocay body formation.

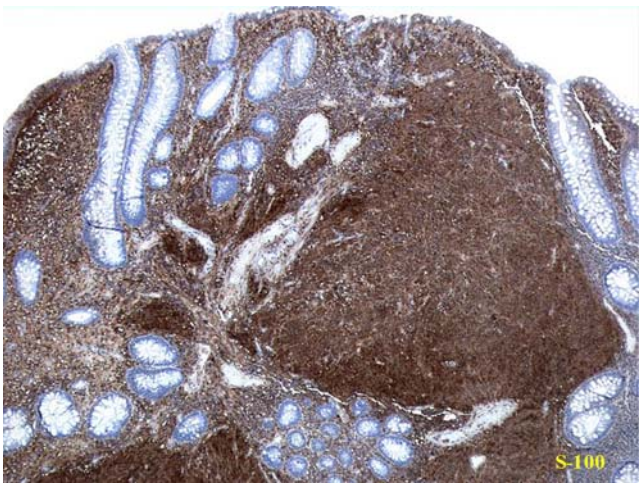

Fig. 6 The tumor cells exhibited strong and diffuse immunoreactivity for S-100 protein (anti-S-100×200)

The epithelioid variant, interestingly, occurred most frequently in the descending and sigmoid colon. These tumors were S100 positive but CD34 and CD117 negative and all behaved in a benign fashion.

Melanin pigment has been described in schwannomas [5] and amianthoid type calcification has also been encountered. The combination of melanin pigment and psammomatous calcification within a schwannoma occurs in two settings. Schwannomas, thus characterized, are associated with Carney's complex, which is named for Aidan Carney who first described the constellation of features attributed to this disease. It is a multiple neoplasia and lentiginosis syndrome of variable expression and nearly complete penetrance [1, 14, 15]. The complex consists of myxomas in the heart, breast, uterus and skin, lentigines, endocrinopathy (Cushing's syndrome from a bilateral pigmented nodular adrenocortical hyperplasia, or acromegaly) and a variety of tumors including psammomatous melanotic schwannoma, growth hormone-producing pituitary adenomas, epithelioid blue nevi, calcifying large cell Sertoli cell tumor of testis, tumors of the thyroid, and ductal adenoma of the breast [3, 4, 12]. In a detailed analysis of 40 psammomatous melanotic schwannomas, Carney noted that several cases occurred without the features of the complex [2]. Those that were associated with Carney's complex tended to be younger with an average age of 20 years, whilst those without the complex were around 33 years of age. There was an equal frequency of tumors in the gastrointestinal tract with or without the complex. He also noted that the tumors may precede the onset of the complex. Within the gastrointestinal tract, these tumors occurred from the esophagus to the rectum with the majority occurring in the stomach [2].

Microscopically, they are composed of solid sheets of spindled, dendritic, and epithelioid cells. The nuclei vary from being fusiform to round with vesicular of hyperchromatic chromatin, small nucleoli, and occasional nuclear pseudoinclusions [2]. In occasional cases, the nuclei may be large and binucleate, trinucleate and even cells with 10, or more nuclei may be encountered. The cytoplasm is generally eosinophilic and melanin is present in variable amounts. Verocay bodies are unusual. The psammomatous calcification tends to be round or oval and occasional mulberry shaped foci are present. The psammoma bodies are scattered relatively uniformly throughout the tumors. Approximately, 50% of psammomatous melanotic schwannomas may also contain areas of fat. Osseous metaplasia and thick walled vessels may also be noted. Overt cytological atypia is not seen and very occasional to rare mitotic figures are noted [2].

The differential diagnosis includes primary or metastatic melanoma, gastrointestinal stromal tumor (GIST), pigmented neuroendocrine tumor, and epithelioid leiomyoma. The absence of cytological atypia (mitoses, large inclusion-like nucleoli, hypercellularity, and foci of necrosis) militates

against a diagnosis of melanoma despite the melanoma immunohistochemical markers being positive. A psammomatous malignant melanoma has been described [7]. These authors reasoned that their lesion (which had neural-like features) was a melanoma on the basis that the lesion arose within an intradermal nevus, displayed a high mitotic rate, showed necrosis and marked pleomorphism in the deep aspects of the tumor [7]. GIST, pigmented neuroendocrine tumor, and epithelioid leiomyoma have a characteristic immunophenotype that allows distinction from a psammomatous melanotic schwannoma.

This case highlights an unusual presentation of a rare tumor, a psammomatous melanotic schwannoma presenting as incidental colonic polyps in an asymptomatic patient who lacked the stigmata of Carney's complex.

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CASE REPORT

Postmortem diagnosis of Fabry disease with acromegaly and a unique vasculopathy

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Abstract A 44-year-old Japanese man with elevated growth hormone levels and gradual deterioration of mental and renal function was admitted to the hospital. With his deteriorated general condition and renal failure, the patient developed pulmonary thromboembolism and died of respiratory failure. Autopsy examination was conducted, which revealed abnormal accumulation or intracytoplasmic storage of lipid-rich material in the small blood vessels, kidney, heart, and nervous system. After postmortem pathologic studies, including light-microscopic histochemistry, electron microscopy, and biochemical analysis of the stored lipid contents, a final diagnosis of Fabry disease was made. Histopathologic examination revealed a unique vasculopathy characterized by the presence of abnormal intracytoplasmic lipid inclusions and vascular remodeling. With regard to the clinical presentation of acromegaly, hyperplasia but not adenomatous transformation of the acidophils of the anterior pituitary gland with immunohistochemical detection of growth hormone within the cells was noted. In this case, the complication of acromegaly with hyperplasia of the acidophilic cells of the anterior pituitary gland and the unique vasculopathy causing significant organ failure, mainly of the kidney, heart, and central nervous systems, possibly as a result of microcirculatory failure, are considered to be not incidental findings but to be intimately involved in the pathogenesis of Farby disease.

Keywords Fabry disease · Pituitary · Growth hormone · Microcirculation · Ceramide trihexoside

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Introduction

Fabry disease is an X-linked recessive hereditary disorder associated with mutation of the *GLA* gene (NM-000169) [2, 14]. In early 1898, Anderson in England and Fabry in Germany separately described the disease as angiokeratoma corporis diffusum [1, 8]. In McCusik's Online Mendelian Inheritance in Man database review, Fabry disease is defined as a congenital lipid storage disease because of the deficiency of the lysosomal enzyme alpha-galactosidase (ceramide trihexosidase; EC 3.2.1.22) [6]. The deficiency of this enzyme causes accumulation of ceramide trihexoside

(trihexosylceramide) and ceramide digalctoside in various tissues of the body [24]. The common clinical phenotype of Fabry disease is characterized by cutaneous angiokeratoma, burning pain in the extremities, sensory and autonomic nervous impairment, and accelerated renal and cardiovascular failure [16, 19, 20]. With wider recognition of the disease, clinical and pathologic heterogeneities have been reported among affected individuals [3, 7, 12]. In fact, a cardiac variant of Fabry disease was proposed in individuals with severe cardiac lesions without other profound lesions. The clinical diagnosis of Fabry disease, therefore, may be difficult without a typical clinical presentation or genetic analysis.

We report a case of Fabry disease that presented with an acromegaly-like appearance and cognitive dysfunction, as well as a unique vasculopathy. The objective of the present report is to (1) emphasize the importance of autopsy for accurate diagnosis and (2) provide information on the clinical and pathologic heterogeneities of Fabry disease.

Clinical history

A 44-year-old Japanese man was found unconscious on the street. He was transferred by ambulance to the emergency room and admitted to our hospital. Clinical information about the patient was limited because the patient had been homeless for a long time. At admission, physical examination revealed generalized hypothermia (29.9°C) and loss of consciousness. Routine laboratory analyses revealed elevation of the blood urea nitrogen and serum levels of creatinine potassium, consistent with renal failure. A chest X-ray revealed cardiomegaly and a homogeneous radiopacity in the right upper lobe. After admission, his renal function and consciousness level continued to deteriorate despite aggressive treatment. When he eventually showed partial recovery, we noticed that he had severe cognitive dysfunction. In fact, his score on the Hasegawa Dementia Scale—Revised was 4 out of 30, suggestive of severe dementia. Based on the limited information, he was diagnosed to possibly have developed acromegaly when he was in his teens. Thickening of the heel pad was observed. Brain computed tomography (CT) revealed multiple cerebral infarcts in the border zone area in the right frontal lobe and showed mild enlargement of the sella tunica without any apparent pituitary mass. However, single-photon emission CT revealed diffuse cerebral hypoperfusion in the cerebral cortex as well as in the brainstem and basal ganglia. Electroencephalography revealed an occasional theta or delta burst. The serum level of growth hormone markedly elevated (16.3 ng/ml, normal value <0.42). However, the serum level of prolactin, adrenocorticotropic hormone (ACTH), free thyroxin, free T3, thyroid-stimulating hormone (TSH), and somatomedin were all normal. Four months after his first admission, the patient died of respiratory failure because of pneumonia.

Family history

His father and mother had died of esophageal cancer and myocardial infarction, respectively. His elder brother had suffered from an episode of acute renal failure and also some sort of cognitive dysfunction. He had committed suicide when he was in his 30s. His younger sister had also been recognized to have some cognitive dysfunction and had reportedly died of bronchial asthma when she was in her 20s. A living younger sister is in good health. No definitive diagnosis of Fabry disease had been made in any of the family members.

Materials and methods

Postmortem study, histology, light microscopy, and immunohistochemistry

Autopsy was carried out 14 h and 30 min after the patient's death. Briefly, after through macroscopic inspection, representative tissue specimens from several organs were obtained, fixed in 15% formalin, embedded in paraffin, and processed using conventional histological techniques. The tissue sections, except for those from the brain and spinal cord, were stained with hematoxylin and eosin (H&E); H&E and luxol fast blue (LFB) staining was performed for the sections of the brain and spinal cord. Immunohistochemical analysis of paraffin-embedded tissue sections of the pituitary gland was carried out using the Envision method (Dako, California), with or without the specified pretreatment [4]. Primary antibodies for growth hormone, TSH, follicle-stimulating hormone, luteinizing hormone, prolactin, ACTH, Kp-1 (CD68), and keratin (KL-1) were used. Frozen sections of some formalin-fixed tissue specimens, including those from the kidney and heart, were stained with Sudan Black B for the detection of lipid.

Electron microscopy

Retrieved heart and kidney fragments from formalin-fixed tissue specimens were refixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated using graded alcohol solutions, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (JEOL 1200 EXII) [11, 13].

Results

Postmortem study, histology, light microscopy, and immunohistochemistry

The pathologic findings in the present case are summarized in Table 1 (Fig. 1a–e). In regard to the external appearance, the patient was 169 cm in height and only 38 kg in body weight, possibly because of malnutrition. The heart weighed 580 g, and sections of the heart revealed evidence of left ventricular hypertrophy. The kidney revealed marked atrophy (left, 90 g; right, 100 g). Both lungs were congested (left, 510 g; right, 790 g in weight), and a pulmonary thromboembolus was found in the right S9. Some other incidental findings were found in the testes, thyroid gland, and liver (Table 1). The size and weight (0.8 g) of the pituitary gland were within normal limits. Horizontal slices of the cerebrum revealed recent infarcts in the frontal and parietal lobes. Old cystic infarcts were also observed in the same. The findings were consistent with border zone infarction in the region of supply of the anterior and middle cerebral arteries (Fig. 1b). Small infarcts were also seen in the thalamus and putamen.

The most striking feature on microscopic examination was the marked presence of lipid inclusions in various types of cells (Fig. 1a,c). The inclusions were detected, as vacuoles using H&E staining (Fig. 1a,c) showed strongly positive staining with Sudan black B, suggesting lipid deposition. In the vasculature, lipid deposits were observed in the endothelial cells, pericytes, and smooth muscle cells (Fig. 1a,c). In the nervous system, marked lipid deposition was observed in the amygdala, parahippocampus, inferior temporal gyrus (deep cortical layers), hippocampus, subiculum, and the ganglion cells of the posterior spinal cord root ganglia and celiac ganglia. Sparse intracytoplasmic deposits were also seen in the neurons of the substantia nigra and dorsal motor nucleus of the vagus. No deposits were seen in the frontal, parietal, or occipital cortex or in the cerebellum.

Numerous deposits visualized by LFB staining were visualized in the cytoplasm of the smooth muscle cells of the parenchymal and leptomeningeal. Furthermore, lipid deposits were also observed in the histiocytes in the lymph nodes, spleen, liver, bone marrow, and kidney, as also in the epithelial cells of the renal glomeruli and renal convoluted tubules. Lipid deposits were also seen in the smooth muscle cells of the bronchi and renal pelvis, and the cytoplasm of cardiomyocytes (Fig. 1a).

In the anterior lobe of the pituitary gland, the acinar architecture was relatively well preserved. However, hyperplasia of the acidophilic cells of the anterior pituitary gland with positive immunostaining of the cells using antibody raised against human growth hormone was observed (Fig. 1d,e). No lipid accumulation was seen in either the acinar cells or the vasculature of the pituitary gland.

Analysis of the accumulated lipid

A large amount of trihexosylceramide was extracted from the formalin-fixed kidney tissue. This finding was consistent with the diagnosis of Fabry disease [23, 24].

Table 1 Summary of autopsy findings

Findings
1. Fabry disease
Generalized intracytoplasmic accumulation of lipid (trihexosylceramide) in the:
(a) myocardium with cardiac hypertrophy (heart weight, 580 g),
(b) endothelial cells, pericytes, and smooth muscle cells of the body vasculature with multiple small brain infarcts (brain weight, 1425 g),
(c) neurons and ganglion cells in the posterior spinal roots, celiac ganglion, hippocampus, and striatum nigrum,
(d) histiocytes in the lymph nodes, spleen, liver, bone marrow, and kidney,
(e) epithelial cells in the renal glomeruli, renal convoluted tubules, with marked atrophy of kidney (kidney weight: left, 90 g, right, 100 g),
(f) smooth muscle cells in the bronchi and renal pelvis.
2. Acromegaly-like condition
High serum growth hormone level: 16.3 ng/ml (normal level, 0.42 ng/ml), thickened heel pad, hyperplasia of the anterior pituitary gland acidophilic cells containing human growth hormone as confirmed by immunohistochemistry, although the size and weight of the gland were within normal limits (pituitary gland weight, 0.8 g)
3. Pulmonary congestion and edema with pulmonary thromboembolism and pulmonary infarction in the lower lobe (lung weight: left, 510 g; right, 790 g; pleural effusion: left, 20 ml; right, 300 ml)
4. Atrophy of the testis (testis weight: left, 16.3 g; right, 13.8 g)
5. Thyroidal follicular adenoma (small)
6. Hepatic cavernous hemangioma (small)

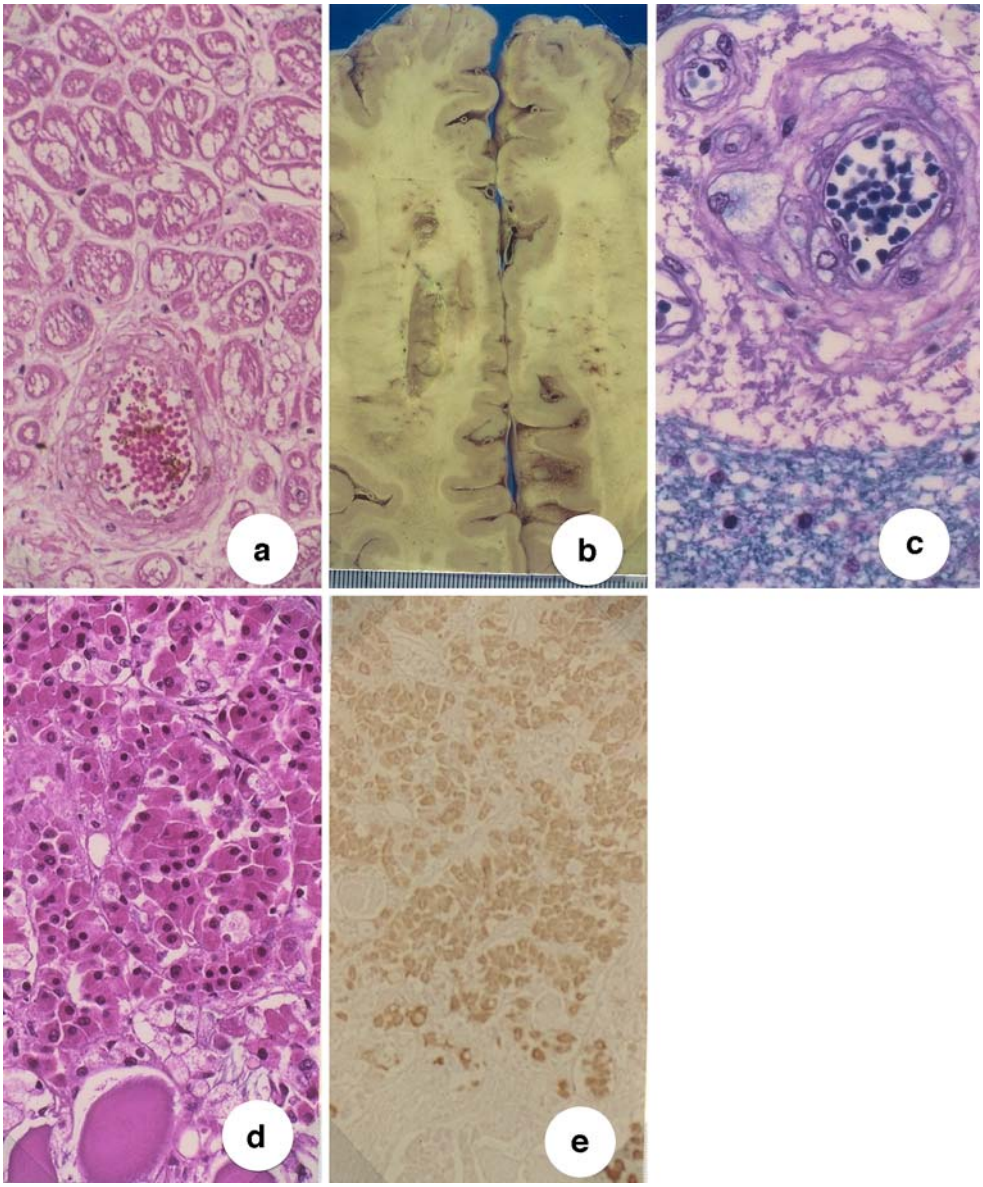

Fig. 1 Photomicrographs of gross and microscopic appearances from several organs. **a** Histological sections of the myocardium. Lipid-rich contents could be extracted during processing from each of the striated cardiac muscle cells showing intracytoplasmic vacuole. Congested vessels with small vacuoles in the endothelial and medial smooth muscle cells are observed. H&E stain, 190×. **b** Macroscopic view of a horizontal (parallel to the computer tomographic scan axis) section of the brain. Many small infarct foci with *gray color* and softening are seen on the cut surface. **c** Histological section of the Virchow Robin space in the brain cortex. Many small vacuoles are seen in the vascular constituent cells. Narrowing of the vascular lumen by the swollen endothelial cells and thickened medial layer and aggregated blood cells. H&E–LFB stain, 190×. **d** Histological section of the anterior (upper part in the figure) and middle part with small Rathke's cysts of the pituitary gland. Predominant acidophil cell proliferation with maintenance of the acinar architecture is; no proliferation of chromophobe or basophilic cells is noted. H&E stain, 190×. **e** Anterior pituitary gland histological and consecutive section used for **d**. Many alveolar cells showed immunopositivity for antibody raised against the human growth hormone. 190×

Electron microscopy of the heart and kidney tissue specimens

In the myocardial tissue specimens, numerous myelinosome like-laminated membranous cytoplasmic bodies or zebra bodies with laminated alternate electron-dense and lucent layers were seen in the cytoplasm of the cardiomyocytes, as well as in the endothelial cells, pericytes, and smooth muscle cells in the vascular wall (Fig. 2).

Furthermore, in the kidney tissue specimens, many myelinosome-like-laminated membranous cytoplasmic bodies or zebra bodies with laminated alternate electron-dense and lucent layers were seen in the cytoplasm of the renal tubular epithelial cells, stromal fibroblasts, macrophages, endothelial cells of the vasculature, and smooth muscle cells in the vascular wall (Fig. 3). Feathery structures with condensed electron-dense layers were seen in some cells in obliterated renal convoluted tubules.

In both the cardiac and renal tissue specimens, small vasculature revealed stenosis or obstruction, possibly because of massive accumulation of intracytoplasmic inclusion, membranous cytoplasmic bodies, or zebra bodies in the endothelial cells (Figs. 2 and 3). Occasionally, liberated free membranous cytoplasmic bodies or zebra bodies were found floating in the vascular lumen, with some aggregation of blood cells (Figs. 2 and 3).

Discussion

We present a case of Fabry disease presenting with an acromegaly-like condition and cognitive dysfunction. A

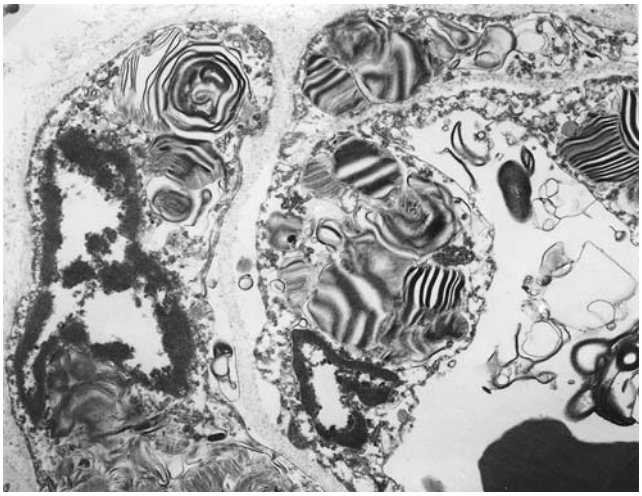

Fig. 2 Electron micrographs of myocardial tissue. High-power view of a small blood vessel of possibly capillary type shown. Many small laminated bodies, myelinosome-like structures, and zebra bodies are scattered in the swollen cytoplasm of the endothelial cells and a pericyte. Note in the lumen, similar small laminated bodies and myelinosome-like structures intermingling with the aggregated red blood cells are seen. 18,000×

unique vasculopathy was observed in several organs. According to the results of autopsy, the patient died of several critical conditions, including severe renal failure, respiratory failure because of pneumonia, pulmonary congestion, and anasarca.

In general, glycosphingolipid accumulation is observed in the endothelial and smooth muscle cells of the body vasculature, cardiomyocytes, epithelial cells of the glomeruli, renal interstitial cells, renal tubular epithelial cells, sweat glands, nervous system (neurons, astrocytes, and ganglion cells of the autonomic nervous system), and cornea [15, 25]. Circulatory disturbance resulting from the lesions in the vasculature is the most common finding in cases of Fabry disease. Vasculopathy is especially frequently reported in the kidney and nervous system, and even vascular dementia has been reported in Fabry disease [15]. Postmortem pathological findings of severe or unique vasculopathy in Fabry disease were recently emphasized again and published by Schiffmann et al. [21] and Schiffmann [22]. Their case with enzyme replacement therapy (ERT; such as intravenous infusions of alpha-galactosidasse A) has been shown to markedly reduce the cellular storage or lysosomal inclusions in vascular endothelial cells [21, 22]. Presently, our ultrastructural study exactly showed similar small structures intermingling with the aggregated red blood cells in the vascular lumen and vascular remodeling. Diagnosis of Fabry disease in the patients' early life and additional or detailed further investigation with clinical attempt of ERT [21, 22] are necessary for their improvement of prognosis.

The living condition of the patient with no immediate relations and the severe cognitive dysfunction would have made the potential clinical diagnosis of Fabry disease extremely difficult in the present case. Recent studies have indicated that some patients may exhibit only the cardiac or renal clinical phenotype of Fabry disease without the typical clinical features [3, 7, 12]. In such cases, it may be difficult to make an accurate diagnosis. In the present case, little clinical information about the patient was available because he lived as a vagrant. He lacked the typical angiokeratoma, severe burning pain in the extremities, and autonomic disturbances. However, careful analyses of the histological sections immediately raised the possibility of some lipid storage disease. In particular, we suspected the diagnosis of Fabry disease in the present case based on the observation of lipid-rich intracytoplasmic inclusions in the heart, kidney, and central nervous system. Further analyses of the accumulated lipid (trihexosylceramide) and electron microscopy of the kidney and myocardial tissue specimens confirmed the diagnosis. This case report thus serves to emphasize the importance of histopathologic examination to confirm the diagnosis in suspected cases.

The present case was diagnosed as having developed acromegaly in his teens. Thickenings of the heel pad and elevated serum growth hormone levels were observed. However, no pituitary adenoma was detected at the time of autopsy. In rare instances, an acromegaly-like condition has been reported without follow-up detailed postmortem examination [5, 9, 10, 26].

In the pituitary gland, some proliferation of acidophilic cells contained the growth hormone, as detected by immunohistochemistry. Histologically, the acinar architecture of the proliferated anterior pituitary gland cells was

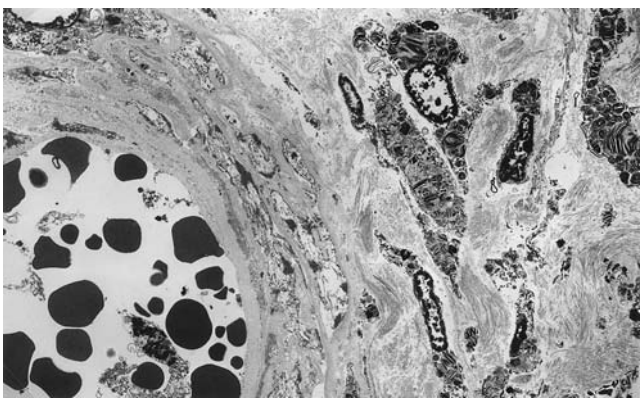

Fig. 3 Electron micrographs of the kidney tissue. Low-power view of the interstitial or stromal tissue of the kidney. Many small laminated bodies, myelinosome-like structures, and zebra bodies are scattered in the cytoplasm of the stromal fibroblastic cells and histiocytes. Similar structures are seen in the medial cells and endothelial cells of the small blood vessels. In the lumen, similar small structures are seen intermingling with the aggregated red blood cells. 3,500×

preserved. Lipid accumulation and the characteristic vasculopathy were inconspicuous in the pituitary gland. The profile of this proliferated anterior pituitary gland cells are suggestive of not adenoma but hyperplasia. Furthermore, the histological characteristics were also suggestive of hyperplasia rather than adenoma or neoplasia. An acromegaly-like condition is extremely rare in Fabry disease in which growth hormone–releasing hormone-producing tumors have not been reported [9, 26]. We speculate that the acromegaly-like condition in our present case was associated with some alterations of the growth hormone regulation pathway and/or vasculopathy in the pituitary gland–hypothalamus axis or in the target organs of growth hormone action (striated muscle, bone, etc.). In fact, the testicular atrophy and thyroidal follicular adenoma observed in our case may also be related to this speculative pathogenic mechanism [17]. Because endocrine abnormalities are considered to be rare, further study may be warranted to investigate the endocrine abnormalities in Fabry disease.

In the present case, direct accumulation of lipids in certain types of cells may be expected to have caused functional deterioration of the cells. A pervious report emphasized the mechanism of luminal narrowing of the blood vessels as being related to the deposition of glycosphingolipids in the vessel walls [15]. In the ultrastructural examination in our study, we found not only intraendothelial deposition of materials (zebra body, myelinosomes, laminated materials—storage material of lipid–sugar compound) but also breakdown materials in the vascular lumen. Noninvasive examination has also suggested endothelial dysfunction in cases of Fabry disease [18]. These pathologic abnormalities might predispose to thromboembolism and stenosis or occlusion of the vascular lumen and ultimately cause failure of the microcirculation in the kidney, heart, and central nervous systems.

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CASE REPORT

Basaloid adenocarcinoma. A new variant of pulmonary adenocarcinoma

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Abstract The 2004 WHO classification of lung tumours recognised basaloid carcinoma as a variant of squamous and large cell carcinoma. We report a unique case of primary pulmonary adenocarcinoma with a basaloid component. An 82-year-old man underwent pulmonary lobectomy for a 2.8 cm tumour. The patient is disease-free 13 months after diagnosis. Histologically, an invasive carcinoma having a glandular and a solid component was observed. The former was an adenocarcinoma with mucus containing spaces lined by columnar mucinous cells and basaloid cells. The solid component was an organoid proliferation of basaloid-type cells, as in cutaneous basal cell carcinoma. Basaloid cells, but not mucinous cells, were immunoreactive for high molecular weight cytokeratins (CK), CK 7 and, focally, for TTF-1. High Ki67 index, p53 and EGFR expression were also found. This tumour is unique in several respects: (1) The solid areas resemble a conventional basaloid carcinoma, except for the presence of small mucin-containing spaces. (2) The mucinous adenocarcinoma areas contain two layers of columnar and basaloid cells. (3) Both components are neoplastic based on cell morphology, invasive properties and phenotypic profile. These findings indicate that a basaloid variant of adenocarcinoma is also existing in the spectrum of basaloid carcinomas of the lung.

Keywords Lung · Adenocarcinoma · Basaloid · Mucinous · New variant

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Introduction

Basaloid carcinoma of the lung (BCL) has originally been described by Brambilla et al. [3] as a peculiar type of primary pulmonary neoplasia with a striking morphological resemblance to the more common basal cell carcinomas of the skin, upper aerodigestive tract and anal canal [1, 6]. Although a minority of the originally described cases (3/38) had focal signs of glandular differentiation within the classical solid or lobulated patterns of growth, BCL was regarded as a variant of squamous or of undifferentiated large cell carcinomas of the lung, and as such this tumour type was classified in the recent WHO classification of lung tumours [18]. In this scheme, BCL is listed in both categories of squamous and large cell carcinomas [18]. The described cases with focal glandular differentiation had morphological similarities with adenoid cystic carcinoma, although the pattern of growth was largely solid and small acinar formations were barely detectable in the

solid lobules. In addition, ultrastructural evidence of microvilli formation in the cell surface facing acinar lumina was also provided [3].

The existence of pulmonary and extra-pulmonary tumours with combined (mixed) features of the squamous and glandular lineages is well known, including for example adenosquamous carcinoma, mucoepidermoid carcinoma, mixed squamous carcinoma and adenocarcinoma of the lung [18]. In all such cases, two distinct components are generally growing one aside the other (collision tumour) or intermingled one into the other. However, no case has so far been described in the English literature of a primary pulmonary tumour combining basaloid features with any known type of adenocarcinoma [7, 18]. A single case of such an association was reported in the parotid gland under the heading of basal cell adenocarcinoma [9] and another apparently similar one (though with myoepithelial differentiation of the basaloid cells) was recently described in minor salivary glands [20].

We report a unique case of mucinous adenocarcinoma of the lung having an associated component of BCL, both in the classical form of solid, lobulated growth pattern and—within the glandular component—as layers of neoplastic basaloid cells, lining the dilated mucinous glands externally to the columnar cells. The term basaloid adenocarcinoma of the lung is proposed for this new variant.

Clinical history

An 82-year-old man, heavy smoker (20 cigarettes per day from age 20 to 68), was admitted to the Division of Thoracic Surgery at the University of Turin at San Luigi Hospital in January 2006 due to cough and thoracic pain. He had worked for 40 years as a clerk in a textile industry and is now retired. In the medical history, the patient received surgery for varicocele (1958), sigmoid diverticulosis (1991) and renal cyst (2002). The chest X-rays revealed a pulmonary sub-pleural opacity; subsequently, a chest CT scan confirmed the presence of a 2.8 cm mass in the sub-pleural location of the left lower pulmonary lobe. No mediastinal lymph node involvement was detected at CT scan and major bronchoscopy was negative for endobronchial lesions. The patient underwent pulmonary lobectomy and was discharged 10 days later. No additional post-operative treatment was performed in consideration of the T1N0 surgical stage of the tumour and the age of the patient. After 13 months, the patient is alive and completely disease-free.

Materials and methods

The surgical specimen was fixed in 10% buffered formaldehyde and representative tumour tissue samples embedded in paraffin. Serial 4 μ thick sections were stained with a standard hematoxylin and eosin for conventional diagnostic purposes, and with Alcian blue and periodic acid-Schiff (PAS) reaction (with and without diastase pre-treatments) to reveal mucins. Parallel sections were also used for immunohistochemistry, employing a biotin-free detection system (En-Vision, DakoCytomation, Glostrup, Denmark), after appropriate antigen retrieval procedures based on microwave oven heating in citrate or EDTA buffer solutions. The primary antibodies used, their type and/or clones, working dilutions and sources are listed in Table 1. Appropriate positive and negative controls were introduced for each immunoperoxidase stain.

Fluorescent in situ hybridisation (FISH) for epidermal growth factor receptor (*EGFR*) gene was performed on serial sections of the tumour, which were de-paraffinised, re-hydrated, pre-treated with 0.2 N HCl and then sodium thiocyanate at 80°C for 30 min, and finally incubated with pepsin (25 mg/ml) at 37°C for 10 min (pre-treatment kit, Vysis, Abbott Molecular, IL, USA). After rinsing in 2X SSC (at room temperature for 5 min) and dehydration, the Spectrum Orange LSI EGFR/Spectrum Green CEP7 probe (Vysis) was applied and the hybridisation performed after sealing the coverslip with rubber cement. The slides were incubated at 80°C for 10 min for co-denaturation of chromosomal and probe DNA and were then placed in a humidified chamber at 37°C for 18 h. Then the rubber cement seal was stripped off, the coverslip gently removed and the slides immediately incubated in 2X SSC/0.3% NP-40 buffer at 73°C for 2 min. After rapid washing in 2X SSC at room temperature and air drying in darkness, the section was counterstained with DAPI (4′,6′-diamidino-2-phenylindole), and the slides examined under an Oympus BX41 microscope, equipped with single and dual bandpass filters for spectrum orange, spectrum green and DAPI, and with ×40 and ×100 oil objectives. The procedure and result interpretation followed published protocols [5, 19].

Results

Grossly, a firm peripheral nodule with pleural fibrotic reaction and retraction was detected. On cut surface, a well-demarcated solid grey node was found measuring 2.7×2 cm with colloid areas in the absence of macroscopically evident necrosis or haemorrhage. Histologically, the carcinoma had a double component with totally different patterns, a glandular one and solid/lobulated one, which merged one into the other. Both components were non-capsulated and had an irregular infiltrative margin into the pulmonary parenchyma with a dense fibrotic reaction around the neoplastic islands.

The glandular component represented approximately 60% of the tumour area and superficially resembled

Table 1 Immunoprofile of the two different components of basaloid adenocarcinoma

Marker	Clone	Dilution	Source	Basaloid area	Glandular area
panCK	KL1	1/50	Immunotech	+	+
HMWCK	34BE12	1/100	DakoCytomation	+	+ basal cells only
CK5	D5/16 B8	1/50	DakoCytomation	+	+ basal cells only
CK7	LP5K	1/100	Neomarkers	++	+ single mucin cells
CK20	ks20.8	1/100	Neomarkers	–	± single mucin cells
MUC-1	Ma695	1/300	Medac	++	+ basal cells only
TTF-1	8G7G3/1	1/100	Zymed	+ focal	–
Surf	SPB01	1/50	Neomarkers	–	–
CgA	LK2H10	1/1,000	Neomarkers	–	–
SYN	SY38	1/100	DakoCytomation	–	–
CD56	1B6	1/150	Novocastra	–	–
BSP	polyclonal	1/1,000	Chemicon	–	–
OPN	Mab1061	1/1,000	Chemicon	–	–
CatK	CK4	1/100	Novocastra	– (stroma +)	– (stroma +)
VEGF	polyclonal	1/250	Neomarkers	–	+ focal, mucin cells
EGFR	111.6	1/50	Neomarkers	++	+ basal cells only
p53	DO7	1/400	DakoCytomation	++ (90%)	+ (60% mucin cells) ++ (90% basal cells)
Ki67	MIB-1	1/300	DakoCytomation	65%	<5% mucin cell layer 20% basal cell layer

CK: cytokeratin, *panCK*: wide spectrum CK, *HMWCK*: high molecular weight CK (types 1, 5, 10, 14), *surf*: surfactant B, *CgA*: chromogranin A, *SYN*: synaptophysin, *BSP*: bone sialo-protein, *OPN*: osteopontin, *catK*: cathepsin K.
Sources: Immunotech: Marseille, France; DakoCytomation: Glostrup, Denmark; Neomarkers: Neomarkers/LabVision, Fremont CA,USA; Medac: Wedel, Germany; Zymed: San Francisco, USA; Novocastra: Newcastle, UK; Chemicon: Temecula, CA.

classical acinar adenocarcinomas. Glandular spaces of different sizes, sometimes enlarged as a result of gland confluence with abundant, strongly PAS stain positive, mucus in the lumen were observed (Fig. 1, top right). Well-differentiated, polarised, columnar mucinous cells lined the tubular or micro-cystic structures. In some glands, the mucinous cell layer was interrupted and/or the mucinous cells were rather cuboidal or roundish. Occasional signet ring-shaped cells abutting the luminal prospect of the glands were also present. Such mucinous glands infiltrated the pulmonary parenchyma and residual bronchial and alveolar spaces were traceable within the tumour entrapped in a moderate fibrotic reaction made of lymphocyte-rich dense collagen. At a closer view, however, all neoplastic glands were basally lined by apparent basaloid type cells, arranged in two to four layers within the basal membrane and beneath the columnar mucinous cell layer (Fig. 1e). These cells were polygonal or roundish with a hyper-chromatic nucleus of larger size than that of the columnar cells and—above all—markedly more irregular in shape (Fig. 1f–h). These cells were all similar to those growing in the solid/organoid component (see below). In such basaloid cell layer, some mitoses were present, as opposed to the columnar mucinous cells in which no mitotic figure has been identified.

The second component represented approximately 40% of the tumour area and, at low power, had the lobulated organoid growth pattern typical of basaloid carcinoma of the skin and other locations (Fig. 1, top left and right). Variously confluent islands with peripheral palisading or, more rarely, cords of poorly differentiated, medium size cells with hyper-chromatic nuclei, no visible nucleoli and scant cytoplasm were present throughout in this part of the tumour (Fig. 1a,b). In addition, part of the neoplastic islands had a cribriform appearance, as a result of small cystic or micro-glandular space formations, generally towards the center of the neoplastic nest or lobule. These spaces were irregularly lined by PAS-positive mucinous columnar cells and contained rare histiocytes (Fig. 1c,d), suggesting the possibility that some of these neoplastic nests were glandular spaces overgrown and almost totally replaced by a basaloid cell population. Numerous mitoses were observed in the solid and cribriform component, including occasional atypical mitoses. More or less extensive foci of necrosis, especially at the center of solid neoplastic lobules, were present.

In the whole tumour, a dense collagen reaction with lymphocytic infiltration, areas of hyalinosis and irregularly shaped calcifications was observed. The neoplasia grew contiguous to the visceral pleura, but did not infiltrate the surface. No signs of angioinvasion or infiltration of the bronchial surgical margins were observed. Hilar and mediastinal lymph nodes were free of metastases, and the tumour was staged pT1-N0. The surrounding non-neoplastic

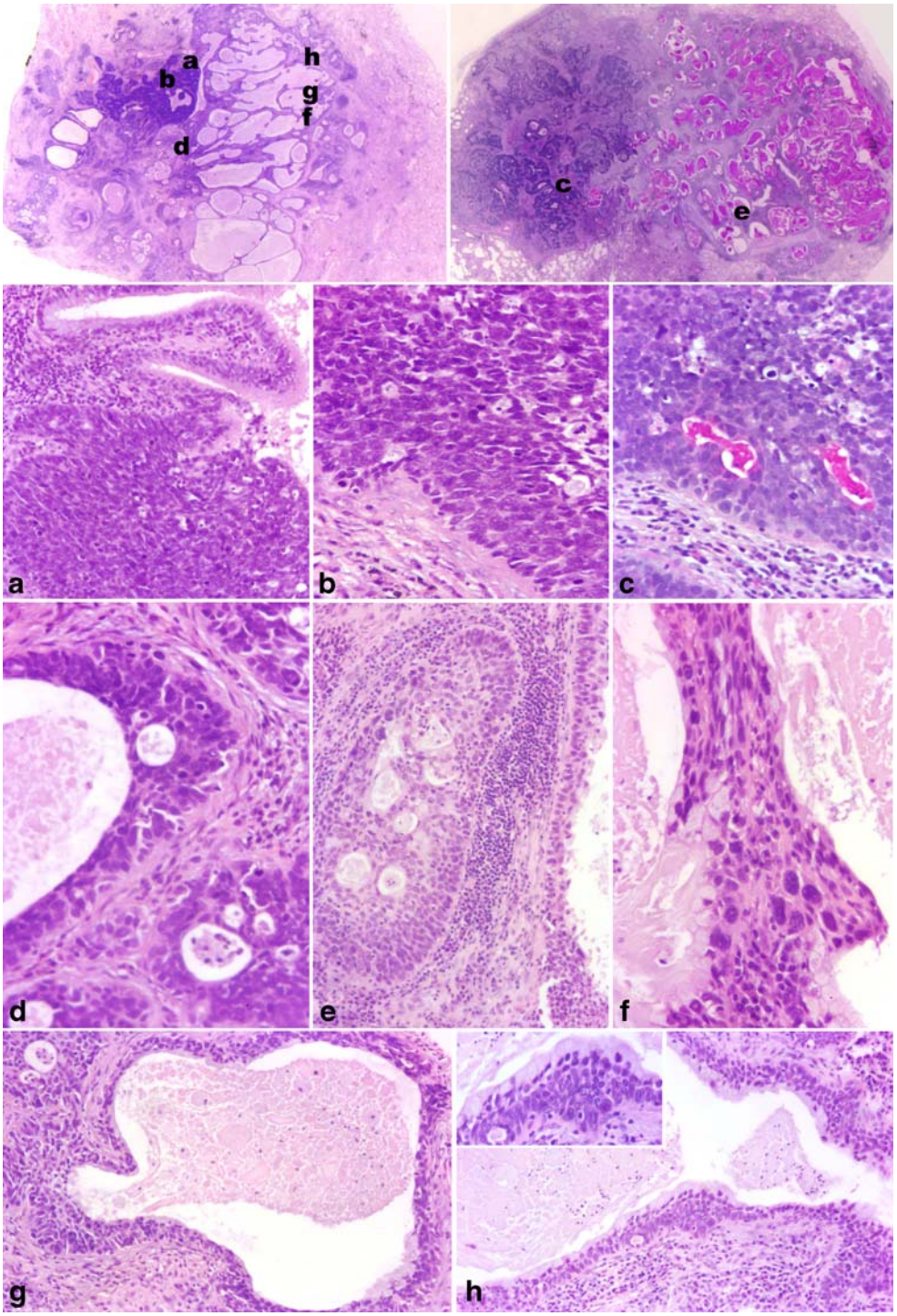

Fig. 1 Basaloid adenocarcinoma of the lung. Whole mounts of the tumour stained with hematoxylin and eosin or with PAS stain (top figures). In both sections, from left to right, a transition from a basaloid component towards a classical mucinous (cysto)adenocarcinoma component is observed. The various patterns of growth are shown in pictures *a* to *h*, and the original location of the representative fields are indicated in the whole mounts by the same letter (**a**–**h**). The basaloid carcinoma ulcerates the bronchial mucosa (**a**) and solid islands with peripheral palisading infiltrate the lung parenchyma causing a fibrotic reaction (**b**). In some areas, small acinar spaces containing PAS-positive material are recognised within the basaloid islands (**c**). Elsewhere, the spaces are dilated and lined by mucinous cells (**d**, **e**). A double component of mucinous and basaloid cells is observed along glandular and mucus-containing cystic spaces (**g**–**h**). The cells of the basaloid layers are larger and more atypical than the well-differentiated polarised mucinous cells (**f**). (**a**, **b**, **d**–**h** hematoxylin and eosin; **c** PAS stain)

pulmonary parenchyma had signs of emphysema and mild respiratory bronchiolitis, probably related to tobacco smoke.

The immunophenotypic profile had some differences between the two components and was consistent with the features of basaloid carcinoma on the one side and of mucinous carcinoma of the lung on the other. Both components were strongly reactive for wide spectrum cytokeratin (CK). The basaloid component also strongly expressed high molecular weight CKs (nos. 1, 5, 10, 14) (Fig. 2a) with this marker also present in the basal cell layer of the glandular component, but absent in the mucinous columnar cell layer (Fig. 2e,f). CK 7 (but not CK 20) was strongly expressed in the basaloid component (Fig. 2b), but in scattered mucinous cells only of the glandular component. Scattered CK 20 reactive cells were seen in the mucinous component. TTF-1 was only focally expressed in

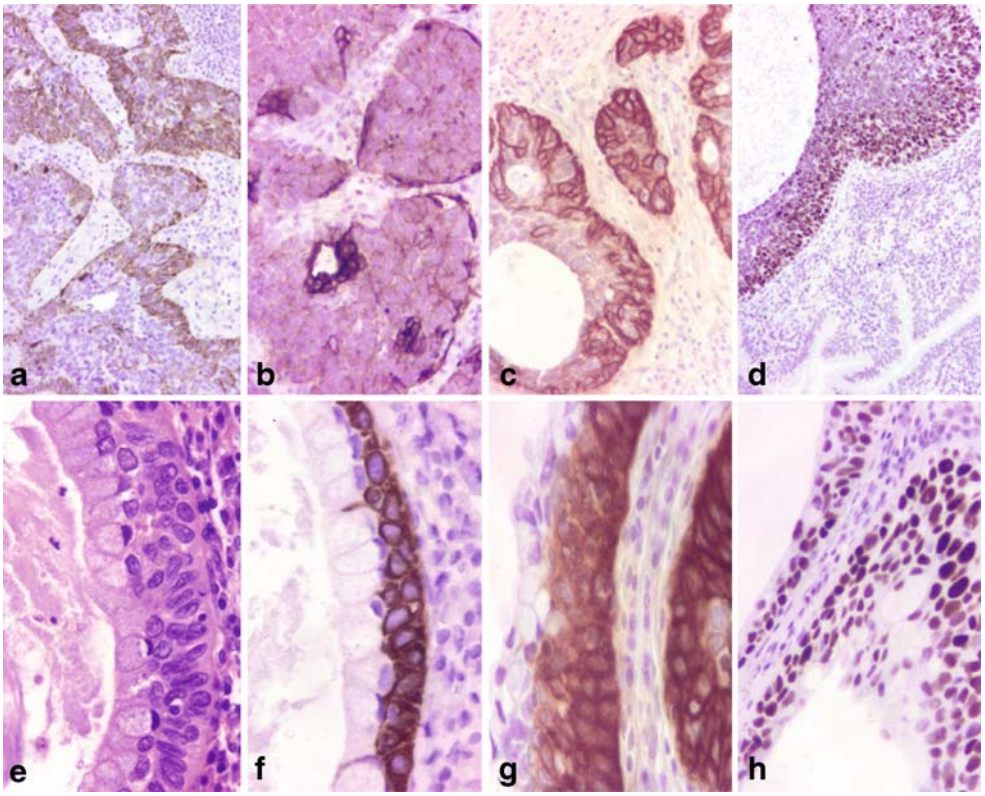

Fig. 2 Basaloid adenocarcinoma of the lung. Immunoprofile of basaloid (**a**–**d**) and glandular (**e**–**h**) components. High molecular weight cytokeratins (**a**) and cytokeratin 7 (**b**) are strongly positive in most neoplastic cells of the basaloid areas. The same cells also express EGF receptor at the cell membrane level (**c**) and most nuclei are positive for p53 (**d**). The glandular component shows a peculiar double layer of inner well-differentiated mucinous cells and of outer basaloid cells (**e**). Only the latter react for high molecular weight cytokeratins (**f**) and EGF receptor (**g**). Conversely, p53 is expressed in the nuclei of both cell types, although the mucinous cells are less intensely stained than the basaloid cells (**h**). (**a**–**d** and **f**–**h** immunoperoxidase stain; **e** hematoxylin and eosin)

the basaloid component and did not stain any mucinous cell. No reactivity for surfactant protein was observed. MUC-1 expression was restricted to the basaloid cells, but absent in the mucinous glands. The neuroendocrine markers chromogranin A, CD56 and synaptophysin were negative. Molecules implicated in invasion and metastatic processes, such as bone sialoprotein, osteopontin and cathepsin K were negative in both neoplastic components. Stromal reactivity for cathepsin K was found, as recently described in the fibrotic stromal tissue of invasive pulmonary adenocarcinoma [15].

Vascular endothelial growth factor (VEGF) was unreactive in the basaloid cells, weakly expressed in mucinous columnar cell and in the intra-tumoural vascular endothelia. Epidermal growth factor (EGF) receptor was strongly positive in peripheral areas of the basaloid carcinoma component (Fig. 2c) and in the basal cell layer of the glandular areas, being mucinous cells completely unreactive (Fig. 2g). Conversely, p53 expression was found in both tumour cell populations, although the glandular cells were weakly expressing p53 in 60% of nuclei (Fig. 2h), as opposed to a strong and diffuse immunoreactivity in most basaloid cell nuclei (Fig. 2d,h). A high proliferative rate was detected by means of Ki67 immunostaining in the basaloid component with approximately 65% of nuclei undergoing cell division, as opposed to the adenocarcinoma component in which the columnar mucinous cells had less than 5% and the basal cell layers approximately 20% of proliferating cells, respectively. FISH analysis of the *EGFR* gene was negative for amplifications and polysomies in both components. It is interesting to note that a low trisomy, according to the definition of Cappuzzo et al. [5] for chromosome 7, was observed in both neoplastic components.

Discussion

In this report, we described the pathological features of a unique primary non-small cell pulmonary carcinoma characterised by basaloid cell growth in both a typical basaloid carcinoma area and within a mucinous adenocarcinoma component having two layers of basal and mucinous columnar cells lining enlarged mucin-laden spaces. We propose the term “basaloid adenocarcinoma” for this particular tumour type.

The tumour is unique in several respects: (1) The solid areas look like a basaloid carcinoma (of the skin or of the lung), except for some cribriform appearance due to the presence of small acinar mucin-containing spaces, in the absence of keratinised squamous cells. (2) The glandular areas represent a well-differentiated mucinous adenocarcinoma, as commonly observed in various locations with columnar mucinous cells lining dilated acinar and micro-cystic formations. (3) In such glandular component, layers of basaloid cells similar to those

described in the basaloid carcinoma component, grow external to the mucinous cell layer and within the basal membrane. (4) Both components are neoplastic based on the cell morphology, invasive properties and the p53/Ki67 profile. These points will be briefly commented on.

Firstly, the basaloid component had an architecture, which at low power was strikingly similar to the classical basaloid carcinoma described as a variant of squamous or large cell lung cancer [18]. Roundish or irregular lobules of basaloid cells having a peripheral palisading were present throughout with an invasive border into the pulmonary parenchyma and immunophenotype described for basaloid carcinoma (including strong expression of high molecular weight cytokeratins). In classical basaloid sub-types of squamous and large cell carcinomas of the lung, the presence or absence of squamous keratinised cells has been indicated as a diagnostic criterion for entering the tumour in one of these sub-types, respectively. No mention is made in the WHO classification to the extent of basaloid differentiation (vs squamous component) required to qualify a tumour as basaloid carcinoma, although in the original report [3] half cases were not pure basaloid carcinomas and the basaloid areas accounted for at least 60% of the tumour. It can be assumed that, in general, basaloid carcinomas have a predominant basaloid growth pattern. The present case was not characterised by the predominance of basaloid features (present in less than half of the tumour area). However, being basaloid cells recognised either in the classical lobulated/solid growth pattern or in a basaloid layer beneath the adenocarcinoma component, it seemed to us that such basaloid component deserved mention in the pathology report. Being relatively extensive, the term basaloid adenocarcinoma was favoured over adenocarcinoma with basaloid differentiation, although this is a largely semantic issue in our opinion. At variance with classical basaloid carcinoma, no squamous keratinised cells were observed and small acinar mucus-containing spaces were present within the basaloid islands, a pattern somewhat similar to that described in Fig. 4 of the original paper of Brambilla et al. on basaloid carcinoma of the lung [3]. These acini contained small amounts of mucins and glandular cells, which were overgrown by the basal cells. This pattern may superficially resemble an adenoid cystic carcinoma (see below). A similar condition was reported in rare basaloid carcinomas of the skin in which focal signs of "duct differentiation" (probably of the sweat gland type) were detected at the center of basaloid islands [10]. No areas of squamous differentiation with keratinisation were traced throughout the tumour, thus excluding that the present tumour was a basaloid variant of squamous carcinoma (code 8083/3 of the WHO classification) [18]. The presence of a well-differentiated adenocarcinoma component also excluded the alternative diagnosis of basaloid variant of large cell carcinoma (code 8123/3 of the WHO classification) [18].

In addition, the basaloid component merged with the glandular component, which showed dual differentiation along mucinous and basaloid cell lineages, both lining the enlarged mucin spaces. The mucinous cells had the typical appearance of mucinous adenocarcinomas as commonly observed in colorectal, ovarian or pancreatic locations and, more rarely, in the lung. The morphological features and the immunoprofile (focal expression of CK 7 and CK 20 and absence of TTF-1) were consistent with those reported in cases of primary pulmonary mucinous adenocarcinoma [16]. External to the mucinous cell layer, a growth of basaloid cells all similar to those described in the basaloid carcinoma component, was regularly represented. These cells were arranged in a few layers apparently confined within the basal membrane, in a pattern superficially resembling the pagetoid spread of breast cancer cells along mammary ducts or the hyper-plastic basal cells of enlarged prostatic ducts [14], except for the fact that such pattern of growth developed in the context of an invasive adenocarcinoma. With regard to the invasive component, a stromal reaction was observed with dense collagen, lymphocytic infiltration, hyalinosis and calcifications. These modifications of the stroma were mentioned, including calcifications [3].

Finally, both cell types had the features of malignant cells. Columnar cells generally maintained the cell polarity and had hyper-chromatic nuclei with minimal atypias. Basaloid cell in both areas had enlarged hyper-chromatic nuclei with occasional highly atypical nuclei and numerous mitoses. The possibility of a hyper-plastic nature of the basaloid cells lining the neoplastic glands was excluded based on the morphology of such cells, the high proliferative index and strong expression of p53. The tumour had an elevated Ki67 proliferative index (65%) in the basaloid component, only. Conversely, p53 was strongly expressed in both cell types and EGFR immunoreactivity was detected in basaloid cells of both component, but not in the mucinous columnar cells. It is interesting to note that the FISH analysis of *EGFR* gene did not reveal any amplification or polysomy, but in both neoplastic components, a low trisomy of chromosome 7 (as defined by Cappuzzo et al.) [5] was observed, suggesting a common genetic background of the different cell populations represented in this tumour.

Despite the presence of worrisome prognostic factors, after radical surgery and in the absence of any additional treatment, the patient is currently free of disease 13 months after the diagnosis. The follow-up is too short for this T1N0 surgically resected tumour to draw any prediction on the intrinsic aggressivity of the disease because 80% of stage IA surgically treated are alive at 5 years and basaloid median survival is about 20 months.

The tumour described in this study may well be the result of a collision between two independent carcinomas of the lung with one component growing into the other. Incidentally, if this were the case, in any event, we would face a currently un-described tumour type ("mixed or combined basaloid carcinoma"?). Although a definite proof of the collision vs divergent differentiation origin of this neoplasm is definitely lacking, there are arguments favouring both hypotheses. The two components of the tumour seem, in part, separate in the low power view of the tumour and merged in the central area. The transition zone shows neoplastic islands containing mucinous cells, which give the impression of being externally overgrown by neoplastic basaloid cells. Such cells have more marked atypias and a higher proliferative potential than the bland-looking tumour cells of the mucinous component. Although all the above features would favour the hypothesis of a collision tumour, other characteristics support the possibility of a divergent differentiation within an adenocarcinoma. These include the fact that basal, reserve cells also exist in the glandular epithelium, and the basaloid tumour cells may be related to these glandular cells, also taking into account the arrangement of basaloid cells along the mucinous cell layer. Such growth pattern along the basal membrane in the glandular component seems to us hard to be interpreted as a merging of two different tumours: the invasive properties of either component would probably have lead to a more irregular infiltration of tumour and stromal areas rather than to an ordered basally located basaloid cell arrangement, recalling the "pagetoid spread-type" of breast cancer. Finally, the presence of single mucin-containing cells in the classical basaloid component, which was already shown in initial basaloid cases description [3], and the similar molecular profile in terms of p53 expression and low trisomy of chromosome 7 (observed in both neoplastic cell populations) better support the hypothesis that the whole tumour is a basaloid variant of adenocarcinoma.

The unique features of this tumour initially raised the suspicion of a metastatic tumour. In fact, no similar tumour had so far been described in the lung. Basaloid variant of squamous or large cell carcinoma was excluded for the above reasons. The described basaloid pattern rather recalled a well-differentiated neuroendocrine tumour [2] and, indeed, the possibility of a combined adenocarcinoma–large cell neuroendocrine carcinoma was originally suggested by morphology alone. Obviously, the absence of neuroendocrine markers (chromogranin A, synaptophysin etc.) immediately excluded this hypothesis. Finally, adenoid cystic carcinoma can be suggested due to the presence of small acinar and cyst formations associated to a more solid growth pattern [2]. The differential diagnosis may be difficult and require immunohistochemistry [8]. The structure of classical adenoid cystic carcinoma, as described for example in the esophagus [12], larynx, uterine cervix [17], salivary glands, mammary gland [4] and prostate [11, 13], and of cloacogenic carcinomas of anal canal [5], is however not exactly overlapping with that of the current tumour, especially with regard to the double-layered glandular spaces lined by mucinous and basaloid cells. Among other possible differential diagnoses, combined squamous and glandular tumours are to be considered; in various organs, these have been described as mucoepidermoid or adenosquamous carcinomas [1, 18]. The currently known tumours of this type typically show a squamous (keratinised) component rather than a pure basaloid cell component, as seen in the present tumour. In addition, so far, published adenosquamous carcinomas are characterised by a mixture of glandular and keratinised cells that either line the lumen of glandular spaces both abutting to the lumen or grow admixed in solid nests. This is not the case of the currently reported tumour in which the adenocarcinoma component has a clear-cut double layer of inner columnar mucinous cells facing the mucin-laden lumen and of outer basaloid-type cells, stratified at the periphery rather than facing the lumen. We are not aware of such a pattern of growth in other adenocarcinomas.

In conclusion, we support the fact that a basaloid variant of pulmonary adenocarcinoma is also existing, thus expanding the currently known spectrum of basaloid carcinomas of the lung. This variant is characterised by the concurrent presence of clear-cut glandular areas, variously admixed with a basaloid cell population. This combination may well represent an example of collision tumour or of dual divergent differentiation. However, the presence of PAS-positive mucin material also within the basaloid areas, the overlapping profile of p53 expression in both components and the low trisomy of chromosome 7 observed in both neoplastic cell populations better support the hypothesis that the whole tumour is an adenocarcinoma with extensive basaloid cell differentiation (that has more or less extensively overgrown the mucinous component in part of the neoplastic glands).

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LETTER TO THE EDITOR

Silicotic mediastinal lymphadenopathy can cause left vocal cord paralysis and dysphagia

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Dear Editor,

Left vocal cord paralysis can complicate differential diagnosis, especially when associated with dysphagia. The added presence of cough, hemoptysis, and bronchial stenosis pinpoints the damage in the mediastinum. Despite chest X-ray, bronchoscopy, and bronchial biopsy, a definitive diagnosis may only be possible at autopsy.

Case report

A 71-year-old white woman sought out her physician because of hoarseness and difficulty in swallowing. Her previous history included treatment for lung tuberculosis about 20 years previously and hormone substitution for hypothyroidism. The patient was a non-smoker. Laryngoscopy demonstrated a complete paralysis of the left vocal cord. A barium swallow revealed slow passage time, a hypotonic esophageal wall, irregular peristalsis, dilatation of the esophagus and diverticula next to the tracheal bifurcation. Chest X-rays showed calcification in the left thyroid gland. Computer tomography of the neck and the upper mediastinum revealed a few mediastinal, hilar, and carinal calcified lymph nodes about 1.5 cm in diameter.

Seven years later, the patient was admitted to hospital because of persistent cough with increasing expectoration and hemoptysis. Occupational history revealed that the patient had worked for 10 years in a brick factory in the post-war era with exposure to silica dust. Physical examination showed acral cyanosis and watch glass nails. The Mantoux test was positive. Leukocyte count (15,000/μl) and sedimentation rate test (60/66 mm) were elevated. Chest X-ray and tomograms showed a band-like pneumonic infiltrate in segment 3 of the right upper lobe with a stenosis of the segment 3 bronchus. Bronchoscopy revealed severe stenosis of the segment 3 bronchus by focal black mucosal tissue. A biopsy was taken, which was complicated by massive bleeding. The biopsy revealed a bronchus wall with slight fibrosis, a mild chronic unspecific inflammation, and anthracosis. After a combined antibiotic therapy (gentamycin and ampicillin) with only initial improvement of the chest X-ray, a tuberculostatic triple drug therapy was introduced. Two weeks later, the patient died unexpectedly during the hospital stay.

Autopsy findings

Autopsy revealed silicotic mediastinal lymphadenopathy with destruction of the segment 3 bronchus of the right upper lobe (Fig. 1) and mediastinal nerves like the left recurrent laryngeal nerve (Fig. 2). Histologically, coalesced lymph nodes were found with necrosis, focal calcification, concentric bundles of collagen and macrophages with anthracosis, and numerous silica particles made visible by

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polarization microscopy. The lungs demonstrated only small silicotic nodules with adjacent perifocal emphysema. Tuberculous caseation with giant cells could not be found. The thyroid gland was diagnosed with Riedel's thyroiditis. Furthermore, recurrent lung embolism could be detected. The patient died of acute pulmonary embolism leading to right heart failure. The deep veins of the legs were without pathological changes. Reevaluation of the bronchial biopsy with polarization microscopy revealed few silica particles.

Discussion

Silicosis is one of the oldest occupational diseases and still kills thousands of people each year. It is a global phenomenon and is not restricted to low-income countries [16, 26]. This incurable, debilitating, sometimes fatal, yet preventable disease is caused by inhaling respirable crystalline silica dust ($Si0_2$) [3]. Silicosis progresses even after cessation of silica inhalation [15]. Silica dust is released during operations in which rocks, sand, concrete, and some ores are crushed, broken, ground, dispersed, sieved, screened, mixed, stored, or bagged. Therefore, persons in a wide variety of different occupations and workplaces are at particular risk of contracting silicosis (e.g., in mining, quarrying, tunnel construction, sandblasting, bricklaying, masonry, metal casting, ceramics, stone carving, gem working, farming, greenhouse gardening, highway repair, dental laboratories, flax scutching, whetstone cutting) [3, 26]. However, silica exposure is not restricted to the workplace, but can be due to living in silica rich environments (soils) in semidesert or desert climates, as proven by reports on the development of silicate pneumoconiosis and even silicosis in inhabitants of the Himalaya, Saudi Arabia, and the southwestern United States [2, 14, 22]. Moreover, silicate pneumoconiosis and silicosis is not restricted to human beings, but can also be found in animals like hens, pigs, water buffaloes, camels, and other species [2, 8, 19–21]. The form and severity in which silicosis manifests itself depend on the extent of exposure to silica dusts and on the origin, crystallinity, and state of surface of the silicas [7, 26]. Crystalline silica (in the form of quartz or crystobalite) has a particularly strong pathogenic effect and is even classified by the International Agency for Research on Cancer as a group 1 human lung carcinogen [26]. Furthermore, freshly fractured silica, such as that generated during abrasive blasting, is more toxic than aged silica [25]. Crushing silica yields Si and SiO radicals on the cleavage planes that react with water to produce the damaging hydroxyl radical (–OH) [25]. Therefore, the pathogenicity of and biological response to silica is a function of its surface [4]. Besides pulmonary silicosis with simple (nodular) silicosis (with fibrotic nodules up to one centimeter in size), acute silicosis (silicoproteinosis), complicated pneumoconiosis (progressive massive fibrosis with nodules larger than 1 cm), and true diffuse interstitial fibrosis [17], silicosis is associated with a great number of autoimmune disorders such as progressive systemic sclerosis, systemic lupus erythematosus, rheuma-

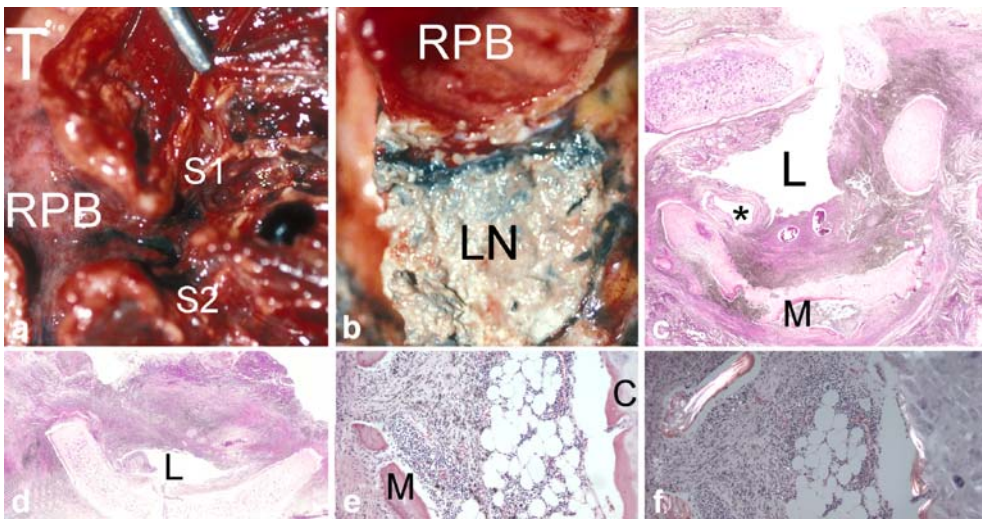

Fig. 1 Destruction of the right S3 bronchus. **a** Dorsal view of the trachea (*T*) and the right principal bronchus (*RPB*) with the division into the segmental bronchi S1 and S2. Note the anthracotic bronchial mucosa. **b** Section through the right principal bronchus (*RPB*) with ventrally situated enlarged coalesced silicoanthracotic lymph nodes (*LN*). **c** Section of the S3 bronchus with destruction of the bronchial cartilage, dilated submucosal vessel (*asterisk*) (massive bleeding at biopsy) and focal osseous metaplasia (*M*) of the cartilage. H & E, ×12,5. **d** Further histological section of the S3 bronchus of the right lung displaying severe stenosis. Lumen (*L*). H & E, ×12,5. **e** Bronchial cartilage (*C*) with osseous metaplasia (*M*), bone marrow and destruction by silicotic tissue. H & E, ×100. **f** Destruction of the metaplastic bone and the bronchial cartilage by silicoanthracotic tissue. Note the birefringent silica particles. H & E, ×100, polarized microscopy

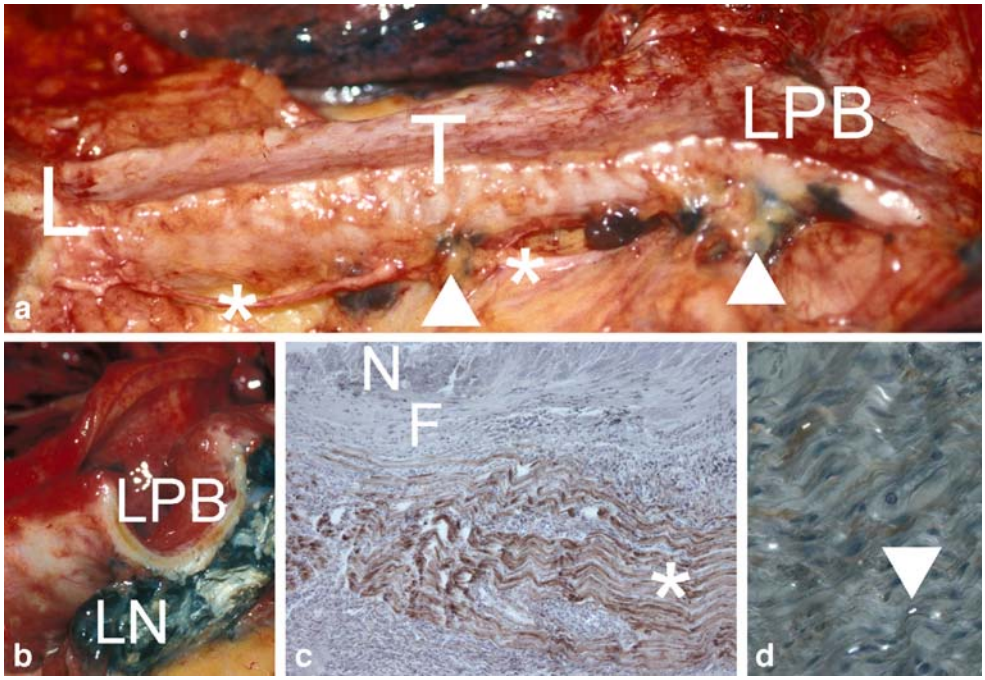

Fig. 2 Destruction of the left laryngeal nerve. **a** Dorsal view of the larynx (*L*), the trachea (*T*), the left principal bronchus (*LPB*), and the left recurrent laryngeal nerve (*asterisks*). Adhesions and destruction of the left recurrent laryngeal nerve (*arrowheads*) at silicoanthracotic lymph nodes. **b** Section through the left principal bronchus (*LPB*) with ventrally situated enlarged and coalesced mediastinal lymph nodes (*LN*). **c** Immunohistochemical staining of the left recurrent laryngeal nerve (*asterisk*) with anti-S100. DAB, ×100. Note the destruction of the nerve bundles and the adjacent fibrosis (*F*) and necrosis (*N*). **d** Separation of S100-positive nerve structures by silicoanthracotic tissue. Immunohistochemistry, anti-S100, DAB, ×630, polarized microscopy. Note the birefringent silica particles (*arrowhead*)

toid arthritis, dermatomyositis, glomerulonephritis, and vasculitis [24]. The link between these various diseases may be the fact that the pathogenesis of silicosis involves uncontrolled immune processes which may start with the recognition and uptake of silica by alveolar or interstitial macrophages via the MARCO receptor and the subsequent activation of these immune cells [9]. According to Huaux [9], it is now generally accepted that silica-induced pulmonary lesions are modulated and triggered by the immune system and its mediators (macrophages, mast cells, B lymphocytes, TNF alpha). These pathogenic routes may highlight new potential therapeutic targets and strategies [9].

Hoarseness due to paralysis of the left vocal cord with or without associated dysphagia can be caused by diseases of the heart (cardiomegaly, mitral stenosis with gross enlargement of the left atrium), the aorta (aortic arch aneurysm), the esophagus (carcinoma), the thyroid (carcinoma), or the mediastinal lymph nodes (bronchial carcinoma, sarcoidosis, tuberculosis, lymphoma, silicosis) [5, 6, 13, 18]. These pathological processes can destroy, compress or stretch the vagal nerves, the thoracic sympathetic outflow—both nerves necessary to control swallowing—and the laryngeal recurrent nerves. The left laryngeal recurrent nerve in particular is more commonly affected than the right by a pathological mediastinal process owing to its longer intrathoracic course around the aortic arch [23]. Lardinois et al. [10] presented a case of left laryngeal nerve palsy due to encasement of the nerve by scar tissue and a dense, irregularly shaped lymph node. Under videomediastinoscopy, the nerve was carefully dissected with scissors and freed from the scar and lymph node encasement. After 15 weeks of postoperative follow-up, total and lasting recovery of the voice was observed. In our case, however, the aggressive silicotic tissue had already destroyed the mediastinal lymph nodes and adjacent anatomical structures like the segment 3 bronchus of the right upper lobe, the left laryngeal recurrent nerve, and other vagal and sympathetic nerves. This destruction resulted in hoarseness, dysphagia, and bronchial stenosis. As our patient had worked for 10 years in a brick factory in the post-war era (1947–1957) with exposition to silica dust, this combination of occupational history and clinical findings led us to suspect silicosis. In the post-war era, occupational and health safety regulations in Germany were non-existent or much weaker than nowadays. Despite the absence of visible silicotic changes in the parenchyma of the lungs in the chest X-ray and computer tomography, the possibility of silicosis could not be dismissed. Silicotic mediastinal lymphadenopathy can be present even without obvious silicotic fibrosis of the lung parenchyma [11, 12, 18]. It is unclear why in some rare cases silicosis is exclusively extrapulmonary. According to Adamson and Prieditis [1], silica particle translocation to the lymph nodes

may be increased especially during alveolar epithelial injury. This epithelial injury also potentiates fibrosis which, however, could not be detected in such cases. Silicotic mediastinal lymphadenopathy as an isolated phenomenon is probably due to a low exposure to silica over a long period of time. This dose of silica may be too low for a significant inflammation in the lung tissue, but is enough to accumulate a large amount of silica in the lymph nodes. Furthermore, silicosis often becomes manifest 20 to 40 years after the initial exposure [4] because the inflammation and damage due to silica may progress even after cessation of silica inhalation [15]. Whether Riedel's thyroiditis or a similar fibrotic form of thyroiditis is associated with silicosis—like rheumatoid arthritis in Caplan's syndrome—or is only coincident in this special case cannot be established with certainty. Silicotic mediastinal lymphadenopathy has to be considered in the differential diagnosis of left vocal cord palsy with associated dysphagia, especially in a patient with an occupational exposition to silica.

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ERRATUM

GSTP1 promoter hypermethylation is an early event in breast carcinogenesis

Ji Shin Lee

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Unfortunately the following acknowledgement was not published in the body of the paper.

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REVIEW AND PERSPECTIVE

Mutation analysis of gastrointestinal stromal tumors: increasing significance for risk assessment and effective targeted therapy

Eva Wardelmann · Reinhard Büttner · Sabine Merkelbach-Bruse · Hans-Ulrich Schildhaus

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Abstract Molecular characterization of gastrointestinal stromal tumors (GISTs) plays an increasing role not only for the patient's prognosis but also for treatment options and in the context of resistance to therapy. Several mutational subtypes in *KIT* or platelet-derived growth factor receptor-α (*PDGFRα*) have been identified to be correlated with a different clinical behavior of GISTs. In *KIT* exon 11, deletions in the proximal part are associated with a high metastatic risk, whereas duplications in the distal part lead to a less aggressive phenotype. GISTs of the small bowel with a duplication in *KIT* exon 9 are often high risk tumors. In contrast, *PDGFRα* exon 18 mutated GISTs tend to have a low malignant potential. The authors suggest to include these molecular data together with classical parameters such as mitotic count and tumor size into the risk assessment of GISTs. The first choice for treatment of GISTs is still the surgical resection. In advanced tumors, which cannot be R0 resected, the neoadjuvant treatment with the tyrosine kinase inhibitor imatinib is now well established. Furthermore, an adjuvant treatment of locally R0-resected intermediate and high risk tumors is evaluated in several international clinical trials. For metastatic disease, treatment with imatinib is still the first option, but with new upcoming substances, the molecular characterization of GISTs may become mandatory. Very recently, it has been shown that sunitinib may be especially effective in GISTs with *KIT* exon 9 mutation, whereas these tumors show only an intermediate response to imatinib. A European Organisation for Research and Treatment of Cancer clinical trial randomizing patients according to their mutational status is under preparation. Secondary resistance to imatinib treatment is increasing, at least partly due to secondary mutations in the tyrosine kinase domain of the KIT receptor. Once a lesion has been shown to carry such a mutation, the local excision may be useful, mean while still responding metastases are further controlled by continuing imatinib. Taken together, the molecular characterization of GISTs turns out to play a central role before and during the treatment with tyrosine kinase inhibitors, which have improved the treatment of GIST patients dramatically.

Keywords GIST · *KIT* mutation · *PDGFRα* mutation · Molecular characterization

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the digestive tract. They are characterized by the expression of the type III receptor tyrosine kinase KIT in the vast majority of cases (>90%) [40, 44]. More than 80% of GISTs carry a gain-of-function mutation in the encoding *KIT* gene or—less often—alternatively in the platelet-derived growth factor receptor-α (*PDGFRα*) gene leading to a ligand-independent autoactivation of one of both tyrosine kinases [18, 20, 21]. Approximately, 50% of patients with GISTs seen in cancer hospitals develop recurrences and/or metastases after surgical resection of the primary lesion that are resistant to radio- and chemotherapy [10, 11]. The tyrosine kinase

inhibitor imatinib mesylate (Glivec®, Gleevec®, Novartis, Basel, Switzerland) has revolutionized the treatment of GISTs leading to response rates of up to 75% of patients suffering from advanced GIST disease. Several international clinical trials have proven that, to date, imatinib is the gold standard in treatment of these patients [5, 19, 48].

Diagnosis of GIST

Most GISTs occur in the stomach (about 60%) followed by the small bowel (30%). Rarely, GISTs may present in the esophagus or in the large bowel (<5%), the latter preferentially in the rectum [37]. A small subgroup of GISTs occurs without connection to the tubular gastrointestinal tract and is designated as extra [36]. Histomorphologically, GISTs may exhibit three different phenotypes: the spindle cell type (70%), the epithelioid cell type (10%), or the mixed cell type (20%), the latter with an intermediate cell type or as composition of distinct spindle or epithelioid cell areas (Fig. 1a–d) [14]. Multinucleated tumor giant cells are preferentially identified in the epithelioid cell type [41].

GISTs are thought to derive from the interstitial cells of Cajal or a common precursor as they share the immunohistochemical co-expression of the stem cell antigens cluster of differentiation 34 (CD34) and KIT. Histopathological evaluation including a well-done immunohistochemistry is sufficient in the most instances for making a correct GIST diagnosis. Difficulties in the differential diagnosis may occur preferentially in gastrointestinal tumors with epithelioid phenotype. Immunohistochemical examination has to rule out not only sarcomatoid carcinomas but also other mesenchymal tumors such as clear cell sarcoma, which rarely presents in the gastrointestinal tract [15, 45, 54] and can be identified by its strong expression of S-100 protein and by fluorescence in situ hybridization detection of the characteristic EWS–ATF1 translocation. Furthermore, CD34 is expressed in more than 70% of GISTs, but may also be found in solitary fibrous tumors that may exhibit an epithelioid or spindle cell phenotype [2]. The latter are constantly completely negative for the KIT receptor.

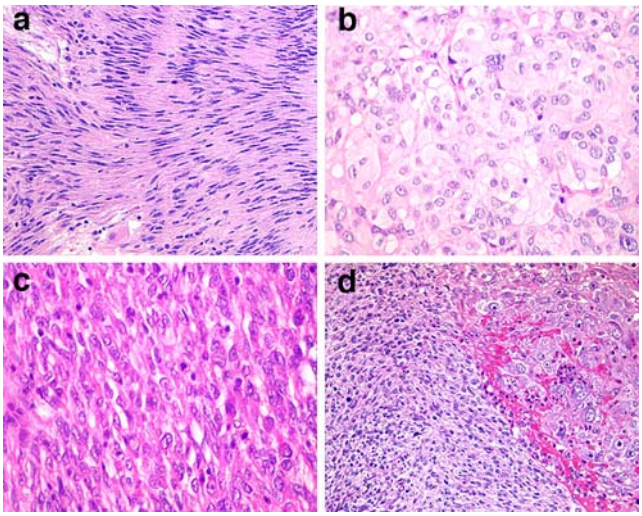

Fig. 1 Histomorphological subtypes of GISTs. **a** Spindle cell type. **b** Epithelioid cell type. **c** Mixed cell type composed of cells with intermediate cell phenotype. **d** Mixed cell type with distinct separated spindle and epithelioid cell morphology (×20 original magnification, H&E)

Less than 5% of GISTs are negative for both CD34 and KIT [25, 42, 47]. Some of the KIT-negative GISTs belong to the subgroup driven alternatively by the PDGFRα receptor (PDGFRA) [18, 22, 33]. Especially in these cases, which may lack immunohistochemical expression of the KIT receptor, molecular characterization is helpful to identify these tumors as GIST. Novel immunohistochemical markers for GISTs have been described, such as protein kinase C (PKC) theta [3, 24] or hypothetical protein spicifically expressed in GISTs named “discovered on GIST-1” (DOG-1) [53]. The first one showed in our laboratory a lower sensitivity than KIT, as only half of all the tumors included in a small immunohistochemical study were immunopositive. DOG-1 is not yet commercially available. Therefore, further studies are required to evaluate the utility of these markers in diagnostic procedures.

Molecular characterization

Mutational analysis should be carried out by using fresh snap-frozen tumor tissue. However, under most circumstances, only formalin-fixed and paraffin-embedded tissue is available, which can also be used. Tumor tissue should be enriched by removal of normal tissues (e.g., mucosa, surrounding connective tissue or liver parenchyma in the case of metastases). All specific polymerase chain reaction products should be sequenced. Screening methods, as single-strand conformation polymorphism, denaturing high-pressure liquid chromatography, or gel electrophoresis, can be used before sequencing.

More than 80% of GISTs carry a gain-of-function mutation in the *KIT* gene or—less often—in the *PDGFR*α gene [38, 39]. These mutations lead to a ligand-independent autoactivation of the encoded type III receptor tyrosine kinases KIT or PDGFRα.

In *KIT*, four hot spots have been identified, i.e., exon 9, exon 11, exon 13, and exon 17 [43]. Exon 11, encoding the juxtamembraneous domain, is affected in up to 65% of all KIT-mutated GISTs [38, 39]. This region is thought to have an autoinhibitory function resulting from its α-helical conformation, which is disrupted by changes in the amino acid sequence [31]. The spectrum of exon 11 mutations

consists of point mutations as well as of large in-frame deletions and insertions. On the protein level, large complex mutations may result in a combination of an amino acid exchange and a deletion of one or more amino acid residues. Exon 9 encodes the fifth extracellular immunoglobuline-like loop probably responsible for proteolysis of the receptor protein and shows mutations in 10 to 15% of GISTs. Mutations in this region are almost always insertions of 6 bp leading to a duplication of the codons A-502 and Y-503 [1, 28]. Genomic changes in one of both tyrosine kinase domains are exceedingly rare with less than 1% of *KIT* mutations. For exon 13 (encoding the tyrosine kinase domain 1), one point mutation (K642E) is most frequent; for exon 17 (encoding the tyrosine kinase domain 2), different point mutations most often occur between codons D-816 and Y-823 [26, 30].

Recently, several groups could show that one third of these GISTs without any *KIT* mutation are driven by an alternative tyrosine kinase very similar to *KIT* [18, 22, 49]. Belonging to the same type III receptor tyrosine family as KIT, PDGFRα shares 35% of the amino acid sequence with KIT and activates nearly the same signal transduction pathways as KIT. The encoding gene is located just beneath the *KIT* gene on the long arm of chromosome 4 (4q12) and consists of 22 exons (one more than *KIT*). PDGFRα mutations are most often located in the tyrosine kinase domain 2 encoded by exon 18 (up to 90% of all *PDGFRα*-mutated GISTs), and point mutations are the most common mutational subtype (predominantly D842V) followed by deletions in the same region of the exon. Less often, mutations are found in exon 12 encoding the juxtamembraneous domain or in exon 14 encoding the first part of the tyrosine kinase domain (Fig. 2) [29].

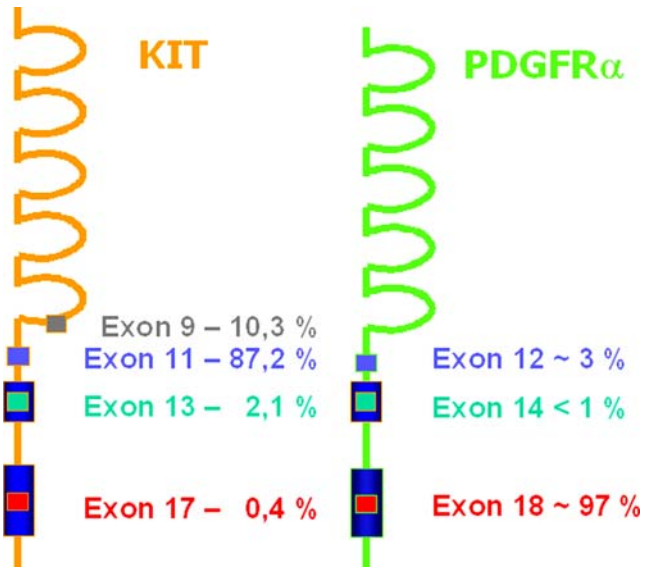

Fig. 2 Mutational hot spots of GISTs in *KIT* and *PDGFRα* gene. Data are given as percentage of all cases mutated in one of both genes

About 15–20% of GISTs carry wild type sequences in all hot spots of *KIT* and *PDGFRA* (wt-GISTs). The pathogenesis of these tumors is still poorly understood.

Prognosis

The prediction of the biological behavior of GISTs is difficult based on pathomorphogical data alone. To date, three parameters are known to be relevant: location of the primary tumor, largest tumor diameter, and mitotic count per 50 high power fields (HPF). The classification system proposed by Miettinen et al. [34] uses all three parameters leading to the three categories: probably benign, uncertain or low malignant potential, and probably malignant (Table 1). Another widely accepted classification [14] considers the risk assessment of an aggressive biological behavior. According to size and mitotic count/50 HPF GISTs are subgrouped into tumors with very low, low, intermediate, and high risk (Table 2). Recently, Miettinen and Lasota [35] modified their risk stratification in a large series of primary GISTs with special regard to the different prognosis of gastric versus small bowel tumors. Table 3 shows which different risk groups could be identified by evaluation of nearly 2,000 GISTs with long-term follow-up. The relevance of the Ki67 index as proliferation parameter has been evaluated by several groups, but its reproducibility is too low to be included as a classification criterion.

Several authors have proven the usefulness of these classifications by correlating the clinical follow-up with subgrouping. However, a part of GISTs behaves unpredictably, leading to the need of further prognostic parameters. Larger actual studies have found that, besides the classical

Table 1 Evaluation of GIST malignancy according to Miettinen et al. [32]

Categories	
Probably benign	
Intestinal tumors	Maximum diameter ≤2 cm and ≤5 mitoses/50 HPFs
Gastric tumors	Maximum diameter ≤5 cm and ≤5 mitoses/50 HPFs
Uncertain or low malignant potential	
Intestinal tumors	Maximum diameter >2 cm but ≤5 cm and ≤5 mitoses/50 HPFs
Gastric tumors	Maximum diameter >5 cm but ≤10 cm and ≤5 mitoses/50 HPFs
Probably malignant	
Intestinal tumors	Maximum diameter >5 cm or >5 mitoses/50 HPFs
Gastric tumors	Maximum diameter >10 cm or >5 mitoses/50 HPFs

Table 2 Risk assessment of an aggressive clinical behavior in GIST according to Fletcher et al. [13]

	Tumor size (cm)	Mitotic count (HPFs)
Very low	<2	<5/50
Low	2–5	<5/50
Intermediate	<5	6–10/50
	5–10	<5/50
High	>5	>5/50
	>10	Any mitotic count
	Any size	>10/50

parameters, molecular typing might give further information concerning aggressiveness of GISTs. They identified special mutational subtypes in exon 11 of *KIT* associated with a high metastatic risk as, for example, the loss of the amino acid residues W-557 and K-558 in exon 11 [32, 50]. Miettinen et al. [38] found in a large series of gastric GISTs that tumors with point mutations in KIT exon 11 behave better than those with deletions. Others describe that duplications in the distal part of *KIT* exon 11 are correlated with a better prognosis [1, 28]. *KIT* mutations in exon 9 encoding the extracellular domain of the receptor protein are found preferentially in GISTs of the small bowel and predict a worse prognosis than GISTs with other *KIT* mutations [1]. On the other hand, GISTs caused by an activating mutation in exon 18 of *PDGFRα* are preferentially located in the stomach and are characterized by a low malignant potential [27, 29, 49]. A comprehensive classification system including the "classical" histomorphological parameters as well as the mutational status is still missing. However, most oncologists already use the mutational status as a predictive parameter for therapy planning. We have included a summary of the most relevant pathomorphologic and molecular parameters for risk assessment in Table 4.

Table 3 Metastatic risk of gastrointestinal stromal tumors (modified according to Miettinen and Lasota [33])

Group	Size (cm)	Mitotic count (HPFs)	Metastatic risk			
			Stomach	Jej./ileum	Duodenum	Rectum
1	≤2	≤5/50	ϕ	ϕ	ϕ	ϕ
2	>2–5	≤5/50	Very low	Low	Low	Low
3a	>5–10	≤5/50	Low	Moderate	High	High
3b	>10	≤5/50	Moderate	High	High	High
4	≤2	>5/50	ϕ*	High[a]	–	High
5	>2–5	>5/50	Moderate	High	High	High
6a	>5–10	>5/50	High	High	High	High
6b	>10	>5/50	High	High	High	High

Based on previously published long-term follow-up studies on 1939 GISTs

– No cases available

[a] Denotes tumor categories with very small numbers of cases

Treatment

The first step in the treatment of GISTs is the surgical resection of the primary tumor. For small tumors, a local excision with clear margins as confirmed by the pathologist is sufficient. In larger tumors with local resectability, the clear margins should measure at least 1 to 2 cm [10, 23]. In metastatic and unresectable GISTs, primary surgery has been shown not to be a sufficient therapy. GISTs are resistant to radio- and chemotherapy. In advanced cases, the gold standard is a targeted therapy with tyrosine kinase inhibitors leading to response rates of up to 80% [12, 19]. Actually, imatinib mesylate (Glivec®) is the first line drug administrated orally (400 mg daily). Imatinib binds to the adenosine triphosphate (ATP)-binding pocket in the tyrosine kinase domain of the KIT receptor thereby inhibiting the ATP binding and thus the enzymatic activity of the receptor [46].

In advanced tumors, an extensive surgical approach with resection of several organs has been shown to be ineffective, as most patients develop early local recurrence with a very unfavorable course. In these cases, a neoadjuvant strategy to reduce the tumor load before surgical resection is under study (for example, NCT00112632, Munich, Germany). Furthermore, several international studies evaluate if an adjuvant treatment with imatinib increases the event-free and overall survival in cases with intermediate or high risk GISTs (for example, the European Organisation for Research and Treatment of Cancer 62024, SSGXVIII, ACOZOG-Z9000, and Z9001).

The molecular status of GISTs turns out to be relevant for the response to targeted treatment with imatinib. Larger clinical studies have shown that tumors with a *KIT* exon 11 mutation respond in a higher percentage than GISTs with an exon 9 mutation. Tumors without *KIT* mutations in these exons often have only low responsivity [8, 17]. *PDGFRα* mutations may lead to a primary resistance to imatinib, especially the point mutation D842V in exon 18, which is the most frequent mutation in this gene. On the other hand, *PDGFRα*-mutated GISTs, in most cases, behave less aggressive than tumors with *KIT* mutation [6, 29].

With new upcoming tyrosine kinase inhibitors other than imatinib, the molecular characterization gets further importance. It has been shown that other drugs such as sunitinib (SU11248, Sunitinib, Sutent®, Pfizer Oncology) are especially effective in GISTs with exon 9 mutation [13]. Furthermore, the mutational status is relevant for the most effective dosage of imatinib. Whereas response rates for

Table 4 Relevant risk parameters of an aggressive clinical behavior in gastrointestinal stromal tumors including molecular data

Parameters	Risk ↓	Risk ↑
Surgery	R0	R1, rupture
Location	Stomach	Small bowel, E-GIST
Size	≤5 cm	>5 cm
Mitotic count	≤5/ 50 HPFs	>5/50 HPFs
Mutated gene	*PDGFRα*	*KIT*
Mutational type (*KIT* exon 11)	Duplication	Deletion (esp. when including codons W557 and K558)

exon 11-mutated GISTs are comparable for 400 mg versus 800 mg/daily, patients with exon 9 mutations show a significant longer progression-free survival when treated with 800 mg instead of 400 mg/daily [9]. Until now, the treatment options throughout Europe are still different. The approval of 800-mg imatinib daily or sunitinib as first line treatments of GIST patients with KIT exon 9 mutation are still missing in several countries. However, most data highlight that, in the future, GISTs with high risk criteria or proven malignant behavior should be characterized molecularly as soon as possible to choose the right treatment regimen.

Novel KIT multi-tyrosine kinase inhibitors such as AMG 706 and AMN 107 (nilotinib) as well as downstream targeting small molecules, as for example, the mTOR inhibitors RAD001 (everolimus) and rapamycin (sirolimus), the PKC inhibitor PKC412, and the vascular endothelial growth factor receptor inhibitor bavacizumab (avastin), are in clinical trials. Lastly, the heat-shock protein-90 inhibitor IPI-540 is also in phase I evaluation in imatinib-refractory patients with GIST. Subgroup analyses will show in the future how these small molecule inhibitors will improve the treatment of GISTs further.

Resistance

With longer duration of imatinib treatment in patients with advanced GISTs, the risk of resistance to therapy increases. Several groups describe an increasing frequency of secondary mutations in *KIT* or rarely in *PDGFRα* [7, 51]. These point mutations are located preferentially in or near the ATP-binding pocket and often involve the known imatinib contact points (Fig. 3). New *KIT* mutations are often found in only some of several lesions, whereas other metastases only carry the primary mutation and are still responding to imatinib [51, 52]. Several different new mutations in addition to the primary mutation may occur indicating polyclonal resistance as already shown in chronic myelogenic leukemia under imatinib treatment [4, 16]. This observation may lead to a surgical approach even in

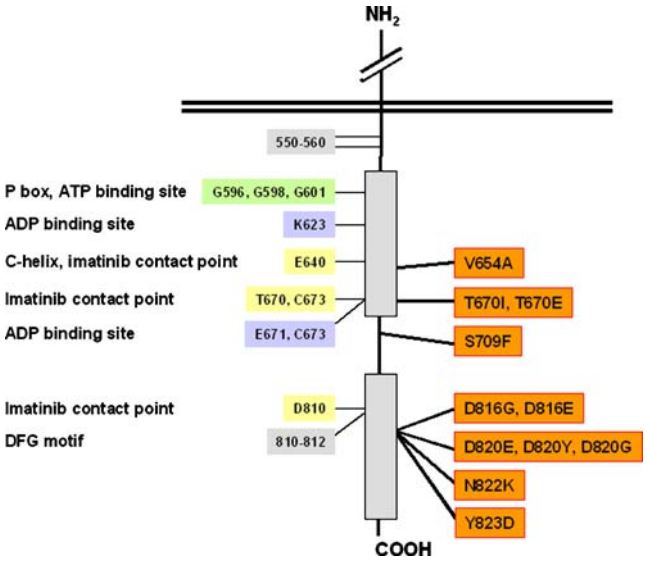

Fig. 3 Co-localization of imatinib binding points and secondary *KIT* mutations under imatinib treatment of GISTs leading to secondary resistance

advanced GIST disease. In these cases, functionally active metastases suspective for secondary mutations may be resected while other still responding lesions do not require surgery while the imatinib treatment is continued [23]. Several novel tyrosine kinase inhibitors that may be even more effective in GISTs resistant to imatinib are in clinical trials as mentioned above.

Several other mechanisms for secondary resistance are still under research, as for example, gene amplification or activation of other tyrosine kinase pathways. Exact data of these issues are still missing.

Conclusions

The treatment of gastrointestinal stromal tumors has changed dramatically since the past few years due to new insights into the molecular mechanisms responsible for prognosis, treatment response, and resistance against therapy. The surgical R0-resection of a localized GIST is still the first choice of treatment. In unresectable, R1-resected, or metastatic GISTs, targeted treatment with the tyrosine kinase inhibitor imatinib is the gold standard. With newly developed small molecule inhibitors such as sunitinib and everolimus, novel stratification criteria might be needed especially regarding GISTs becoming resistant. The molecular characterization of GISTs is not only helpful for diagnostic purposes in cases with low or no KIT receptor expression but might also help to predict clinical prognosis as several subgroups with different risk of aggressive clinical behavior can be identified. Tumors with high risk criteria can be treated individually on the basis of their molecular background. In the future, the molecular

analysis of GIST in the hot spot regions of *KIT* and *PDGFRα* will be a further parameter that has to be determined in addition to the established pathomorphological criteria, at least, in high risk lesions. It might also help to clarify the optimal treatment regimen especially in cases of secondary resistance.

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RECOMMENDATION

Recommendations for the reporting of prostate carcinoma

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Introduction for ADASP reporting guidelines

It has been evident for decades that pathology reports are very variable even within a single institution. Standardization of reporting is the optimal way to insure that information necessary for patient management, prognostic and predictive factor assessment, grading, staging, analysis of outcomes, and tumor registries are included in pathology reports.

The Association of Directors of Anatomic and Surgical Pathology (ADASP) has chosen a pathologist expert in each field to assemble a group from within the pathology community (with clinician input if desired) to write specific cancer protocols. These were then approved by the ADASP council and subsequently by the membership. The American College of Surgery (ACS) Commission on Cancer (COC) accredits cancer centers in the USA. Recently, the COC decided to require elements, deemed as essential by the College of American Pathologists (CAP), to be described in all pathology reports in their accredited cancer centers as of January 2004. Importantly, they do not require that the specific CAP protocols or synoptic reports be utilized. ADASP has updated all of its protocols to comply with the COC requirements in the form of uniform checklists. The checklists use the staging criteria sited in the American Joint Committee on Cancer (AJCC) 2002 staging manual (sixth edition) but include a variety of other references listed in each of the checklists. Moreover, the checklists are formatted for ease of use. They may be used as templates for uniform reporting and are designed to be compatible with voice-activated transcription.

The different elements in these revised ADASP Diagnostic Checklists have been divided into Required and Optional. The term Required in this context only signifies compliance with the COC guidelines. ADASP realizes that specimens and practices vary and it will not be possible to report these elements in every case. However, ADASP hopes that pathologists will find these checklists to be useful in daily clinical practice while facilitating compliance with the new COC requirements.

The checklists are in standard PDF file format and may be easily downloaded from the ADASP Web site. They are not to be reproduced, altered, or used for commercial purposes without consent from ADASP.

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ASSOCIATION OF DIRECTORS OF ANATOMIC AND SURGICAL PATHOLOGY (Updated September 2006, Version 1.4).

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I. Features the Association recommends to be included in the final report because they are generally accepted as being of prognostic importance, required for therapy, and/or traditionally expected [1–8].

A. Gross Description

1. Description of Specimen Received—Fresh, in formalin, intact, cut, margins inked, or not, etc.
2. Specimen Labeling—Labeled with (name, number), designated as prostate, and procedure (core biopsy, transurethral biopsy, transurethral resection, enucleation, radical prostatectomy, radical cystoprostatectomy, other)
3. Size—Theoverall size of the excised specimen should be measured in three dimensions: TURP specimens should be weighed; needle biopsy cores should be counted and measured in length (state if multiple small fragmented cores without need for measuring)
4. Additional Organs Attached—(e.g., seminal vesicles, vasa deferentia, bladder neck in radical prostatectomy specimens).
5. Tumor Description
   - Presence of lesion(s) or absence of lesion(s)
   - Location of the lesion(s) (e.g., in radical prostatectomy: posterior, posterolateral, lateral, anterior and apex, mid, base)
   - Size of the lesion(s) (greatest diameter if radical prostatectomy)
   - Consistency of the lesion(s) (e.g., firm, fleshy)
6. Lymph Nodes—Number and appearance of lymph nodes if received
7. Frozen Section—Whether a frozen section was performed and the diagnosis that was made

B. Diagnostic Information

1 Histological Type

- ___Adenocarcinoma (acinar, not otherwise specified)
- ___Prostatic duct adenocarcinoma
- ___Mucinous (colloid) adenocarcinoma
- ___Signet-ring-cell-like carcinoma
- ___Adenosquamous carcinoma
- ___Small cell carcinoma
- ___Sarcomatoid carcinoma
- ___Undifferentiated carcinoma, not otherwise specified
- ___Other (specify): _____

2 HistologicalGrade. All acinar adenocarcinomas should be graded using the Gleason grading system [9, 10]. Ductal adenocarcinomas are typically assigned a Gleason score $4+4=8$ yet are diagnosed as "ductal adenocarcinoma (Gleason score $4+4=8$)" to convey the unique clinical and pathological features of this tumor. There is no consensus as to the grading of mucinous carcinomas. Approximately one-half of urological pathologists grade them as Gleason pattern 4 (e.g., Gleason score $4+4=8$ if pure mucinous tumor). The remaining experts grade the tumor based on the underlying tumor architecture, mentally subtracting away the mucin. Regardless of the method used to grade mucinous carcinomas, the majority of these tumors end up being assigned a Gleason score $4+4=8$. True signet-ring-cell carcinomas containing vacuoles of mucin that are primary in the prostate are exceedingly rare. Rather, there exists signet-ring-cell-like carcinomas, which contain clear vacuoles without mucin. These tumors are graded by their underlying architecture. Small cell carcinomas are not graded as they have unique clinicopathological features and most importantly are treated differently than Gleason pattern 5 adenocarcinoma. The carcinomatous component of sarcomatoid carcinoma and adenosquamous carcinoma should be assigned a Gleason score.

For all types of specimens (needle, TURP, enucleation, radical prostatectomy), when there is a minor secondary component (<5% of tumor) and where the secondary component is of higher grade, the latter should be reported. For instance, a case showing >95% Gleason pattern 3 and <5% Gleason pattern 4 should be reported as Gleason score $3+4=7$. Conversely, if a minor secondary pattern is of lower grade, it need not be reported. For instance, where there is >95% Gleason score 4 and <5% Gleason 3, the score should be reported as Gleason score $4+4=8$. These aggressive cases with only a few glands of Gleason pattern 3 should be distinguished from cases with a more prominent secondary Gleason pattern 3, which are assigned a Gleason score of $4+3=7$.

In needle biopsy specimens, it is recommended that separate Gleason scores be assigned for each specimen container. This is most critical for the situation where the grade for the tumor in one container is Gleason score $4+4=8$ and the others are of lower grade. In these cases, the tumor behaves according to the highest grade (e.g., Gleason score $4+4=8$) and not the composite (overall) score, which would be lower. Further support for assigning separate Gleason scores for different containers is that the Gleason grade factored into currently existing tables and nomograms (e.g., Partin tables, Kattan nomograms), which predict the stage and prognosis of prostate cancer, utilized the highest Gleason score in a case and not the composite (global) score. Providing a global or composite score reflecting the overall Gleason score in the entire specimen is optional.

In needle biopsy specimens where more than two patterns are present and the worst grade is neither the predominant nor the secondary grade, the predominant and highest grade should be chosen to arrive at a score (e.g., 75% pattern 3, 20% pattern 4, and 5% pattern 5) is assigned

a Gleason score $3 + 5 = 8$. It is assumed that a minor component of high-grade cancer on needle represents a sampling artifact, where it is likely that there will be a significant amount of the high-grade cancer in the prostate.

In TURP or enucleation specimens where one cannot identify separate tumor nodules, only one Gleason score is assigned. In radical prostatectomy specimens, each dominant tumor nodule is assigned a separate Gleason score. It is not necessary to assign a separate score to each small, multifocal, low-grade cancer focus in the setting of a larger higher-grade dominant nodule. In the case where there is no dominant tumor nodule, it can be stated that there are multifocal tumor nodules with a comment as to their grades. In TURP, enucleation, and radical prostatectomy specimens, the Gleason score is based on the primary (most common) and secondary (next most common) pattern. If there is a third pattern or if the second pattern occupies less than 5% of the specimen, then this pattern is reported as a tertiary pattern.

3. Tumor Extent. In core biopsies, the absolute number of involved cores should be reported out of the total number of cores received. In cases with fragmented cores where one cannot accurately derive the number of involved or total number of intact cores, one can merely state the overall percentage of the fragmented specimen involved by cancer. In addition, one should provide one other more detailed measurement of cancer, such as the linear extent of involvement in millimeters (either per core or total) or the percentage of cancer in each involved core. There is no consensus whether one should give the linear extent or percentage of involved core by either counting gaps of uninvolved tissue in the measurement or by "collapsing" the tumor by ignoring the intervening gaps of benign tissue. As different foci of cancer along a core most likely represent the same tumor as opposed to multifocal cancer, it is preferred to record the tumor extent including the uninvolved tissue in the measurement as it more accurately represents the minimal extent of the cancer in the prostate. To distinguish small discontinuous foci from continuous cancer, one can report the following: "Small foci of cancer discontinuously involving *X*% of the length of the core", where "*X*" is measured from one end of the cancer to the other on the core regardless of intervening benign tissue.

In TURP and enucleation specimens, the percentage of tissue involved by carcinoma is reported, with 5% the cut-off between T1a and T1b disease.

There is no uniform data that tumor volume in radical prostatectomy specimens is an independent predictive parameter of prognosis once other standard parameters are recorded. Nonetheless, it is recommended that some measurement of tumor volume be recorded even if it is a subjective quantification of "minimal, moderate, and extensive." If more precise measurements are required by the clinician, an "eyeball" estimate of the percentage of the specimen involved by cancer is sufficient.

4. Margins of Resection—The entire surface of a radical prostatectomy specimen should be inked to evaluate the surgical margins. Usually, surgical margins should be designated as "negative" if tumor is not present at the inked margin and as "positive" if tumor cells touch the ink at the margin. When tumor is located very close to an inked surface but is not actually in contact with the ink, it is considered negative. Positive surgical margins should not be interpreted as extraprostatic extension. Intraprostatic margins are positive in the setting of capsular incision (so-called pT2+ or pT2x disease). The specific locations of the positive margins are useful to report, and there should be some indication of the extent of margin positivity [e.g., unifocal versus multifocal or focal versus extensive or number of positive sites (blocks) or linear extent in millimeters].

The apical and bladder neck surgical margins should be submitted entirely, preferably with a perpendicular sectioning technique. Microscopic involvement of bladder neck muscle fibers in radical prostatectomy specimens should not be equated with a pT4 designation. The latter generally requires gross involvement of the bladder neck. A recent study has shown that patients with microscopic bladder neck involvement have recurrence rates similar to patients with seminal vesicle involvement (pT3b).

5. Extraprostatic Extension (EPE). This is the preferred term for the presence of tumor beyond the confines of the prostate gland. Tumor abutting on or admixed with fat constitutes EPE and, in general, is the only method to reliably diagnose EPE on needle biopsy. However, if one relies on the identification of tumor in fat to diagnose EPE on radical prostatectomy specimens, EPE will be underdiagnosed. One should also diagnose EPE when tumor extends beyond the condensed smooth muscle of the prostate to involve the looser connective tissue and thinner less compact smooth muscle outside of the prostate. One can also use the overall scanning magnification to assess whether tumor has extended beyond the normal contour of the gland. Reporting EPE at the apex is controversial as the boundaries of the prostate gland in this region are vague; benign prostatic acini are seen admixed with skeletal muscle in this region. One option is merely to state whether tumor is present and whether the margins are positive or negative in the apical region while not attempting to determine if tumor is organ-confined in this area.

The other option is to assume that the urologist has gone as wide as possible and the inked margin at the apex is outside of the prostate. Tumor not extending to the ink is considered organ-confined and tumor at the inked margin is considered as showing EPE, unless benign prostatic glands are also seen at the inked margin whereby capsular incision is diagnosed. The specific location(s) of EPE are useful to report. Descriptors of EPE (unifocal versus

multifocal or focal versus nonfocal or focal versus extensive or linear millimeters or number of blocks) may be used.

6. Lymph Node Status. Indicate the number of nodes involved and the total number of nodes evaluated.

7. Angiolymphatic Invasion. This finding is independently predictive of prognosis in radical prostatectomy specimens and should be recorded. The Association does not recommend the routine use of immunohistochemical stains to detect intravascular invasion.

8. Perineural Invasion. Perineural invasion in needle biopsy cores has been associated with EPE in most correlative radical prostatectomy studies, although its value as an independent prognostic factor has been questioned. Perineural invasion has been found to be an independent risk factor for predicting an adverse outcome in patients treated with external beam radiation. As it is easily measured and appears to have prognostic significance on biopsy, regardless of whether it is an independently prognostic parameter, its presence should be recorded on needle biopsy specimens. Perineural invasion has no prognostic significance in radical prostatectomy specimens and should not be mentioned in the pathology report.

9. Prostatic Intraepithelial Neoplasia (PIN)—Generally, low-grade PIN is not reported. The presence of isolated high grade PIN (HGPIN) should be reported in all biopsy specimens. The risk of cancer on repeat biopsy within 1 year of a needle biopsy diagnosis of HGPIN is not sufficiently different from the risk of cancer on repeat biopsy after a benign diagnosis on needle biopsy [11]. Consequently, immediate repeat biopsy after a needle biopsy diagnosis of HGPIN is not necessary. Whether and when a repeat biopsy should be performed remains to be studied. The reporting of HGPIN in prostatectomy specimens is optional.

10. Staging—It is necessary to provide the TNM staging for radical prostatectomy specimens. The subdividing of pathologically organ-confined disease whether the tumor involves <1/2 of one lobe (pT2a), involves >1/2 of one lobe (pT2b), or involves both lobes (pT2c) has been criticized [12, 13]. This aspect of the staging system will be changed in future revisions, and many urological pathologists do not subdivide pT2 tumor using the current system. Adenocarcinoma of the prostate is multifocal in more than 85% of cases. In many of these cases of bilateral and/or multifocal tumor, the other tumors are small, low-grade, and clinically insignificant. Consequently, the distinction between pathologic stages T2a and T2c may reflect several very different conditions: (1) a large single tumor nodule involving both sides; (2) separate large tumor nodules on each side; (3) dominant nodule on one side with multifocal minute tumor on the other side; or (4) bilateral minute multifocal tumor. Prognostically, there are no differences between the subdivisions of pT2 [12, 13]. Stage pT2b tumor almost never exists, as it is almost impossible for a tumor to involve greater than one-half of the lobe without involving the other lobe [12]. However, using the current staging system for subdividing pT2 tumor remains an option. It is now recommended by the American College of Surgeons, and will be updated in the new TNM Staging System such that pathologists do not fill in a "pM" (metastasis) category, as that is the domain of the clinician.

11. Submission of Tissue for Microscopic Evaluation in TURP and Radical Prostatectomy Specimens [14]. TURP specimens should be sampled with eight cassettes. In a younger man (e.g., <65 years old), consideration should be given to more extensive sampling. Some experts suggest submitting additional cassettes based on the weight of the TURP. In general, random chips are submitted. If an unsuspected carcinoma is found in tissue submitted and it involves 5% or less of the tissue examined, the remaining tissue is generally submitted for microscopic examination, especially in younger patients.

Radical prostatectomy specimens may be totally submitted or partially sampled in a systematic fashion. For partial sampling in the setting of a grossly visible tumor, the sections with visible tumor along with the entire apical and bladder neck margins and samples from the base of each seminal vesicle should be submitted. If there is no grossly visible tumor, a number of systematic sampling strategies may be used. One that yields excellent prognostic information involves submitting the posterior aspect of each transverse slice along with a mid-anterior block from each side. The anterior sampling detects tumors that predominantly involve the transition zone. The entire apical and bladder neck margins and base of each seminal vesicle should also be submitted.

Appendix

ASSOCIATION OF DIRECTORS OF ANATOMIC AND SURGICAL

Final Anatomic Diagnosis Checklist
Prostate Carcinoma
(Ver 1.4, 12-06)

Accession No.:	**Part No(s).**	**Date:**
Patient Name:		
ORGAN,	**SITE,**	**OPERATION:**
Prostate Gland		Radical Prostatectomy
Prostate Gland		Radical Prostatectomy with right and left pelvic and wlymphadenectomy
Other		

-Primary Tumor Diagnosis: *Required*

- Adenocarcinoma (not otherwise specified)
- Mucinous adenocarcinoma
- Prostatic duct adenocarcinoma
- Small cell carcinoma
- Adenosquamous carcinoma
- Transitional cell carcinoma
- Squamous cell carcinoma
- Other:_____

A. Gleason Score: ____+ ____ = ____/10 with tertiary pattern ____ (if present)

Gleason Grading System

1 Single, separate, closely packed, uniform fairly large glands with a margin delineating the edge of tumor

2 Single, separate fairly large glands that are less uniform and more loosely arranged, with a less definite margin

3 Single, separate, but variably sized and shaped glands that may be widely separated and have a poorly delineated margin
Sharply circumscribed, rounded tumor with a cribriform pattern of the same size as normal glands

4 Fused glands
Poorly formed glands
Irregular or large cribriform glands
Hypernephromatoid pattern

5 Single cells
Sheets of cells
Cords of cells
Tumor with central comedonecrosis

NOTE: It is required that only the dominant tumor nodule is assigned a Gleason score with an option to assign a Gleason score to other major tumor nodules. The dominant nodule is typically the largest tumor, which is also the tumor with the highest stage and highest grade. In the unusual occurrence of a non-dominant nodule (i.e. smaller nodule) that is of higher stage or highest grade, one should also assign a grade to that nodule.

B. Location of Tumor: Required

- Tumor predominantly involves the _____ lobe (specify laterality)
- Tumor involves both lobes.

NOTE: Only dominant tumor(s) should be categorized. Small multifocal tumor foci should not be factored in unless they are the only or highest grade or highest stage tumor in the prostate

C. Extent of Tumor

- Tumor is confined to the prostate (lacks extra-prostatic extension),
- Tumor demonstrates extra-prostatic extension with
  - unilateral extra-prostatic extension (specify laterality) .
  - bilateral extra-prostatic extension.
- Tumor invades into muscular wall of the seminal vesicle(s) (specify laterality)
- Extra-prostatic extension cannot be determined since the outer border of the prostate is microscopically not intact (capsular incision is present, _____ specify laterality)

-Margins of Excision: Required

(specify status of bladder base, apical margins, vas deferens margins, and peripheral margins)

- Bladder base margin is free of tumor
- Apical margin is free of tumor
- Bladder base and apical margins are free of tumor
- Vas Deferentia margins are free of tumor
- Tumor is present at the apical margin
- Tumor is present at the bladder base margin
- Tumor is present at left/right vasa deferens margin
- Tumor is present at the peripheral margin in an area of extra-prostatic extension at aspect of specimen (specify site (s) of involvement such as anterior, posterior, left, right, etc)
- Tumor is present at the peripheral margin in an area of capsular incision at aspect of specimen (specify site(s) of involvement such as anterior, posterior, left, right, etc)

NOTE: All of the following lymph node groups will not be identified in most cases. However, appropriate designations are provided below.

-Lymph Nodes, right pelvic:

A. Number examined _____
B. Number positive _____
C. Comment _____

-Lymph Nodes, left pelvic:

A. Number examined _____
B. Number positive _____
C. Comment _____

-Lymph Nodes, right obturator:

A. Number examined _____
B. Number positive _____
C. Comment _____

-Lymph Nodes, left obturator:

A. Number examined _____
B. Number positive _____
C. Comment _____

-Additional Tumor Features: Optional

A. **Tumor involves approximately _____ %** of the prostate gland (alternate measurements of tumor volume can be used).
B. **Vascular invasion:** Identified Not Identified
C. **Extent of extra-prostatic spread:** Focal Non-focal
D. **Extent of margin positivity:** Focal Non-focal

-Addition findings and comments:

- Prostatic Intraepithelial Neoplasia (PIN), high grade
- Adenosis
- Basal cell hyperplasia
- Atrophy
- Treatment related changes
- Other:

-Ancillary Studies: Optional

Special stains are performed, the results are as follows:

A. _____
B. _____
C. _____
D. _____

Interpretation _____

Immunohistochemical studies are performed, the results are as follows:

A. _____
B. _____
C. _____
D. _____

Interpretation:_____

-pTN Stage: Required

A. **Primary Tumor:**

pT2	Tumor confined within the prostate
pT2x (or pT2+)	Tumor is organ confined except in an area of capsular incision where it cannot be determined
pT3	Tumor extends out of the prostate
pT3a	Tumor demonstrates extra-prostatic extension without seminal vesicle invasion
pT3b	Tumor demonstrates extra-prostatic extension and invades seminal vesicle
pT4	Tumor is fixed or invades adjacent structures other than the seminal vesicles such as: bladder neck (grossly), external sphincter, rectum, levator muscles and/or is fixed to the pelvic wall

NOTE: A microscopically positive bladder neck margin is pT3

NOTE: If surgical margin(s) are positive for carcinoma, this should be indicated by the designation R1 in the pT stage: e.g., pT3(R1).

B. **Regional Lymph Nodes**:

PNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node or nodes

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ORIGINAL ARTICLE

TNM staging of midgut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system

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Abstract Criteria for the staging and grading of neuroendocrine tumors (NETs) of midgut and hindgut origin were established at the second Consensus Conference in Frascati (Rome) organized by the European Neuroendocrine Tumor Society (ENETS). The proposed tumor–node–metastasis (TNM) classifications are based on the recently published ENETS Guidelines for the Diagnosis and Treatment of gastroenteropancreatic NETs and follow our previous proposal for foregut tumors. The new TNM classifications for NETs of the ileum, appendix, colon, and rectum, and the grading system were designed, discussed, and consensually approved by all conference participants. These proposals need to be validated and are meant to help clinicians in the stratification, treatment and follow-up of patients.

Keywords Neuroendocrine tumors · Ileum · Appendix · Colon · Rectum · Staging · TNM · Grading · Mitotic index · Ki-67 index

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Background

Based on recognized differences in morphology, function and clinical behavior [1, 2, 21, 30], the current WHO classification provides a prognosis-oriented definition of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) [3, 5, 8, 12, 13, 34].

All GEP-NETs probably have a malignant potential, but their biological behavior differs from tumor type to tumor type [9, 10, 14–17, 22, 25, 26, 36]. Given their rarity [10, 14–17], correct diagnosis and appropriate treatment are often difficult in nonexpert settings and even for appendiceal "carcinoids," probably the best known GEP-NETs with the most benign behavior [31]. Recent data on ileal, appendiceal, and rectal carcinoids, also indicated several variables influencing survival and prognosis [6, 15, 29, 35].

Guidelines for the management of patients with GEP-NETs were developed by the recently established European Neuroendocrine Tumor Society (ENETS) [23, 37]. In two separate meetings a consensus was sought on these guidelines. The papers deriving from the first conference dedicated to foregut tumors, including a detailed tumor–node–metastasis (TNM)/staging and grading proposals, have been published meanwhile [4, 27]. The "Consensus Conference on the ENETS Guidelines for the Diagnosis and Treatment of Neuroendocrine Gastrointestinal Tumors, Part 2: Midgut and Hindgut Tumors" was held in Frascati (Rome, Italy) from November 1 to 4, 2006. In this paper, we present the TNM staging and grading proposals for pure NETs of the lower jejunum/ileum, appendix, and colon/rectum.

Materials and methods

Fifty-seven experts in the field of GEP-NETs from 18 different countries attended the consensus conference. The attendees represented all medical branches involved in managing patients with GEP-NETs. They formed four working groups according to their specific clinical expertise: (1) pathology and genetics (11 participants, all listed as

Table 1 Proposal for a TNM classification for endocrine tumors of lower jejunum and ileum

	TNM
T-primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor invades mucosa or submucosa and size ≤1 cm
T2	Tumor invades muscularis propria or size >1 cm
T3	Tumor invades subserosa
T4	Tumor invades peritoneum/other organs
For any T add (m) for multiple tumors	
N regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M	Distant metastasis
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1[a]	Distant metastasis

[a] M1 specific sites defined according to Sobin LH, Wittekind C [32].

Table 2 Disease staging for endocrine tumors of lower jejunum and ileum

Stage Disease stages	T-primary tumor	N-regional nodes	M-distant metastasis
Stage I	T1	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	Any T	N1	M0
Stage IV	Any T	Any N	M1

coauthors), (2) surgery (8 participants), (3) imaging and radiology (7 participants), (4) medicine and clinical pathology (31 participants, including the coauthor B.W.). Most of the participants also attended the first consensus conference held in Frascati in November 2005.

The conference was divided sequentially into five sessions devoted to specific topics on an anatomical basis (ileal well-differentiated NETs; appendiceal well differentiated NETs; colorectal well differentiated NETs; NETs metastatic to the liver; poorly differentiated neuroendocrine carcinomas of midgut and hindgut origin).

A working booklet with the ENETS guidelines text [23] and specific queries had been prepared in advance by the organizing committee. The work was organized as previously detailed [4, 27]. This procedure was followed for all five sessions. The TNM staging proposal was prepared by the pathology and genetics working group and amended and approved by the plenary session of the consensus conference. The grading system was mainly discussed and defined by the pathology and genetics working group.

Results and discussion

The consensus guidelines have been reported elsewhere. The TNM staging proposal for NETs of midgut and hindgut origin together with a grading system is intended to reflect, like its forerunner for the NETs of the stomach, duodenum and pancreas [27], the prognostic assessment by the pathologist. The intestinal NETs were separated into lower jejunum/ileum, appendix, and colon/rectum, but were not distinguished according to specific functional activity, main tumor cell type, or genetic background.

TNM staging proposal

The currently published TNM format was adopted as working template (see Tables 1, 2, 3, 4, 5, and 6) [32].

Tumor There is no proposed definition for in situ endocrine tumor of the jejunum, ileum, appendix, colon and rectum, because no specific precursor lesion has been described in the literature so far. For the lower jejunum and ileum, the size limits indicated for T1 and T2 are those defined for tumors of "benign behavior" and "uncertain behavior," respectively, according to the WHO site-specific clinico-pathological correlations [5, 8, 34]. For the appendix and colon and rectum tumors, lower size limits were defined for T1 and T2 based on current data [6, 15, 29]. For colon and rectum tumors, T1 was divided into T1A and T1B based on current information on the biology of tumors below 1 cm in size and between 1 and 2 cm [6].

Deeply invasive and large tumors are included in the T3 and T4 categories, taking into account site-specific features. For any T definition, the maximum tumor size should be reported and, in the case of multiple lesions, the largest one. The use of T3 category subdivision (pT3a, b, c, and d) according to distance below or higher than 5 mm from *muscularis propria* as proposed for the adenocarcinoma [33], could be of value. Its application could be implemented once data on endocrine carcinomas will be generated.

Lymph nodes N1 indicates the presence of any single or multiple metastases in the regional lymph node group, according to TNM rules. A minimum of 12 nodes should be identified in a surgical specimen, assessed and, when possible, named according to their location in relation to tumor. Although regional lymph node metastases are a negative prognostic factor in GEP-NETs [11], the signifi-

Table 3 Proposal for a TNM classification for endocrine tumors of the appendix

TNM	
T-primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor ≤1 cm invading submucosa and muscularis propria
T2	Tumor ≤2 cm invading submucosa, muscularis propria and/or minimally (up to 3 mm) invading subserosa/mesoappendix
T3	Tumor >2 cm and/or extensive (more than 3 mm) invasion of subserosa/mesoappendix
T4	Tumor invades peritoneum/other organs
N-regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M-distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1[a]	Distant metastasis

[a] M1 specific sites defined according to Sobin LH and Wittekind Ch [32].

Table 5 Proposal for a TNM classification for endocrine tumors of colon and rectum

TNM	
T-primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor invades mucosa or submucosa
	T1a size <1 cm
	T1b size 1–2 cm
T2	Tumor invades muscularis propria or size >2 cm
T3	Tumor invades subserosa/pericolic/perirectal fat
T4	Tumor directly invades other organs/structures and/or perforates visceral peritoneum
For any T add (m) for multiple tumors	
N-regional lymph nodes	
NX	Regional lymph node status cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M-distant metastases (subspecification as in small bowel)	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1[a]	Distant metastasis

[a] M1 specific sites defined according to Sobin LH and Wittekind Ch [32].

cance of the number of metastatic nodes is not yet known. Therefore, similar to the previous foregut TNM proposal, the N1 status in stage IIIB in Tables 1, 2, 3, 4, 5, and 6 has to be specified with regard to the number of lymph nodes involved to allow validation.

Distant metastasis M1 indicates the presence of any single or multiple metastases at any distant anatomical site (including nonregional nodes). As extrahepatic bone metastases are a negative prognostic factor [7, 21], it is recommended to specify the anatomical site of the metastasis according to the TNM classification rules (PUL, pulmonary; HEP, hepatic; OSS, osseous; etc.) [32].

Staging Stage I encompasses the T1 NETs with limited growth. Stage II identifies tumors that are larger in size or more invasive, either T2 or T3, although always in the absence of metastasis. At stage III, the increased malignancy refers either to invasion into surrounding structures (Stage IIIA) or to the presence of regional node metastases (Stage IIIB). Stage IV always implies the presence of distant metastases.

Table 4 Disease staging for endocrine tumors of the appendix

Stage Disease stages	T-primary tumor	N-regional nodes	M-distant metastasis
Stage I	T1	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	Any T	N1	M0
Stage IV	Any T	Any N	M1

Grading proposal

Grading Studies on well-differentiated NETs of midgut and hindgut origin have shown the usefulness of a grading system (see Table 7) [6, 35, 36]. Well-differentiated endocrine tumors with proliferative activity greater than 2%, but below that usually found in poorly differentiated endocrine carcinomas, may have a prognosis intermediate between the "2% NETs" and poorly differentiated carcino-

Table 6 Disease staging for endocrine tumors of colon and rectum

Stage Disease stages	T-primary tumor	N-regional nodes	M-distant metastasis
Stage IA	T1a	N0	M0
Stage IB	T1b	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	Any T	N1	M0
Stage IV	Any T	Any N	M1

Table 7 Grading proposal for (neuro)endocrine tumors of ileum, appendix, colon and rectum

Grade	Mitotic count (10HPF)*	Ki-67 index (%)**
G1	<2	≤2
G2	2–20	3–20
G3	>20	>20

* 10 HPF (High Power Field)=2 mm^2, at least 40 fields (at 40× magnification) evaluated in areas of highest mitotic density; ** MIB1 antibody; % of 2000 tumor cells in areas of highest nuclear labeling.

mas [18–20, 23, 24]. We decided to follow the same grading system proposal as that devised for foregut tumors, with the aim of distinguishing G2 from G1 and G3 GEP-NETs. The three tumor categories are defined as follows: G1, <2 mitoses per 2 mm^2 (10 high-power fields, HPF, 40× magnification) and/or Ki-67 index ≤2%; G2, 2–20 mitoses per 2 mm^2 and/or Ki-67 index between 3% (intended as >2%) and 20%; G3 with 21 or more mitoses per 2 mm^2 and Ki-67 index >20%.

The G1 and G2 well-differentiated NETs usually display diffuse and intense expression of the two general immunohistochemical neuroendocrine markers, chromogranin A and synaptophysin [28]. Punctate necrosis is per se indicative of a more aggressive tumor and points to a G2 or G3 status, which is then determined by the mitotic count and the proliferation fraction. G3 indicates a poorly differentiated neuroendocrine carcinoma with high mitotic counts/Ki-67 index, fields of necrosis, significantly reduced chromogranin A expression and intense staining for synaptophysin, meeting the current WHO histological criteria [5, 8, 34].

Mitotic count and Ki-67 index As for the foregut proposal, mitoses should be counted on hematoxylin and eosin stained slides in at least 40 HPF when possible. The mitoses should be assessed in areas where they are most frequent after a general slide survey. For Ki-67 assessment, the MIB1 antibody is recommended at the conditions that have been established at the laboratory in question. The Ki-67 index should be assessed in 2,000 tumor cells in areas where the highest nuclear labeling is observed (often but not exclusively at the tumor periphery).

Concluding remarks

The TNM staging system proposed here for midgut and hindgut NETs closely follows its forerunner for foregut tumors [27]. It has the same basis, i.e., the current WHO classifications of GEP-NETs, and results from a consensus conference held by specialists and practicing physicians involved in the management of patients with GEP-NETs. The grading system described here is substantially identical to that proposed for foregut NETs and again attempts to close the gap between the advances of the most recent WHO classifications and the need for a better prognostic assessment of NETs. These proposals, as well as those already published, await confirmation by clinicopathologic work.

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ORIGINAL ARTICLE

Value of staining intensity in the interpretation of immunohistochemistry for tumor markers in colorectal cancer

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Abstract The purpose of this study was to determine whether staining intensity in conjunction with the percentage of positive tumor cells should be used as an indicator of protein expression detected by immunohistochemistry. A tissue microarray of 1,197 colorectal cancers was immunostained for p53, Her2/neu, epidermal growth factor receptor (EGFR), adenomatosis polyposis coli (APC), and β-catenin. Immunoreactivity was described by the percentage of positive tumor cells (percent positivity) and by the staining intensity (weak, moderate, strong). The interobserver reproducibility of both was evaluated by two pathologists. The association of T stage, N stage, tumor grade, vascular invasion, and survival with percent positivity, staining intensity, and the combination of both was assessed. In univariate analysis, protein expression assessed by percent positivity resulted in 11 significant associations between the proteins and clinico-pathological features. Eight of these 11 were also demonstrated using only the degree of staining intensity. However, more than half of the associations identified by percent positivity alone were lost when staining intensity was also analyzed in combination with the percentage of positive tumor cells. A scoring method based on percent positivity, rather than on staining intensity, for p53, Her2/neu, EGFR, APC, and β-catenin is reproducible and appears to be sufficient for establishing associations of the selected tumor markers with most clinico-pathological features.

Keywords Colorectal cancer · Immunohistochemistry · Scoring system · Staining intensity

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Introduction

Diagnosis and research in colorectal cancer (CRC) routinely relies on the interpretation of protein expression detected by means of immunohistochemistry (IHC). The lack of standardized IHC scoring systems has led to a variety of unvalidated methods used to assess immunoreactivity. Scoring systems for tumor markers in CRC are usually based on a measure of the proportion of positive tumor cells and are often combined with a degree of staining intensity [3, 9, 12, 29]. It is recognized that the interpretation of staining intensity is not only highly subjective but may be affected by storage time, variation in protocols, and fixation procedures [2, 13]. Despite these concerns, staining intensity has become an integral component of many IHC scoring methods for tumor markers in CRC [7, 8, 10, 12, 23, 25–27].

We have recently shown that a descriptive, semiquantitative scoring system for IHC based on the percentage of immunoreactive tumor cells (percent positivity) provides a more complete assessment of the predictive and prognostic value of several tumor markers in CRC when compared to an evaluation system using "negative/positive" [16–19]. Additionally, we have studied the interobserver variability of this semiquantitative method among pathologists for several proteins namely, epidermal growth factor receptor

(EGFR), vascular endothelial growth factor, p53, Bcl-2, and apoptotic protease-activating factor 1 (APAF-1), in tumor biopsies and tissue microarray (TMA) punches and have shown that it is reproducible [30, 31, 34].

One of the most important advantages of a semiquantitative scoring method is that it allows the investigator to establish more biologically or clinically relevant cutoff scores for positivity for the protein and the outcome under study rather than relying on an often arbitrary threshold value, such as 10%, to describe a tumor as "positive." Such a method to ascertain cutoff scores has been recently proposed using receiver operating characteristic (ROC) curve analysis and has been applied, along with several other tumor markers, to EGFR and APAF-1 [32–34].

The purpose of this study was to determine whether staining intensity in conjunction with percent positivity should be used as an indicator of protein expression detected by IHC. The associations of p53-, Her2/neu-, EGFR-, adenomatosis polyposis coli (APC)-, and β-catenin-staining intensity with a range of clinico-pathological features, notably T stage, N stage, tumor grade, vascular invasion, and survival, were evaluated in 1,197 mismatch repair (MMR)-proficient CRC.

Materials and methods

Tissue microarray construction

A TMA of 1,420 unselected, nonconsecutive CRCs was constructed [24]. Briefly, formalin-fixed, paraffin-embedded tissue blocks of CRC resections were obtained. One tissue cylinder with a diameter of 0.6 mm was punched from morphologically representative tissue areas of each donor tissue block and brought into one recipient paraffin block (3×2.5 cm) using a homemade semiautomated tissue arrayer. The resulting TMA set comprised three slides.

Clinico-pathological data

The clinico-pathological data for all patients included T stage (T1, T2, T3, and T4), N stage (N0, N1, and N2), tumor grade (G1, G2, and G3), vascular invasion (presence or absence), and survival time [19].

Immunohistochemistry

Four-micrometer sections of TMA blocks were transferred to an adhesive-coated slide system (Instrumedics, Hackensack, NJ). Standard indirect immunoperoxidase procedures were used for IHC (ABC-Elite, Vector Laboratories, Burlingame, CA). One thousand four hundred and twenty CRCs were immunostained for mutL homolog (MLH)1 (clone MLH-1; dilution 1:100; BD Biosciences Pharmingen, San Jose, CA), MSH2 (clone MSH-2; dilution 1:200; BD Biosciences Pharmingen, San Jose, CA), and MSH6 (clone 44; dilution 1:400, Transduction Laboratories). After dewaxing and rehydration in deionized H_2O, sections were subjected to heat antigen retrieval in a microwave oven (1,200 W, 15 min) in 0.001 mol/L ethylenediamine tetraacetic acid at pH 8.0 for MLH1 and MSH2 and in 0.01 mol/L citrate buffer (pH 7.0) for MSH6. Endogenous peroxidase activity was blocked using 0.5% H_2O_2. The sections were incubated with 10% normal goat serum (Dako Cytomation, Missassauga, Canada) for 20 min and incubated with a primary antibody at room temperature (Her2/neu clone PN2A, DAKO, Denmark; p53 clone DO-7, 1:100; DAKO; clone β-catenin-1, dilution 1: 200; Dako Cytomation, APC clone C20, dilution 1:50; Santa Cruz, CA). Subsequently, sections were incubated with secondary antibody (K4005, EnVision+ System-HRP (AEC); Dako Cytomation) for 30 min at room temperature. For visualization of the antigen, the sections were immersed in 3-amino-9-ethylcarbazole+substrate-chromogen (K4005, EnVision+ System-HRP (AEC); Dako Cytomation) for 30 min and counterstained with Gill's hematoxylin. IHC for EGFR was performed using an automated stainer (EGFR clone 3C6, 3 mg/mL; Ventana Medical Systems, Tucson, USA) according to the manufacturer's instructions. TMA slides for each protein were stained on the same day under identical conditions.

MMR results

The 1,420 CRCs were stratified according to MMR status: (1) MMR-proficient tumors expressing MLH1, MSH2, and MSH6, (2) MLH1-negative tumors, and (3) presumed hereditary nonpolyposis colorectal cancer (HNPCC) cases demonstrating loss of MSH2 and/or MSH6 at any age or loss of MLH1 at age less than 55 years [11]. In population-based studies, the mean age of diagnosis of Lynch syndrome is around 55 years. By contrast, sporadic high-level microsatellite instability (MSI-H; MMR-deficient) CRC is far more age-related with a mean age of onset of around 75 years and few cases occurring younger than 60 years. A cutoff of 55 years was set as a reasonable compromise for distinguishing CRCs with loss of MLH1 into likely HNPCC syndrome vs likely sporadic. These immunohistochemical groupings showed a good fit with the known clinicopathological features associated with these subsets of CRC. Particularly, the MLH1-negative group was associated with advanced age, predilection for women and the proximal colon, large tumor size, and poor differentiation. The presumed HNPCC group was young and showed no gender difference, and there was a predilection for the proximal colon as compared with the MMR-proficient group. While it is possible that a small proportion of presumed sporadic MSI-H and HNPCC

cases were incorrectly assigned, the overall findings are likely to be valid in view of the large numbers of samples and the good fit with clinico-pathological features. Only MMR-proficient tumors were included in the study to ensure a homogeneous sample of tumors (N=1,197, 84.4%).

IHC evaluation

Immunoreactivity was evaluated in all 1,420 punches by one experienced pathologist. TMA punches with insufficient tissue or tumor for analysis were excluded from the study. Protein expression was scored in the nucleus for p53 and β-catenin and in the cytoplasm for APC. EGFR and Her2/neu positivity were scored in both cell membrane and cytoplasm. Immunoreactivity was assigned a score based on the proportion of positive tumor cells over total tumor cells (percent positivity) ranging from 0 to 100%. Staining intensity was evaluated as 0=negative, 1=weak, 2=moderate, and 3=strong. If the staining intensity was heterogeneous, then scoring was based on the greatest degree of intensity. MLH1, MSH2, and MSH6 were scored in the nucleus as negative (0%) and as positive (>0%).

Statistical analysis

Interobserver reproducibility of scoring percent positivity and staining intensity

To determine the interobserver reproducibility of percent positivity and staining intensity, a minimum of 100 CRC punches was evaluated by a second pathologist. The intraclass correlation coefficient (ICC) defined as the ratio of the between-subject variance over the between-subject+within-subject variances was used to determine the reliability of percent positivity for each protein. The ICC has previously been used to assess the agreement of IHC scores [14, 31]. An ICC of 0.7 or greater is considered sufficient to establish reproducibility [14]. The interobserver agreement of staining intensity (negative, weak, moderate, and strong) was determined using the kappa coefficient (κ) [15]. The overall κ coefficient measures the reliability of categorical data while taking into account the probability that both observers achieved the same scores by chance [1]. The weighted κ may be used as a measure of inter-rater agreement for ordinal variables and quantifies the relative difference between them. The greater the difference is between the scores, the lower the weighted κ. The interpretation of κ is commonly made as follows: values between 0.81 and 1.0 represent "almost perfect" agreement, 0.61 and 0.80 "substantial" agreement, 0.41 and 0.60 "moderate" agreement, 0.21 and 0.40 "fair" agreement, and 0 and 0.20 "slight" agreement [15].

Univariate analysis

Logistic regression analysis was used to determine the association of percent positivity or staining intensity with T stage (early=T1+T2/late=T3+T4), N stage (absence [N0] or presence [>N0] of lymph node involvement), tumor grade (low=G1+G2/high=G3), and vascular invasion (absence or presence). Survival analysis was carried out using Cox proportional hazards regression.

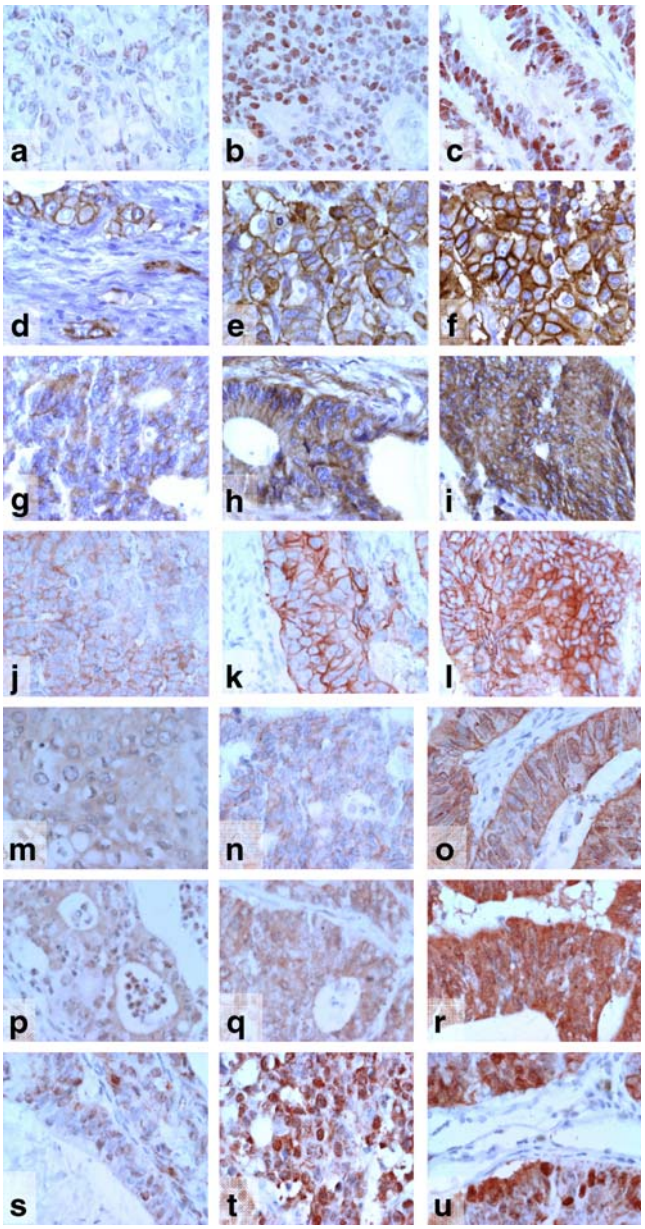

Fig 1 **a**–**l** Weak (*left*), moderate (*center*), and strong (*right*) staining intensity of nuclear p53 (40×; **a**–**c**), membranous EGFR (40×; **d**–**f**), cytoplasmic EGFR (40×; **g**–**i**), and membranous Her2/neu (40×; **j**–**l**). **m**–**u** Weak (*left*), moderate (*center*), and strong (*right*) staining intensity of cytoplasmic Her2/neu (40×; **m**–**o**), cytoplasmic APC (40×; **p**–**r**), and nuclear β-catenin (40×; **s**–**u**)

Table 1 Interobserver agreement for percent positivity (percent scores) measured by the intraclass correlation coefficient (ICC) and for staining intensity measured by the overall and weighted kappa coefficient (κ)

Protein	Measurements of interobserver agreement		
	Percent scores	Staining intensity	
	ICC	Overall κ (95%CI)	Weighted κ (95%CI)
p53 (n)	0.91	0.2 (0.11–0.3)	0.5 (0.43–0.57)
APC (c)	0.85	0.41 (0.23–0.58)	0.53 (0.36–0.68)
β-Catenin (n)	0.78	0.34 (0.22–0.47)	0.52 (0.39–0.64)
Her2/neu (m)	0.71	0.53 (0.36–0.69)	0.67 (0.52–0.82)
Her2/neu (c)	0.68	0.57 (0.43–0.71)	0.62 (0.47–0.76)
EGFR (c)	0.54	0.12 (0.0–0.25)	0.26 (0.14–0.39)
EGFR (m)	0.48	0.11 (0.01–0.22)	0.32 (0.22–0.42)

n Nuclear, *c* cytoplasmic, *m* membranous

Multivariate analysis

Staining intensity and percent positivity were entered into a multivariate logistic regression model for all binary outcomes, whereas multiple Cox proportional hazards regression was performed for survival analysis. Adjusted *P* values for percent positivity and staining intensity were obtained.

P values less than or equal to 0.05 indicate a significant association of percent positivity or staining intensity with the outcome. All analyses were carried out using SAS version 9.1 (The SAS Institute, Cary, NC).

Results

Interobserver reproducibility of percent positivity and staining intensity

The reproducibility of scores expressed as percent positivity was very strong for p53 (ICC=0.91). The ICCs for APC (ICC=0.85), β-catenin (ICC=0.78), and membranous Her2/neu (ICC=0.71) suggest excellent consistency of scores between observers. The interobserver agreement for cytoplasmic Her2/neu scores was only slightly lower than for its membranous counterpart (ICC=0.68). However, the reliability of membranous and cytoplasmic EGFR expression was only moderate to low. The same observers independently re-evaluated EGFR expression a second time by scoring the number of immunoreactive tumor cells without regard to localization of staining (i.e., by scoring either membrane and/or cytoplasmic EGFR). The interobserver agreement was significantly increased (ICC=0.86) [33].

The agreement of staining intensity between observers using the overall κ coefficient was only moderate for APC (κ=0.41) and membranous and cytoplasmic Her2/neu (κ=0.53 and 0.57, respectively). The reproducibility of staining intensity for β-catenin was determined to be fair (κ=0.34) while that of membranous or cytoplasmic EGFR was poor (κ=0.11 and 0.12, respectively). Analyses with the more generous weighted κ did little to improve these findings (Fig. 1; Table 1).

Association of protein expression with clinico-pathological features

Univariate analysis of percent scores

The evaluation of percent positivity with the clinico-pathological features identified significant associations between p53 and T stage (*P*=0.007) and tumor grade (*P*=0.005), membranous EGFR expression and T stage (*P*=0.005), N stage (*P*=0.002), tumor grade (*P*=0.014), and survival (*P*<0.001), and cytoplasmic EGFR expression and survival (*P*=0.01). APC expression was correlated with T stage (*P*=0.023) and β-catenin expression with tumor grade (*P*=0.035), vascular invasion (*P*=0.008), and survival (*P*=0.004). There were no associations of Her2/neu with any of the clinico-pathological features (Table 2).

Table 2 Association of protein expressed as percent positivity with clinico-pathological features by univariate analysis (*P* value)

	T stage	N stage	Tumor grade	Vascular invasion	Survival
p53 (n)	0.007	0.365	0.005	0.358	0.401
EGFR (m)	0.005	0.002	0.014	0.128	<0.001
EGFR (c)	0.193	0.692	0.382	0.522	0.01
Her2/neu (m)	0.857	0.299	0.551	0.475	0.932
Her2/neu (c)	0.322	0.629	0.235	0.139	0.15
APC (c)	0.023	0.138	0.976	0.205	0.208
β-Catenin (n)	0.619	0.073	0.035	0.008	0.004

n Nuclear, *c* cytoplasmic, *m* membranous

Table 3 Association of staining intensity with clinico-pathological features by univariate analysis (*P* value)

	T stage	N stage	Tumor grade	Vascular invasion	Survival
p53 (n)	0.026	0.127	0.017	0.991	0.61
EGFR (m)	0.005	0.009	0.049	0.192	<0.001
EGFR (c)	0.108	0.447	0.736	0.908	0.017
Her2/neu (m)	0.101	0.51	0.657	0.632	0.738
Her2/neu (c)	0.671	0.487	0.2	0.384	0.364
APC (c)	0.029	0.866	0.714	0.942	0.689
β-catenin (n)	0.424	0.541	<0.001	0.067	0.175

n Nuclear, *c* cytoplasmic, *m* membranous

Univariate analysis of staining intensity

All eight significant associations of staining intensity with the clinico-pathological features were previously established by percent positivitiy. The associations of membranous EGFR and tumor grade, β-catenin, vascular invasion, and survival were not present (Table 3).

Multivariate analysis of percent positivity and staining intensity

The combined analysis of percent positivity with staining intensity identified five associations between the proteins and clinico-pathological features that were previously found using only the percentage of positive cells. In addition, the remaining associations found to have statistical significance in univariate analysis of percent positivity (p53 with T stage, membranous EGFR with T stage, N stage, and tumor grade, APC with T stage, and β-catenin with tumor grade) were no longer observed in combination with the degree of intensity. In only 1 of the 35 analyses (2.8%) did staining intensity provide additional information about the association of the protein with the outcome (β-catenin with N stage; Table 4).

Discussion

The results of this study confirm that the evaluation of percent positivity for nuclear p53, cytoplasmic APC, nuclear β-catenin, and membranous and cytoplasmic Her2/neu expression is highly reproducible among pathologists. The assessment of EGFR expression resulted in strong interobserver agreement when cytoplasmic and/or membranous immunoreactivity were scored together rather than in their separate localizations. The intensity of staining was not reproducible for the proteins in this study.

In the univariate analysis, protein expression assessed as percent positivity resulted in 11 significant associations between the proteins and clinico-pathological features. Eight of these 11 were also demonstrated using only the degree of staining intensity. However, more than half of the associations identified by percent positivity alone were lost when staining intensity was also analyzed.

Scoring systems for tumor markers in CRC are typically based on some measure of the number of positive tumor cells and often combined with a degree of staining intensity. However, Atkins et al. [2] demonstrated using an anti-EGFR antibody in head and neck cancer, non-small cell lung carcinomas, and colorectal adenocarcinoma that the degree

Table 4 Association of IHC expressed as percent positivity and clinico-pathological features in multivariate analysis with staining intensity (adjusted *P* values)

	T stage	N stage	Tumor grade	Vascular invasion	Survival
p53 (n)	0.09[a]	0.643	0.01[b]	0.745	0.409
EGFR (m)	0.095[a]	0.19[a]	0.341[a]	0.676	0.021[b]
EGFR (c)	0.777	0.815	0.266	0.339	0.045[b]
Her2/neu (m)	0.438	0.32	0.46	0.804	0.693
Her2/neu (c)	0.125	0.7	0.327	0.322	0.167
APC (c)	0.137[a]	0.084	0.815	0.125	0.179
β-catenin (n)	0.446	0.025[c]	0.062[a]	0.036[b]	0.005[b]

n Nuclear, *c* cytoplasmic, *m* membranous
[a] Loss of significance
[b] Continued significance
[c] Gain of significance with the addition of staining intensity

of staining intensity varied by tumor type, was partially influenced by the choice of fixatives, and was inversely correlated with storage time of the unstained tissue sections. These factors in addition to the variation in IHC protocols inevitably contribute to the subjective nature of staining intensity. Contradictory results from different reports on the same tumor markers may be partially explained by this subjective assessment of immunoreactivity [23, 26].

We have previously demonstrated that a descriptive, semiquantitative scoring system based on the percentage of positive tumor cells (percent positivity) is reproducible and has several advantages over standard scoring methods based on predetermined cutoff scores [17–19]. First, this scoring system allows a more thorough assessment of the predictive or prognostic significance of tumor markers by evaluating the entire range of protein expression levels (from 0 to 100%). Moreover, by quantifying protein expression at the outset, more biologically and clinically relevant cutoff scores for tumor positivity can be established by, for example, performing ROC curve analysis [34]. This method has been used to select cutoff scores for tumor markers macrophage stimulating factor 1, Raf-1 kinase inhibitor protein, receptor of hyaluronic acid-mediated motility (RHAMM), APAF-1, EGFR, as well as for several others involved in transforming growth factor β signaling in CRC [4, 20, 21, 32, 33]. Additionally, the correlations between various proteins can be assessed. We have recently shown using this scoring method that the percentage of pERK-positive tumor cells is strongly associated with increases in RHAMM expression supporting the hypothesis of a RHAMM–mitogen-activated protein kinase interaction in MMR-proficient CRC [17]. By percent scoring, we have also described how classification and regression tree methods could be used to select proteins playing a role in predicting rectal tumor response to preoperative radiotherapy [30]. Finally, this descriptive scoring method avoids an often complex and interpretative composite scoring system based on the intensity of staining. One such method includes a four-tier scoring of the intensity of staining (0, 1+, 2+, 3+) coupled to either the mean percentage of positive tumor cells or to a categorical measure of the percentage of positive tumor cells (for example, 1–10%, 10–50%, and >50) [3, 6, 9, 12, 29]. A graded scoring system has also been used where the percentage of positive tumor cells is categorized (0=no positivity, 1=1–25%, 2=25–50%, 3=>50%) and multiplied by the degree of intensity (0, 1, 2, 3) to obtain a score that is then dichotomized into “low” or “high” expression (low=score<6 and high=score≥6) [26]. Others have reported only the degree of staining intensity regardless of the proportion of immunoreactivity or considered only staining intensities of 2+ or 3+ as positive for protein expression [24].

The purpose of this study was not to evaluate the prognostic significance of several tumor markers in CRC but rather to determine whether staining intensity is a useful indicator of immunoreactivity in colorectal tumors. In addition, the study focused on whether staining intensity provides independent information on the association of the protein with clinico-pathological features beyond that which can be obtained from the semiquantitative assessment of immunoreactivity. The markers included in this study are well established and/or of current interest as prognostic factors. They were selected to provide a range of subcellular localizations for scoring purposes (cytoplasm, cell membrane, nucleus) as well as representing both tumor suppressors (p53 and APC) and oncogenes (β-catenin, Her2/neu, and EGFR).

TMA technology allowed us to analyze more than 1,000 CRCs using only three slides. One tissue sample (0.6 mm) per tumor was obtained. Although it is argued that a single tissue core may not be representative of the whole tumor, results using one sample appear to approximate those from larger tissue sections as more samples are analyzed. In fact, even larger tissue sections may contain only a small fraction of the entire tumor mass (1/10,000) [24]. Goethals et al. [9] reported that four core biopsies are sufficient to account for tumor heterogeneity. Because the inclusion of several punches per tumor is not always possible, a larger series of tumors should compensate for tumor heterogeneity as was the case in this study. Several studies have shown well-established associations between molecular features and clinico-pathological endpoints in TMAs using only one spot per tumor [5, 22, 28]. Most importantly, evaluating a single tumor punch may lead to a more reliable analysis of interobserver agreement for both percent positivity and staining intensity, as precisely the same area of tumor is scored by each observer [24].

The results of our study suggest that staining intensity is not an independent measure of protein expression for the markers in this study. Additionally, the evaluation of immunoreactivity using a semiquantitative scoring method appears to be sufficient for establishing associations of the selected tumor markers with most clinico-pathological features.

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ORIGINAL ARTICLE

MAD1 (mitotic arrest deficiency 1) is a candidate for a tumor suppressor gene in human stomach

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Abstract Mitotic arrest deficiency 1 (MAD1) is a component of the spindle checkpoint factors that monitor fidelity of chromosomal segregation. We previously confirmed that the level of MAD1 protein was decreased in gastric carcinoma compared with non-tumoral mucosa by conducting proteome-based analyses (Nishigaki R, Osaki M, Hiratsuka M, Toda T, Murakami K, Jeang KT, Ito H, Inoue T, Oshimura M, Proteomics 5:3205–3213, 29). In this study, an immunohistochemical analysis was performed to examine MAD1 expression histologically in gastric mucosa and tumor. MAD1 was detected in the supranuclear portion of normal epithelial, intestinal metaplasia, and adenoma cells, but its expression was not restricted to any specific area in carcinoma cells. Lower levels of expression were noted in 16 (47.1%) of 34 adenomas and in 52 (60.5%) of 86 carcinomas, whereas all normal mucosae and intestinal metaplasias were grouped into cases with higher level of expression. Moreover, the expression of MAD1 was significantly lower in advanced carcinomas than early carcinomas and in intestinal than diffuse type, respectively ($P<0.05$). Exogenous expression of wild-type MAD1, but not the mutant MAD1, inhibited cell proliferation and resulted in G2/M accumulation in MKN-1, a gastric carcinoma cell line. Taken together, our findings suggest that the *MAD1* gene could be a candidate tumor suppressor gene and that down-regulation of MAD1 expression contribute to tumorigenesis in human stomach.

Keywords MAD1 · Gastric carcinoma · Tumor suppressor gene · Immunohistochemistry · Centrosome

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Introduction

Gastric carcinoma remains one of the major causes of cancer-related death worldwide [7], especially in Japan [37]. Although many studies have found various genetic and molecular alterations in patients with gastric cancer [36], the precise mechanisms behind the development and progression of this disease remain unclear. Recent advances in proteomic science have led to a better understanding of the molecular mechanisms of gastric carcinogenesis and its progression. The comprehensive analysis of protein expression profiles, proteomics is one approach in identifying novel diagnostic and/or prognostic markers. Previously, we identified various up- or down-regulated proteins specific to gastric carcinoma by proteomic analysis using human-

resected stomach tissue [29]. Of these proteins, mitotic arrest deficiency 1 (MAD1) was identified as a cancer-specific down-regulated protein. MAD1 is a component of the spindle assembly checkpoint (SAC), which includes other microtubule-interacting proteins (BUB family proteins, CENP family proteins, MAD2, Mps1, Nuf2, HEC1, Zwint-1, ZW10, etc…). SAC proteins preferentially localize to kinetochores of unaligned chromosomes and contribute to the production of a diffusible signal that inhibits mitotic cell cycle progression from metaphase to anaphase [3, 11, 31]. This signal delays anaphase by inhibiting the activity of the anaphase-promoting complex/cyclosome (APC/C), a multisubunit E3 ubiquitin ligase required for the degradation of securin and subsequent activation of separase, which is necessary for the cleavage of cohesin and the separation of sister chromatids [3, 30, 41]. Therefore, it stands to reason that a down-regulation of MAD1's function might be associated with carcinogenesis in human stomach via chromosomal instability. As yet, little has been reported regarding the functions of MAD1 in human cancers. Moreover, the expression and distribution of MAD1 in gastric carcinoma remain unclear.

In this study, we examined the histological expression and distribution of MAD1 in human gastric non-neoplastic mucosae, adenomas, and carcinomas. In addition, we also investigated the mechanism by which MAD1 expression suppressed cell proliferation and verified whether *MAD1* has functions as a tumor suppressor gene in human gastric carcinoma cells.

Materials and methods

Clinical samples

Histological examinations were performed on 86 surgically resected gastric adenocarcinomas and 34 gastric adenomas obtained by endoscopic mucosal resection. In all cases, histological type was classified according to the criteria of the Japanese Gastric Cancer Association [16]. All specimens were extracted from the files of the Division of Organ Pathology, Faculty of Medicine, Tottori University and affiliated teaching hospitals. Approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Tottori University (approval number 283). All specimens were fixed with 10% formalin and embedded in paraffin. Three-micrometer thick sections were examined using immunohistochemistry.

Immunohistochemistry

Dewaxed paraffin-embedded sections were immunostained using the streptavidin–biotin peroxidase complex (SAB) method with a HISTOFINE SAB-PO (M) Immunohistochemical Staining Kit (Nichirei) [12]. As primary antibodies, a mouse monoclonal antibody raised against MAD1 [18] and a goat polyclonal antibody raised against γ-tubulin (Sigma) were used. Immunoreactions were visualized with diaminobenzidine and the sections were counterstained with hematoxylin. MAD1 immunoreactivity was classified into two groups: lower and higher levels of expression. A tumor was judged to have a higher level of expression when at least 10% of the tumor cells or epithelia were immunoreactive. The correlation between MAD1 expression and each histological factor was evaluated using Mann–Whitney's *U* test. $P<0.05$ was considered as statistically significant.

Cell culture

Seven human gastric carcinoma cell lines (MKN-1, MKN-7, MKN-28, MKN-45, MKN-74, TMK-1 and KATO-III) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 292 μg/ml L-glutamine at 37°C in 5% CO_2.

Trichostatin A (TSA, Sigma) and 5-aza-2′-deoxycytidine (Aza, ICN Biochemical) were kept as a 3.3-mM stock solution in ethanol and a 1 mM stock solution in water, respectively, and added to the medium at a final concentration of 500 nM and 1 μM, respectively.

RT-PCR single-strand conformation polymorphism

Mutations in the coding regions of *MAD1* were examined in the seven gastric carcinoma cell lines. Total RNA was isolated and cDNA was synthesized as described previously [9]. The polymerase chain reaction (PCR)–single-strand conformation polymorphism (SSCP) and direct sequencing were performed as described previously [39].

Detection of MAD1 mRNA expression by RT-PCR

The expression level of MAD1 messenger RNA (mRNA) after treatment of Aza or TSA was examined in the seven gastric carcinoma cell lines. Total RNA was isolated, and complementary DNA (cDNA) was synthesized as described previously [9]. The sequences of primers were 5′-ggagaaaatgcagatggagctgagt-3′ and 5′-gagatcctcccttcagtgc-3′, and 28 cycles of PCR were performed. The annealing temperature was at 60°C, and PCR products were confirmed by 2% agarose-gel electrophoresis, followed by ethidium bromide staining.

SDS-PAGE and Western blot analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed as described previously [9]. The membranes were blotted with

the anti-MAD1 monoclonal antibody (1:2,000) [18] or with the anti-β-actin monoclonal antibody (1:2,000; AC-15, Sigma). Signals were visualized with an enhanced chemiluminescence system (ECL Detection System; Amersham Pharmacia Biotech).

Immunoprecipitation

MKN-1, MKN-28, and MKN-45 cells were lysed in immunoprecipitation buffer (25 mM Tris pH 7.5, 100 mM NaCl, 2 mM EDTA, and 0.5% Triton X-100) with protease inhibitors (1 mM PMSF, 10 μg/ml Leupeptin, and 10 μg/ml Aprotinin). The extracts were immunoprecipitated with anti-MAD1 polyclonal antibody [18] or with anti-γ-tubulin monoclonal antibody (Sigma). The precipitates were examined by SDS-PAGE and Western blotting using the two antibodies described above.

Plasmid vector and generation of stable cell lines

To generate mammalian expression vectors for enhanced green fluorescent protein (EGFP)-tagged MAD1, N-terminus-deleted MAD1 (MAD1 ΔN) and C-terminus-deleted MAD1 (MAD1 ΔC), cDNAs encoding a full-length MAD1 (1–718aa), the C-terminus only (295–718), and the N-terminus only (1–303) were produced by PCR from human MAD1 cDNA obtained from ATCC (Fig. 1). The primer sequences used in the constructions are available upon request. Fragments of the wild-type or mutated MAD1 were inserted into pEGFP-C3 (Clontech) at the *Bgl*II and *Eco*RI sites.

MKN-1 cells expressing stable levels of the above genes were generated by transfection with the plasmid vectors using Trans IT-LT1 Polyamine Transfection Reagents (TaKaRa) according to the manufacturer's instructions. Cells were selected for 2 weeks in the presence of 500 μg/ml G418 (Nakarai Tesque).

Cell viability and flow cytometry

Cell viability was measured using Cell Counting Kit-8 (Dojindo) according to the manufacturer's instructions. Results are presented as the mean±SD. Comparisons were made using Student's *t* test. $P<0.05$ was considered statistically significant. To analyze the DNA content, a flow cytometric analysis for EGFP and propidium iodide fluorescence was performed using an EPICS-ELITE 4.0 (Coulter) as described previously [13]. Proportions of cells in the G1, S, and G2/M phases of the cell cycle were determined with the WinMDI 2.8 software program (freeware).

Immunofluorescence microscopy

MKN-7 cells were grown on chamber slides and were fixed with 4% paraformaldehyde at 4°C for 20 min followed by 0.5% Triton X-100 in phosphate-buffered saline (PBS) at 4°C for 20 min. The slides were then incubated with the primary antibodies: rabbit polyclonal anti-MAD1 antibody [18], mouse monoclonal anti-γ-tubulin antibody (sigma), mouse monoclonal anti-GM130 antibody (BD Biosciences), and mouse monoclonal anti-KDEL antibody (Stressgen); followed by flourescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG antibody (Vector Laboratories) and Alexa594-conjugated goat anti-mouse IgG antibody (Molecular Probes). The MKN-7 cells were viewed under a Leica confocal microscope (TCS SP2), and software (Leica confocal software) was used to produce images.

Results

Expression of MAD1 protein in normal gastric mucosa, intestinal metaplasia, adenoma, and carcinoma

Immunohistochemistry was performed to examine the histological expression of MAD1 in human gastric mucosae and carcinomas. In the normal gastric mucosa, frequent MAD1-positive cells were distributed among surface mucous cells and isthmus cells, but such cells were rare in fundic and pyloric glands, especially in deeper zones. Immunoreactivity of MAD1 protein was noted in the

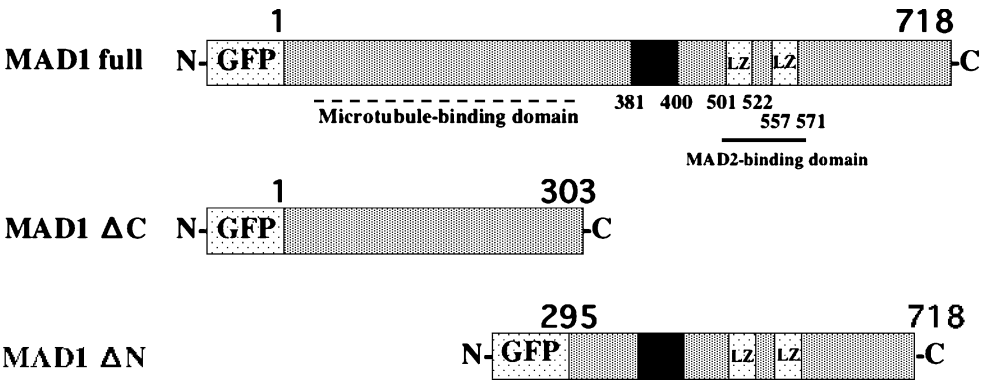

Fig. 1 Schematic representation of full-length MAD1 (MAD1 full), C-terminus-deleted MAD1 (MAD1 ΔC) and N-terminus-deleted MAD1 (MAD1 ΔN). *GFP* Green fluorescent protein, *NLS* nuclear localizing signal, *LZ* leucine zipper

supranuclear portion of the cells in these areas (Fig. 2a), as well as in intestinal metaplasia and adenoma cells (Fig. 2b,c). On the other hand, the subcellular distribution of MAD1 was not restricted to a specific area in carcinoma cells (Fig. 2d). In addition, little immunoreactivity was seen in mesenchymal cells including lymphocytes.

Table 1 shows the frequency of lower expression levels of MAD1 in gastric adenomas and carcinomas. The frequency was significantly higher in carcinomas than in adenomas ($P<0.05$). Moreover, the frequency was significantly increased in the 57 advanced carcinomas than in the 29 early carcinomas and in the 46 intestinal types than 40 diffuse types, respectively ($P<0.05$). On the other hand, none of the normal gastric mucosae or intestinal metaplasia lesions were classified as having lower expression levels (data not shown). These findings suggest that a down-regulation of MAD1 expression is associated with tumorigenesis and cancer progression in human stomach.

Subcellular distribution of MAD1 in gastric epithelia and carcinoma cells

Next, we wished to identify the subcellular distribution of MAD1 in gastric epithelial cells and carcinoma cells. We wanted to do this because the histological studies revealed that MAD1 protein was located in the supranuclear portion of epithelial and adenoma cells, while the immunoreactivity pattern was altered in carcinoma cells. We surmised that the distribution of MAD1 in gastric epithelial cells could be at centrosome, Golgi apparatus, or endoplasmic reticulum (ER) based on the histological findings. To define localization more precisely, γ-tubulin, GM130, and KDEL were used as characteristic markers for centrosome, Golgi apparatus, and ER, respectively. Endogenous expression of these proteins was detected and visualized by dual-colored fluorescent immunocytochemistry using specific antibodies. As shown in Fig. 3a, MAD1 was detected as green and γ-tubulin was detected as red, respectively. All of the green signals were concordant with the red signals when the two were merged. A few red signals (γ-tubulin) were observed alone, although no green signal was detected alone. By contrast, the immunoreactive pattern of MAD1 was discordant with that of GM130 or KDEL (data not shown). These cytological findings suggested that MAD1 forms a complex with γ-tubulin at the centrosome.

To confirm the above hypothesis biochemically, immunoprecipitation and immunoblotting were performed using antibodies against MAD1 and γ-tubulin. MAD1 protein

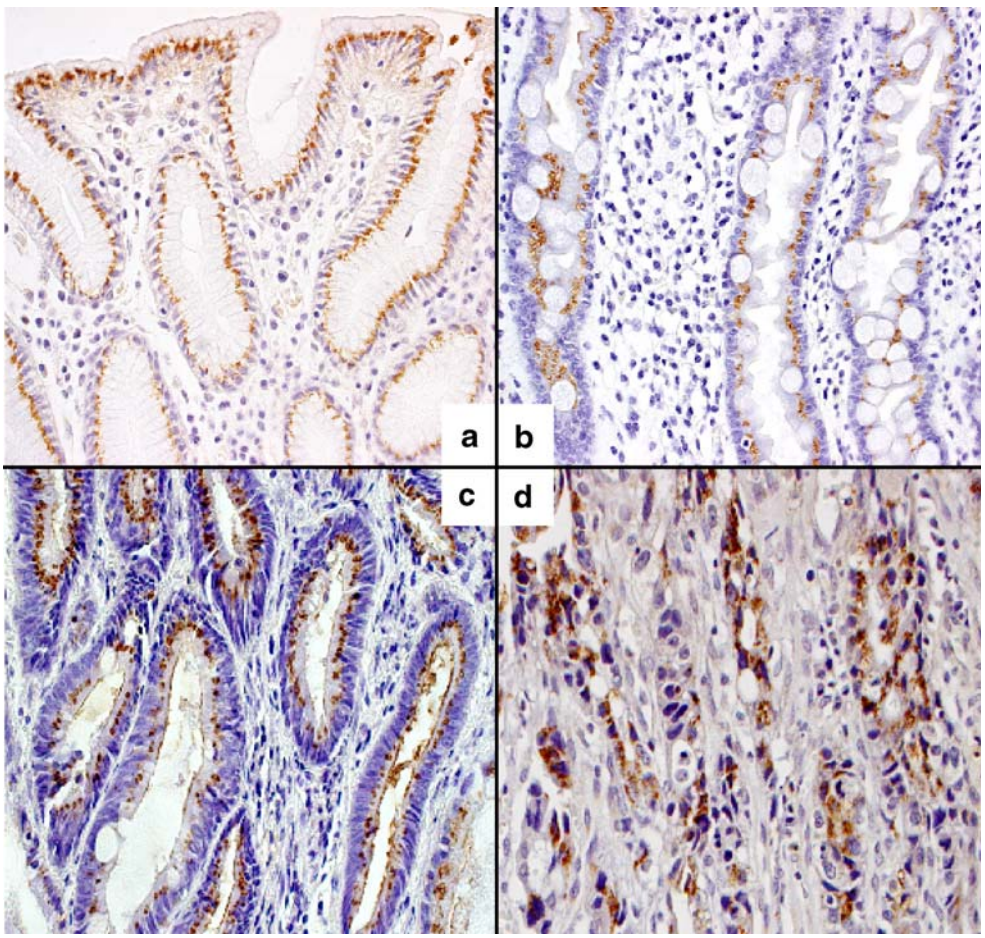

Fig. 2 MAD1 expression in normal gastric mucosa (**a**), intestinal metaplasia (**b**), adenoma (**c**), and adenocarcinoma (**d**). Immunoreactivity of MAD1 is noted in the supranuclear portion in **a**, **b**, and **c**. Immunoreactivity of MAD1 is not restricted to a specific area in carcinoma cells (**d**)

Table 1 Expression of MAD1 in gastric tumors

	Lower expression cases	*P* value
Adenoma (*n*=34)	16 (47.1%)	0.05
Carcinoma (*n*=86)	52 (60.5%)	
Early (*n*=29)	14 (48.3%)	0.05
Advanced (*n*=57)	38 (66.7%)	
Intestinal type (*n*=46)	32 (69.6%)	0.05
Diffuse type (*n*=40)	20 (50.0%)	

was revealed to coprecipitate with γ-tubulin protein, and vice versa in MKN-1, MKN-28 and MKN-45 cells, respectively (Fig. 3b). These results are consistent with MAD1 localization at centrosome not only in gastric carcinoma cell lines but also in non-neoplastic epithelia. Immunohistochemistry was performed to reveal the distribution of MAD1 and γ-tubulin using serial sections of resected stomach. Interestingly, most of the immunoreactivity was found with γ-tubulin in both gastric epithelial and carcinoma cells (Fig. 3c).

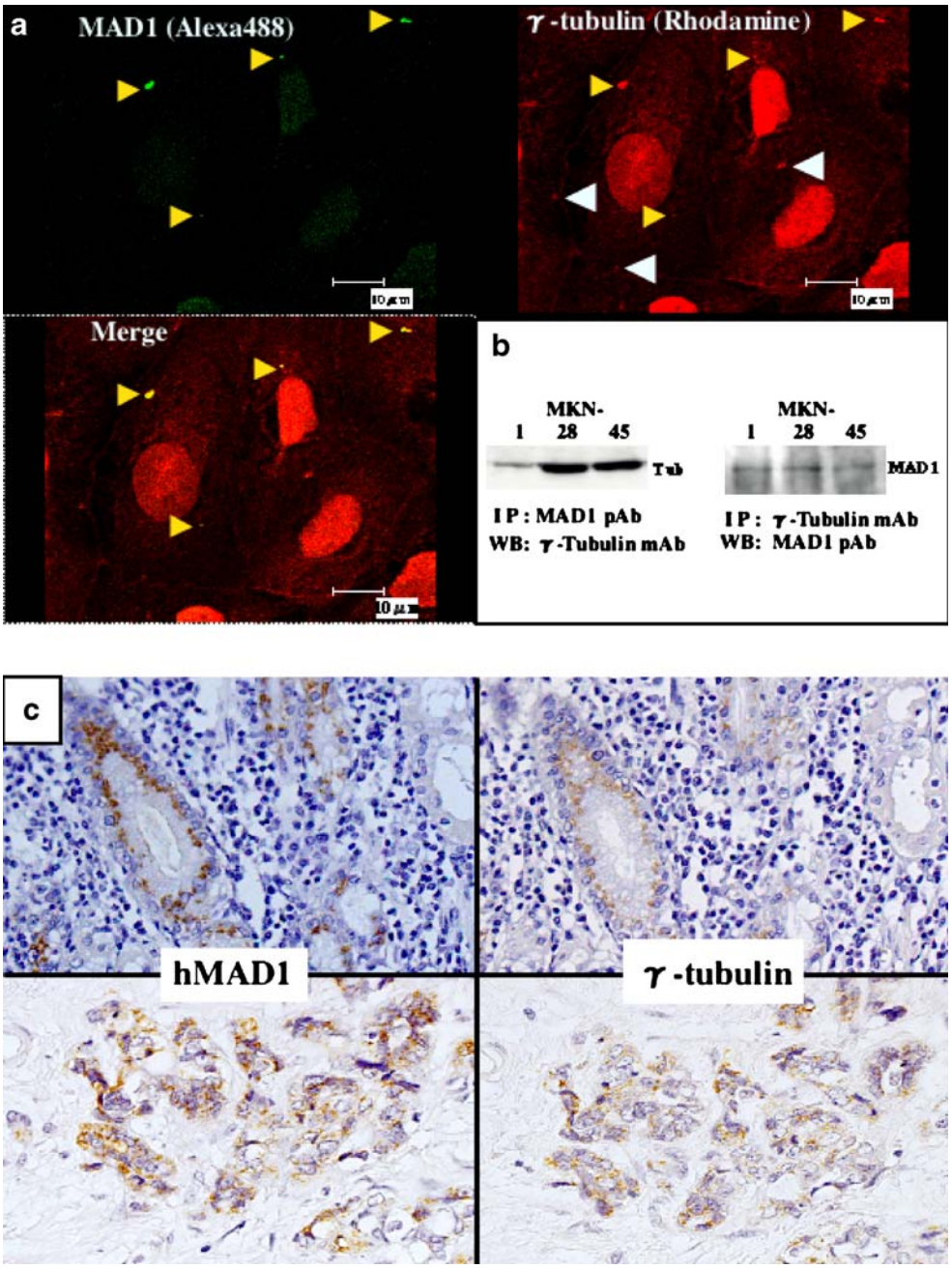

Fig. 3 Association of MAD1 with γ-tubulin. **a** Colocalization of MAD1 and γ-tubulin assessed by confocal microscopy in MKN-7 cells. Immunostaining with anti-MAD1 antibody (*green*) and with anti-γ-tubulin antibody (*red*), areas of colocalization are in yellow (*arrows*). A few red signals (γ-tubulin) are observed alone (*arrowheads*), whereas no green signal (MAD1) is detected alone. **b** Association of endogenous MAD1 with γ-tubulin in cells. Lysates of MKN-1, MKN-28 and MKN-45 cells were subjected to immunoprecipitation with anti-MAD1 pAb or anti-γ-tubulin mAb, fractionated by SDS-PAGE, and immunoblotted with anti-γ-tubulin mAb or anti-MAD1 pAb, respectively. **c** Immunohistochemical analysis of MAD1 and γ-tubulin in non-tumoral gastric mucosa (*upper panels*) and gastric carcinoma (*lower panels*) using serial sections

Genetic vs epigenetic alteration of the MAD1 gene in human gastric carcinoma cell lines

To determine whether human gastric carcinoma cell lines bear mutations within the *MAD1* coding region, seven cell lines were screened by SSCP and direct sequencing. We found a C to T transition at position 663C; this change is without an amino acid alteration (His to His) in MKN-1 cells, and we observed a G to A transition at position 1673G in which arginine 558 was converted to histidine in MKN-1, MKN-45 and MKN-74 cells. These genetic changes, however, are established polymorphisms and do not represent mutations specific for cancer, according to a previous report [39].

Then, we next focused on epigenetic changes as explanations for reduced MAD1 expression. Epigenetic silencing of gene expression can be due to hypermethylation of the gene promoter and/or acetylation of chromatin-associated histones. Aza, an inhibitor of DNA methyltransferase, and TSA, an inhibitor of histone deacetylase, can reactivate gene expression when hypermathylation of CpG islands in the MAD1 promoter or acetylation of gene-associated histones is the cause of reduced expression [1]. As shown in Fig. 4a, the expression of MAD1 protein was consistently reactivated by treatment with either Aza or TSA in all seven cell lines compared to non-treated counterparts. The expression of MAD1 mRNA was also clearly up-regulated by these treatments in MKN-28 and MKN-74 (Fig. 4b). These results suggest that both DNA methylation and histone acetylation normally participate in regulating *MAD1* gene expression.

Exogenous expression of MAD1 suppresses MKN-1 cell proliferation

The cell cycle regulatory activity of MAD1 was examined next using a cell proliferation assay. Using MKN-1, we selected for the stable expression of several MAD1-expressing cell lines. In G418 selected clones, the number of proliferating cells was monitored by Cell Counting Kit-8. As shown in Fig. 5a, proliferation of MKN-1 cells expressing wild-type MAD1 (MKN-1/MAD1 full) was significantly slowed compared to the other clones ($P < 0.01$). The growth curve of MKN-1/MAD1 ΔN was similar to that of the control clone (MKN-1/GFP). Although the growth rate of MKN-1/MAD1 ΔC was slightly suppressed compared to that of MKN-1/GFP or MKN-1/MAD1 ΔN, no significant difference was ascertained.

To understand the mechanisms of MAD1's growth suppression, DNA contents were examined by flow cytometry. Figure 5b shows that exogenous expression of wild-type MAD1 apparently increased cells that accumulated in the G2/M phase. Induction of apoptosis generally results in an accumulation signal in the sub-G1 peak. In our experiments, no evidence of apoptosis was induced by MAD1 expression in MKN-1 cells.

Discussion

In this paper, we report that MAD1 expression was observed in epithelial cells and tumor cells but not in mesenchymal cells including lymphocytes. Hence, the

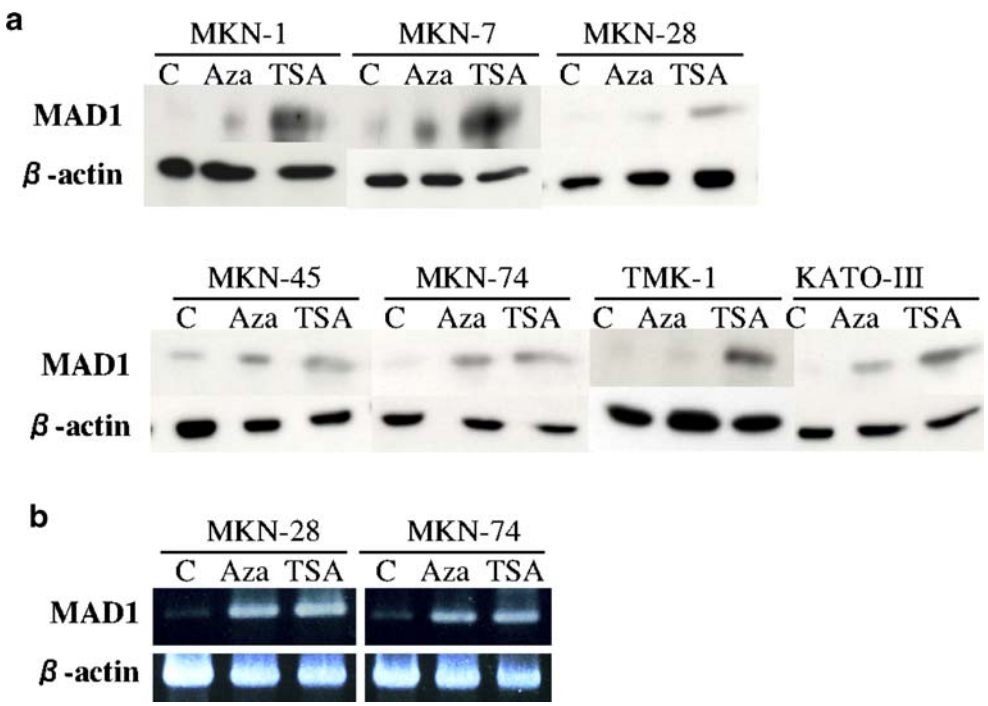

Fig. 4 Expression of MAD1 protein (**a**) or mRNA (**b**) were reactivated by treatment with Aza or TSA in human gastric carcinoma cell lines. Cells were cultured for 48 h in the presence of Aza (500 nM 5-aza-2′-deoxycytidine treatment) or TSA (1 μM Trichostatin A treatment). **c** Untreated control

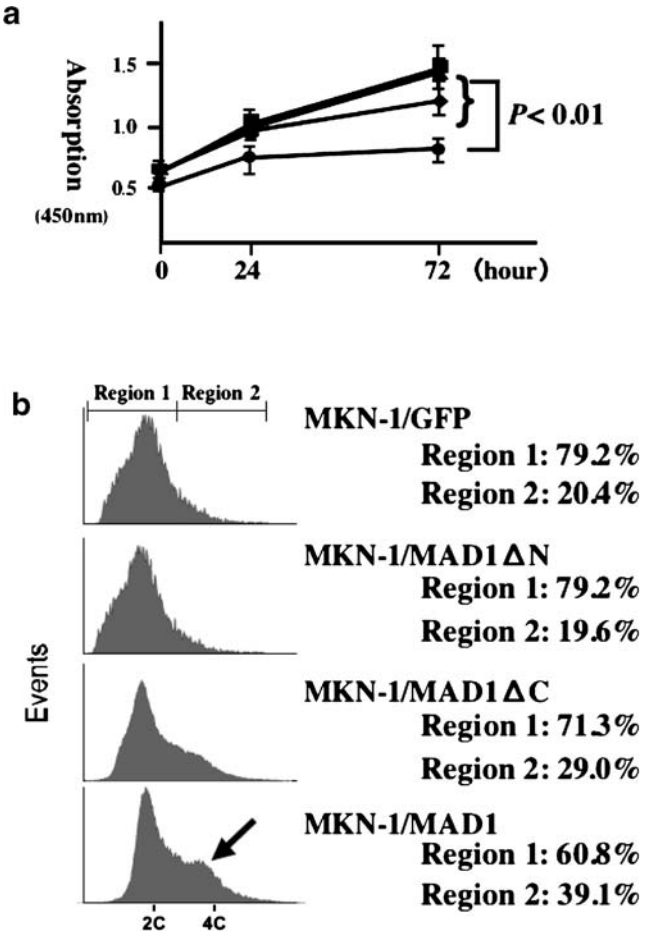

Fig. 5 Suppression of the proliferation of MKN-1 cells by transfection of *MAD1* gene. **a** Growth curves of the cell lines: MKN-1/MAD1 full (*filled circle*), MKN-1/MAD1 ΔC (*filled diamond*), MKN-1/MAD1 ΔN (*filled triangle*) and MKN-1/GFP (*filled square*), respectively. **b** The DNA content of each cell was measured by flow cytometry. Increased cell accumulation in G2/M phase (indicated by *arrow*) was observed in MKN-1 cells transfected with the wild-type *MAD1* gene

down-regulation of MAD1 expression identified by proteomics indicates that the expression level of MAD1 was obviously reduced in the gastric carcinoma cells compared to non-neoplastic epithelial cells. All of our non-tumor mucosae were classified as higher MAD1 expression cases, while about half of the gastric adenomas and two-third of the carcinomas fell into the lowered expression category. In addition, MAD1 expression was lower in advanced carcinomas compared to early carcinomas. There have been reports that MAD1 is required to recruit MAD2 to kinetochores and that a MAD1–MAD2 complex at kinetochores is important for SAC function [2, 4, 18, 26, 27, 35]; thus, our observed the down-regulation of MAD1 expression could explain one reason for tumorigenesis and cancer progression in human stomach.

Histological examinations also revealed that more MAD1 protein was present in the diffuse type than in the intestinal type carcinomas. Kienitz et al. [21] reported that partial repression of MAD1 resulted in chromosomal aneuploidy in a human colon cancer cell line, indicating that a reduction in MAD1 function contributes to chromosomal numerical aberrations. Recently, Iwanaga et al. [14] have shown that mice heterozygously knocked out for one MAD1 allele have high incidence of constitutive tumors. Several reports have also described that intestinal type gastric carcinomas had a significantly higher rate of aneuploidy than diffuse type carcinomas [17, 22, 23, 24, 32]. Taken together, the findings collectively support an interpretation that chromosomal instability in gastric carcinoma cells might be attribute, in part, to reduced MAD1 expression. It would be informative in future studies to elucidate how loss of function in spindle checkpoint proteins, including MAD1, leads prospectively to in vivo aneuploidy.

Our present work contributes to an understanding of reduced MAD1 expression in human gastric carcinoma cells by examining genetic and epigenetic regulation. To date, no data are available on genetic or epigenetic disorder of *MAD1* gene in human stomach. Based on direct sequencing, we found that four nucleotide transitions resulting conserved, H221H, and non-conserved, R558H, amino acids were detected in coding region of MAD1 protein in MKN-1, MKN-45, MKN-74 cells. These genetic changes have been reported as polymorphisms and are unlikely to be cancer-specific alterations [39]. Interestingly, Iwanaga et al. [15] previously identified a single nucleotide polymorphism at codon 558 located within the second leucine zipper in MAD1 in human cells. They reported that MAD1H558 was less proficient than MAD1R558 in binding MAD2 and in enforcing mitotic arrest. Therefore, a polymorphism such as R558H in MAD1 might be an at-risk form that contributes to attenuate spindle checkpoint function in human gastric epithelial cells.

The sequencing analyses revealed no causal mutations reducing MAD1 expression in human gastric cell lines. We, therefore, examined whether epigenetic disorders could explain reduced MAD1 expression. Because *MAD1* was reactivated by Aza or TSA treatment, we inferred that MAD1 expression could normally be under epigenetic control. Recently, it has been reported that a number of tumor suppressor genes, such as *RUNX3* [25], *p16* [38], *DCC* [33], *hMLH1* [6], *DAP kinase* [19], *RASSF1A* [5], *TSLC1* [10], among others are silenced by promoter hypermethylation in gastric carcinoma. Our findings suggest that MAD1 could also have tumor suppressor function and may be similarly regulated as other tumor suppressors through epigenetic mechanisms.

One of our additional salient observations is that MKN-1 cell proliferation was inhibited by exogenous expression of wild-type MAD1 resulting in accumulation in the G2/M phase. Tunquist et al. [40] showed that the microinjection

of MAD1 protein into *Xenopus laevis* embryos caused metaphase arrest. Thus, the suppression of cell growth by the transduction of wild-type MAD1 might be explained by M phase arrest due to activation of the spindle checkpoint function. Because little growth arrest function was seen with N- or C-terminus-deleted MAD1 in MKN-1 cells, these results suggest that full-length MAD1 is required for spindle checkpoint function.

Our present study revealed preferential subcellular distribution of MAD1 at centrosome in the interphase non-neoplastic epithelia and carcinoma cells. Shah et al. [34] reported that MAD1 protein and the other spindle checkpoint factors, is located at kinetochores in prometaphase, and MAD1 is released from kinetochores and relocalized to spindle poles after prometaphase. These results suggest that MAD1 protein found at centrosome in interphase presumably inactive for function. Further studies are needed to elucidate the relationship between sequential subcellular localization and MAD1's SAC activity.

We note with interest that the subcellular distribution of MAD1 in gastric carcinoma cells is extremely different from that in normal epithelial cells. A supranuclear localization of MAD1 protein was noted in normal epithelial cells, intestinal metaplasia, and adenoma. Normal cells generally have one centrosome, duplicated in S phase. By contrast, numerical and distributional abnormalities of the centrosomes have been reported in a variety of solid tumors [8, 20, 28, 42]. The altered immunoreactive MAD1 pattern in gastric carcinoma cells might reflect numerical and distributional abnormalities in the centrosomes. We infer these findings to support that (1) a down-regulation of MAD1 expression might be associated with a disruption to the distribution and/or function of the centrosome, which can contributed to gastric tumorigenesis, and (2) the histological examination of MAD1 protein might assist in distinguishing between gastric adenoma and carcinoma.

In conclusion, our histological examination revealed that the expression of MAD1 protein is down-regulated in gastric tumors probably via epigenetic alteration. This is the first report linking MAD1 abnormality with gastric carcinomas. Because exogenous expression of MAD1 suppressed the proliferation of MKN-1 cells, we suggest that the *MAD1* gene exhibit tumor suppressor-like activity in human stomach.

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ORIGINAL ARTICLE

Morphologic features in the regenerating liver—a comparative intravital, lightmicroscopical and ultrastructural analysis with focus on hepatic stellate cells

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Abstract Different cell types play a role in the liver regeneration. The present study reveals morphological key steps of liver regeneration by correlating intravital, light, and electron microscopic with immunohistochemistry results focusing on hepatic stellate cells (HSCs). In Lewis rats, liver regeneration was induced by a 2/3-hepatectomy. Animals (n=7 each) were killed after 0, 1, 2, 3, 4, 7, and 14 days. Morphological features were investigated by light microscopy, immunohistochemistry [α-smooth muscle actin (α-SMA), Desmin, vascular endothelial growth factor (VEGF)/VEGF receptor, Ki-67, ssDNA], intravital microscopy (sinusoid density, number of hepatocytes, and HSC), and electron microscopy focussed on cell-to-cell interactions. During liver regeneration, HSC were activated at day 3 showing a loss of autofluorescence and simultaneously an increased α-SMA expression and direct cell contact to hepatocytes. HSC activation was followed by increasing VEGF expression and sinusoid density. After 14 days, liver architecture and ultrastructure was restored and HSCs were deactivated showing decreased α-SMA expression as well as increased apoptosis and no more direct cell contact to hepatocytes. HSCs play a central role in the regenerating liver by governing angiogenesis and extracellular matrix remodeling. A direct cell contact to hepatocytes seems to be essential for HSC activation, whereas deactivation is accompanied by loosening of hepatocyte contact and increased apoptosis.

Keywords Liver regeneration · Hepatic stellate cells · Angiogenesis · Electron microscopy · Intravital microscopy · Light microscopy

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Introduction

The liver has the remarkable capability to regenerate representing the precondition for modern liver surgery [11, 13]. After tissue loss of the liver, growth factors, cytokines, and protease promote and control the temporal and spatial proliferation and migration of the various cells to efficiently reconstitute liver mass. The most important findings of liver regeneration were gained from the model of 70% partial hepatectomy by Higgins and Anderson [11]. Hepatocyte proliferation starts about 24 h after resection in the areas of the lobules surrounding the portal triads and then proceeds to the pericentral areas by 36–48 h. The hepatocytes leave their quiescent state and enter the proliferative stadium of the cell cycle. After 72 h, the replication of the hepatocytes is completed. Most nonparenchymal liver cells enter the DNA synthesis 24 h after the hepatocytes, with a peak activity at 48 h, for example, hepatic stellate cells (HSCs) and Kupffer cells [11]. On the other side, sinusoidal endothelial cells (SECs), the open, fenestrated, discontinuous endothelial cells that line the vessels

supplying the parenchymal plates, do not initiate DNA synthesis until 48–72 h after resection. The DNA synthesis of the SECs is peaking on fourth day and continue to proliferate at least until 8 days after partial hepatectomy. This chronological order of cell proliferation results in the formation of avascular hepatic islands, also called hepatocyte clusters, throughout the liver lobule. Subsequent proliferation and migration of the sinusoidal endothelium into the hepatocyte clusters are driven by the release of various angiogenetic growth factors, including vascular endothelial growth factor (VEGF) synthesized by hepatocytes [1, 7]. It seems that not only hepatocytes play an important role in regulating the vascularization of the hepatocyte clusters but also other cells of the liver like HSCs [1, 5].

Surprisingly, very little is known about the role of HSCs in the liver regeneration. HSCs represent a distinct cell type engaged in several important functions under both physiological and pathological conditions. HSCs occur in "quiescent" and "activated" morphologically distinguishable forms. The quiescent HSCs are found in the space of Disse, especially at the branching of sinusoids where they wrapped sinusoidal cells with their long cytoplasmic processes. The quiescent HSCs possess the highest capacity to store vitamin A. In the quiescent state, HSCs are characterized through a low proliferation rate. In response to liver injury, HSCs change their phenotype from quiescent to activated. In the activated state, HSCs lose vitamin A and synthesize a large amount of extra-cellular matrix components. The HSCs are found to be congregating around the avascular hepatocyte islands and adhering to the hepatocytes [9, 14, 16].

In the present study, we investigated the liver regeneration in close intervals by correlating different morphological methods, e.g., light, electron, intravital microscopy, and immunohistochemistry with special reference to the role of HSCs.

Materials and methods

Animals and study design

All operations and handling procedures were in accordance with local animal welfare regulations and approved by the veterinary district administration of Muenster.

In 49 Lewis-rats, liver regeneration was induced by a 2/3 hepatectomy [13]. Animals (n=7 each) were killed after 0, 1, 2, 3, 4, 7, and 14 days. All procedures were carried out under nitrous oxide/isoflurane anesthesia ($N_2O/O_2 = 2:1 + 1.5\%$ isoflurane). Preoperatively, the rats had to fast for 24 h but had free access to water. Postoperatively, animals received water and food ad libitum.

Intravital microscopy

Intravital microscopy was performed with a fluorescence microscope, Zeiss Axioscope, integrated in the Multi-Purpose Microscope system [100 W/2 HBO mercury lamp; eyepieces: 10×/20; objective Zeiss Achroplan 20×/0.5 w Ph 2 for water immersion; zoom lens 0.5–2.0; filter combinations of 450–490/515–565 nm (excitation/emission) for sodium fluorescein and 330–390/>430 nm for bisbenzamide and vitamin A autofluorescence] in epi-illumination technique [9]. Sodium fluorescein (2 μmol/kg body weight i.v., Sigma) was used to enhance contrast for assessing the sinusoid density. Hepatocyte nuclei were identified by admission of Hoechst 33342 (Ho342, 20 μmol/kg b.w., Sigma). HSCs were identified using their inherent vitamin A autofluorescence.

Intravital microscopy was performed under constant conditions of anesthesia and temperature by an adjustable heating system integrated into the table. A polyethylene catheter was inserted into the carotid artery for measurement of arterial blood pressure. For investigation of the liver regeneration, the lower surface of the left liver lobe was used. In the first, sodium fluorescein was injected via the central venous catheter. In the next step, half an acinus was visualized using the filter for sodium fluorescein to identify the sinusoids and to measure the width of the hepatic cord in the later off-line analysis. Then the filters were switched from the filter for sodium fluorescein to the filter for vitamin A autofluorescence and also for bisbenzimide to study the HSCs. It is very important to firstly focus on the acinus during the investigation of the sinusoids for immediately having a sharp picture of HSCs owing to their rapid photobleaching characteristics. Six to ten acini were randomly selected and recorded for the analysis of sinusoid density, hepatic cord width and number of HSC [2, 5, 18]. After the hepatocytes were studied because the same filter had to be used for the hepatocytes as for the HSCs and the fluorescence of the hepatic nuclei illuminated the vitamin A autofluorescence of the HSCs. Also 6–10 acini were randomly selected and recorded for the analysis of the hepatocytes.

All parameters were quantified off-line by a frame-to-frame analysis of video-recorded microscopic images via the computer-assisted image analysis system AnalySIS (SIS, Muenster, Germany). A final magnification of ×750 was employed for assessment of the liver regeneration. The following parameters were analyzed: The number of hepatocyte nuclei were counted per area ([n/mm^2]). The hepatocytes were identified by their regular, circular shape and enduring nature of their Ho342-related fluorescence. The hepatic cord width ([μm]) was measured ten times per acinus. The sinusoid density was defined as the number of sinusoids per half acinus ([n/mm^2]). HSC frequency per area was calculated by counting the number of photo-

bleaching sites per half acinus ([n/mm^2]). HSCs were identified as intense areas of fluorescence, which faded within 10–20 s of epi-illumination. HSCs were counted in the first second of UV epi-illumination because of its rapidly photobleaching characteristics.

Light microscopy

For histological evaluation, all liver specimens were fixed for at least 2 days by immersion in 4% paraformaldehyde phosphate-buffered solution (pH 7.4) and were subsequently dehydrated and embedded in paraffin wax to process sections at a thickness of 10 μm that were conventionally stained by Hematoxylin & Eosin and examined under a Zeiss lightmicroscope by an experienced morphologist. Histological criteria of evaluation were focussed on cell swelling, mitosis, immigration of sinusoidal cells into hepatic clusters, and endothelium structure. For documentation, photomicrographs were taken from the periportal and the pericentral zones of liver lobules belonging to the control group (sham operation) and to the six postoperative groups.

Electron microscopy

For transmission electron microscopy, liver specimens were immersed in 2.5% glutaraldehyde solution for 24 h (0.1 m

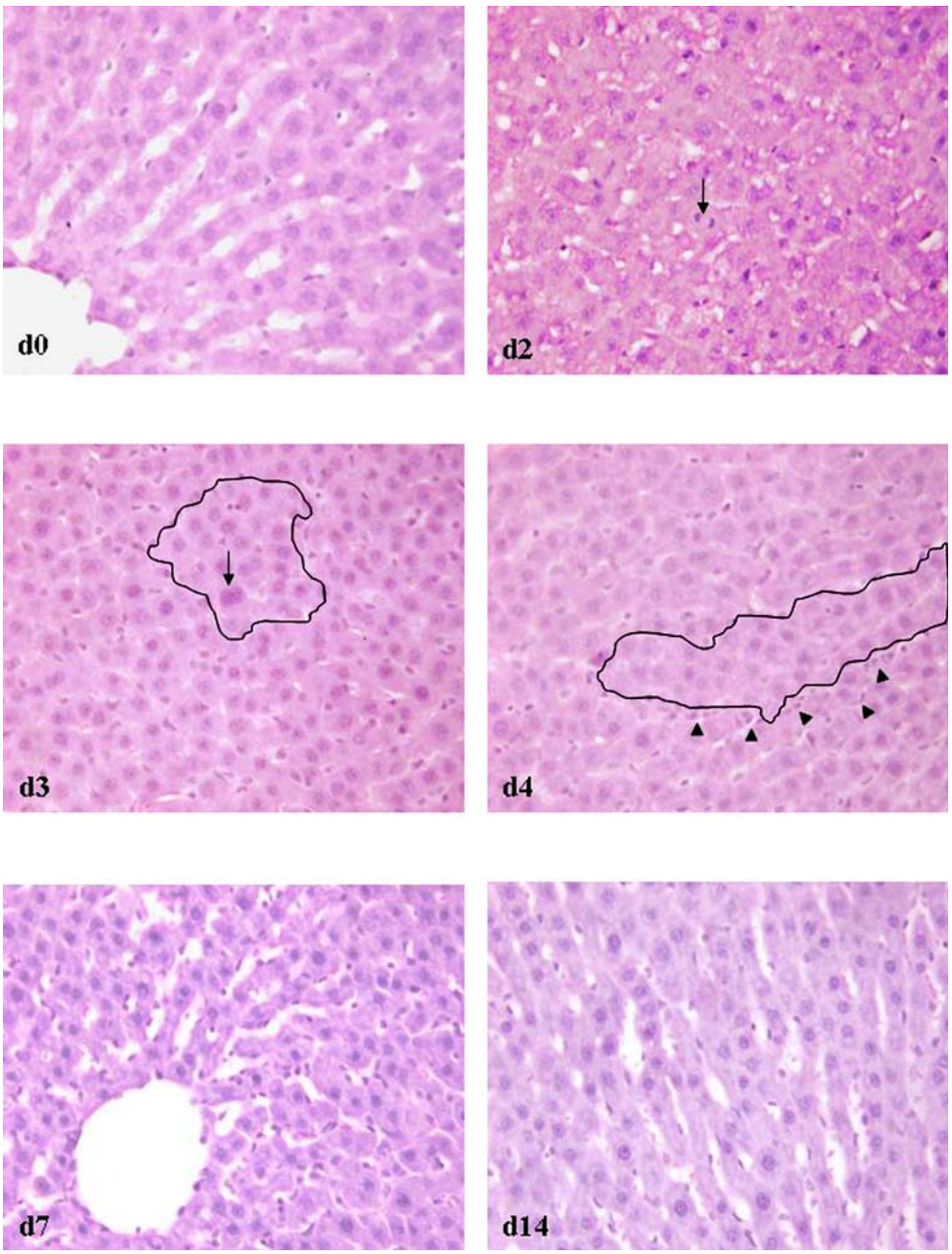

Fig. 1 Light microscopical features of liver regeneration. *d0*: normal liver architecture (×250); *d2*: mitosis of hepatocyte (*arrow* points on telophasis; ×250); *d3*: clusters of hepatocytes without a visible lumen which border is marked by a *line* with cell division (see *arrow*; ×250); *d4*: cluster of hepatocytes which contour is marked by a *line* (×250), *arrows* point on endothelial cell strands without a visible lumen surrounding the cluster; *d7*: normally structured liver tissue with still increased hepatic cord width (×250); *d14*: normal liver architecture (×250). *d* Day after induction of liver regeneration by partial hepatectomy

PBS, pH 7.2), postfixed with 1% OsO_4 in cacodylate-buffer for 1 h (0.1 m PBS, pH 7.2), dehydrated, embedded, and polymerized in epoxy resin (Epon 812) for 24 h. Ultrathin sections were contrasted with uranyl acetate and lead citrate and observed under a transmission electron microscope (CM-10, Philips, Germany). Criteria of ultrastructural investigation were focussed on cell–cell membrane contacts between hepatocytes and nonparenchymal cells.

Immunohistochemistry

The monoclonal antibody, MIB-5 (DakoCytomation, Hamburg, Germany), is specific for the rat homologue of the Ki-67 cell-cycle-associated nuclear antigen and detects cells outside the G0 stage of the cell cycle. MIB-5 was diluted 1:20 and visualized on paraffin sections using the immuno-alkaline phosphatase (APAAP) method with fuchsin as the chromogen. The hepatocellular mitotic index, i.e., the proportion of MIB-5 decorated nuclei per 100 hepatocellular nuclei, was assessed by counting a grid defined field containing approximately 1,000 hepatocytes and nonparenchymal cells per section.

Immunohistochemical assessment of apoptosis was performed on paraffin sections using the labeled streptavidin–biotin immunoperoxidase technique (Streptavidin Alexa Fluor 488, Molecular Probes Eugene, USA) with a rabbit

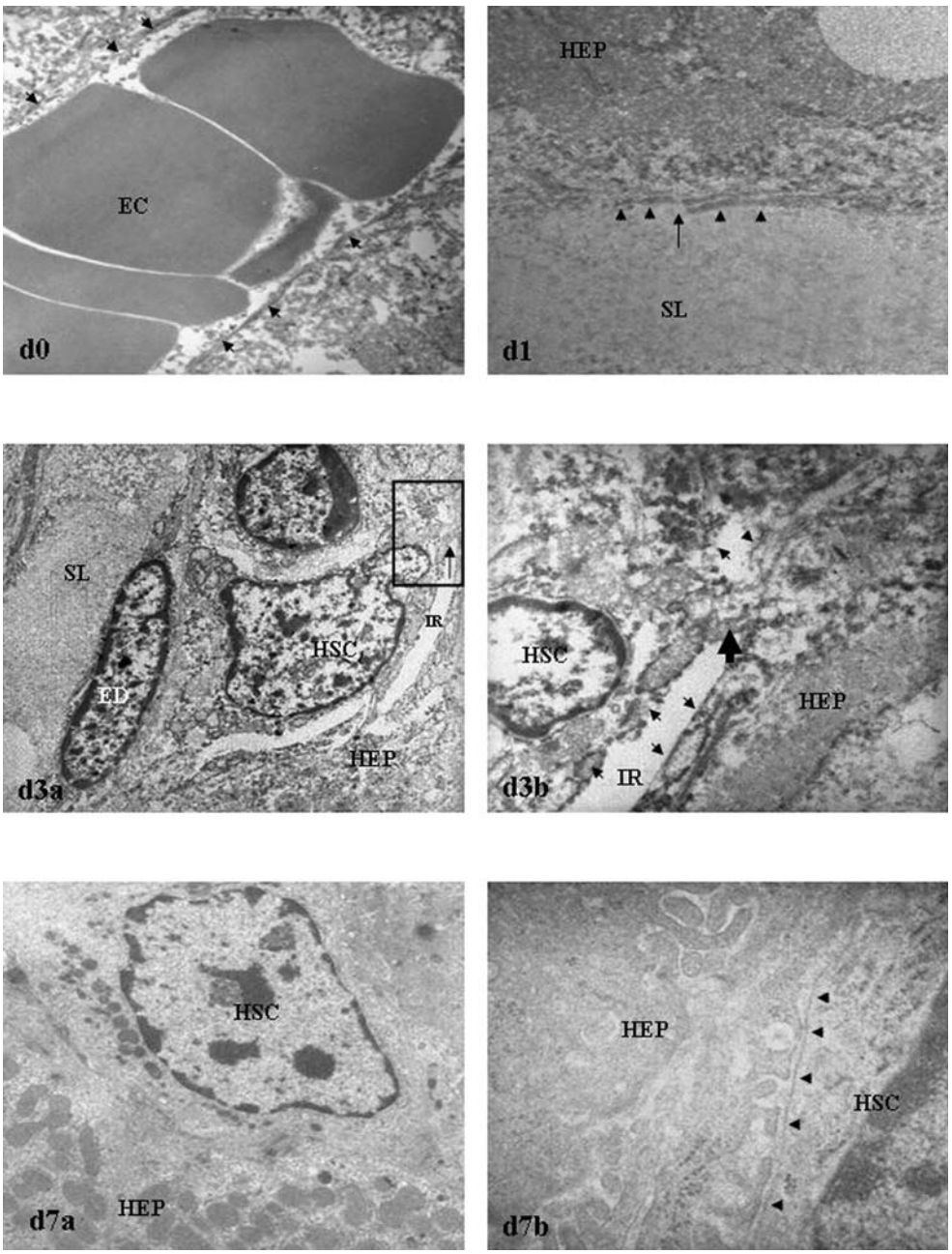

Fig. 2 Electron micrographs of the regenerating liver. *d0*: intact endothelial membrane (*arrow head*, endothelial cell membrane; ×3400); *d1*: damaged endothelial membrane (see *arrow*; *arrow head*, endothelial cell membrane; ×3400); *d3a*: cell–cell contact between hepatic stellate cell and hepatocyte (see *arrow*; ×1,950); *d3b*: detail of 3a showing a cell–cell contact with a linear attachment zone of cell membranes of both cells without junctional complexes (see *arrow*; *arrow head*, cell membrane; ×6,300); *d7a*: absent cell–cell contact between hepatic stellate cell and hepatocyte (×1,950); *d7b*: detail of 7a showing the cell membrane of a HSC and a hepatocyte with a clearly visible intercellular space (*arrow head*, endothelial cell membrane; ×11,500). *d* Day after induction of liver regeneration by partial hepatectomy, *HSC* hepatic stellate cell, *ED* endothelial cell, *HEP* hepatocyte, *SL* sinusoidal lumen, *EC* erythrocyte

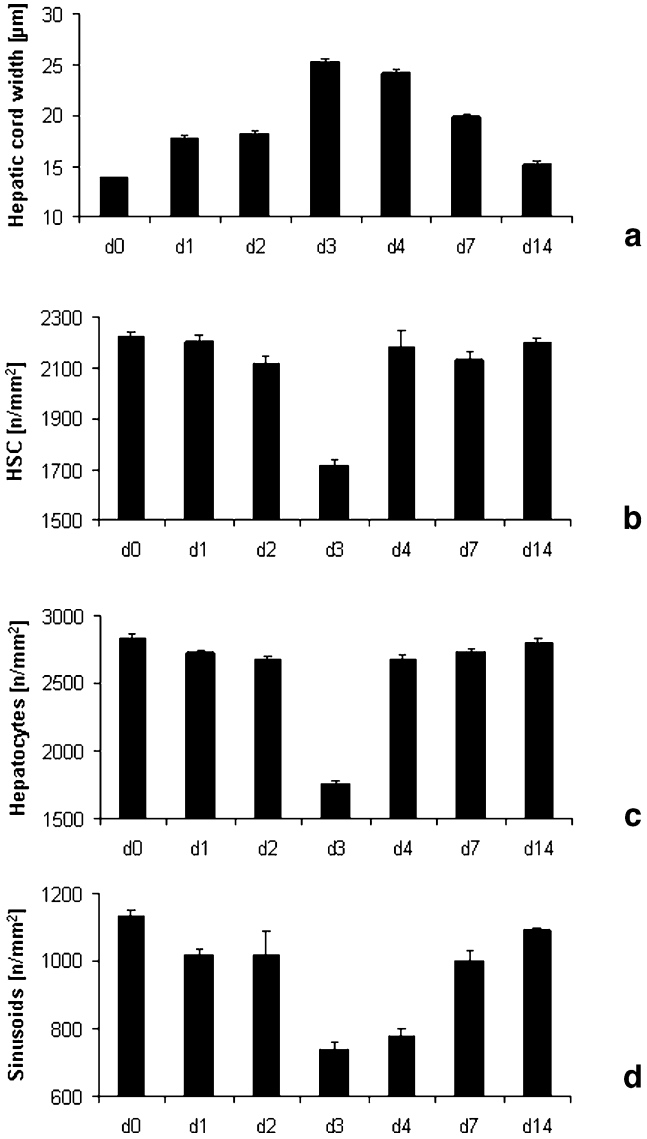

Fig. 3 Intravital microscopic features of liver regeneration. **a** Hepatocyte cord width (µm); **b** number of HSC (n/mm^2); **c** number of hepatocytes (n/mm^2); **d** sinusoid density (n/mm^2). *d* Day after induction of liver regeneration by partial hepatectomy

polyclonal antibody against ssDNA (IBL, Japan) at a 1:2,000 dilution and a biotinylated anti-rabbit antibody (Vector Laboratories, USA) at a dilution of 1:500. Nuclei were stained with DAPI (Sigma Chemical, USA) at a dilution of 1:10,000. Apoptosis index, i.e., the proportion of ssDNA decorated nuclei per 100 hepatocellular nuclei, was semiquantified as described above.

Immunohistochemical detection of VEGF and its receptor (VEGFR) as markers for angiogenesis was performed on paraffin-sections with an anti-VEGF antibody (ab2992, Abcam) and an anti-VEGFR antibody, respectively, and visualized as described above. The immunostaining intensity of VEGF and VEGFR was evaluated according to a four-grade score: 0=negative, 1=partially (<25%) weak-positive stained cells, 2=partially (<25%) moderate or diffuse (>75%) weak-positive cells, 3=diffuse (>75%) moderate or strong positive cells, 4=diffuse strong positive cells.

Immunohistochemical detection of desmin as marker for HSCs and α-smooth muscle actin (α-SMA) as a marker for activated HSCs was performed on paraffin sections with anti-desmin antibody (clone D33, mouse monoclonal) and anti-α-SMA antibody (clone 1A4, Rabbit monocloncal, both DakoCytomation, Hamburg, Germany) using the staining detection as described above. The percentage of nonactivated and activated HSCs was assessed as described above.

Results

Light microscopy

All specimens of the first group showed a regular liver histology with a normal hepatocellular structure and stainability. One day after induction of liver regeneration, tissue specimens displayed hepatocyte swelling and intracellular vesicular steatosis predominantly in the periportal zone. On the postoperative days 2 and 3, numerous mitotic figures in hepatocytes and nonparenchymal cells as well as clusters of small hepatocytes were visible, which were surrounded by sprouts of patent capillaries on day 4. On day 7, liver tissue appeared to be normally structured apart from the occurrence of enlarged lobules compared to the control specimens. Some persisting clusters of small hepatocytes were noticed to be immigrated by aluminal endothelial cell strands. On day 14, the regenerative liver showed a regular liver histology with a normal hepatocellular structure (Fig. 1).

Electron microscopy

One day after liver resection, hepatocyte vesicular steatosis and endothelial cell damage of adjoining sinusoids was most obvious. The cell membrane of the endothelial cell displayed desquamation (see Fig. 2 d1). On the postoperative day 2, mitotic figures of hepatocytes were apparent. On days 3 and 4, hepatocytes often appeared to have developed membrane contacts to adjoining nonparenchymal cells (see Fig. 2 d3a). These cells were situated alongside of the endothelial cells within the space of Disse and presumably belong to the stellate-type cell due to their cell protrusions extending from the central cell body with the nucleus, whereas hepatocytes were identified by their cuboidal shape, their microvilli covering, and their numerous mitochondria. At higher magnification, cell membrane of hepatocytes and HSCs revealed a linear type of contact over an estimated distance of around 1.0 µm (see Fig. 2 d3b). In the sham operation group and on the 7th and the 14th postoperative day, such types of cell contact could not

be observed by electron microscopy (see Fig. 2 d7a and d7b). At the 7th and the 14th postoperative day, all specimens showed hepatocytes with regular ultrastructure without occurrence of vesicular steatosis. Likewise, endothelial cells appeared as cells with morphological features corresponding to the control specimens and HSCs reobtained loose contact to hepatocytes.

Intravital microscopy

Intravital microscopic features of liver regeneration started at day 0 with continuous columns of hepatocytes (2,836.2±29.5/mm^2) with a narrow cord width (13.9±0.3 μm), extended sinusoids (1,135.1±15.4/mm^2), and HSCs (2,221.7±16.6/mm^2) located around all sinusoids. Hepatic cord width increased to a maximum on day 3 (25.3±0.3 μm). The number of hepatocytes and autofluorescent HSCs decreased to a minimum on day 3 (Hepatocytes, 1,760.6±18.1/mm^2, HSC: 1,751.9±20.2/mm^2). Particularly, a strong drop of HSCs was recorded between the second and third day. In contrast to day 0, HSCs were arranged in clusters on the third day. From day 4 onwards, the number of hepatocytes and HSCs continuously increased reaching their original number on day 7. The sinusoid density decreased to a minimum on the third day. On the fourth day, the sinusoid density remained low and recovered on the seventh day. After

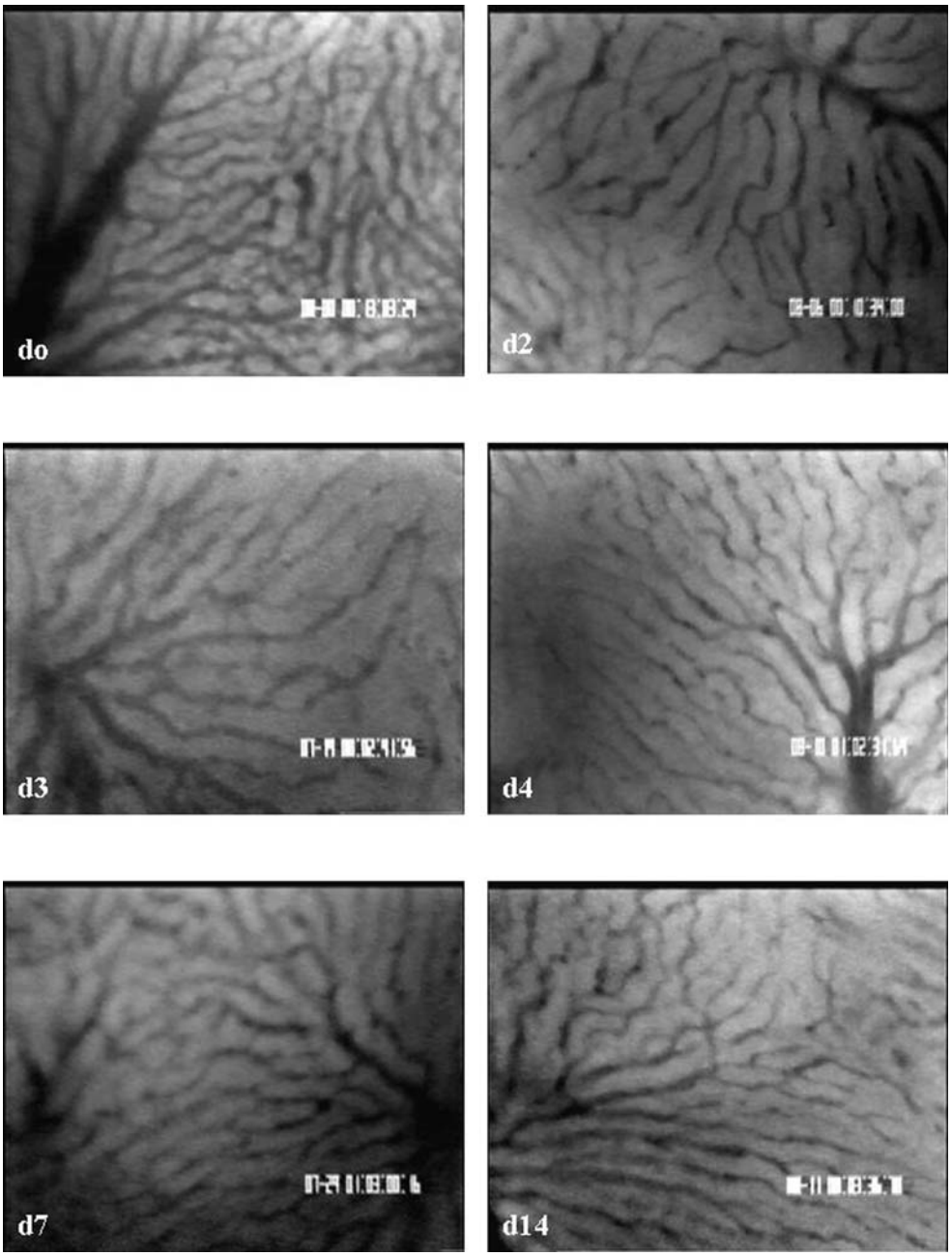

Fig. 4 Intravital microscopical image of sinusoid density and hepatic cord width at days 0, 2, 3, 4, 7, and 14. The sinusoid density decreased to a minimum on the third day and the hepatic cord width increases to a maximum. On the fourth day, the sinusoid density remained low and recovered on the seventh day. The *dark lumen* are representing the sinusoids. *d* Day after induction of liver regeneration by partial hepatectomy

14 days, the original intravital microscopic liver morphology was regained (Figs. 3, 4, 5, and 6)

Immunohistochemistry

Two days after induction of liver regeneration, hepatocyte proliferation—as shown by the Ki-67 expression—increased up to the fourth day. Proliferation of nonparenchymal cells proceeded with a delay of 24 h. On the seventh day, Ki-67 expression showed original values for hepatocytes and nonparenchymal cells. Expression of VEGF (Fig. 8) and its receptor showed a strong increase on the third, fourth, and seventh day. Whereas desmin expression of HSC remained almost constant during liver regeneration, HSCs showed an increased α-SMA expression starting from day 2 to a maximum between days 3, 4, and 7. Hepatocyte apoptosis was increased at day 7, whereas the nonparenchymal cells showed an increased apoptosis at day 7 and 14 in comparison to day 0 (Fig. 7; Table 1).

Discussion

This study demonstrates that HSCs were activated at day 3 during liver regeneration by loosing their autofluorescence and showing an increased α-SMA expression and direct cell

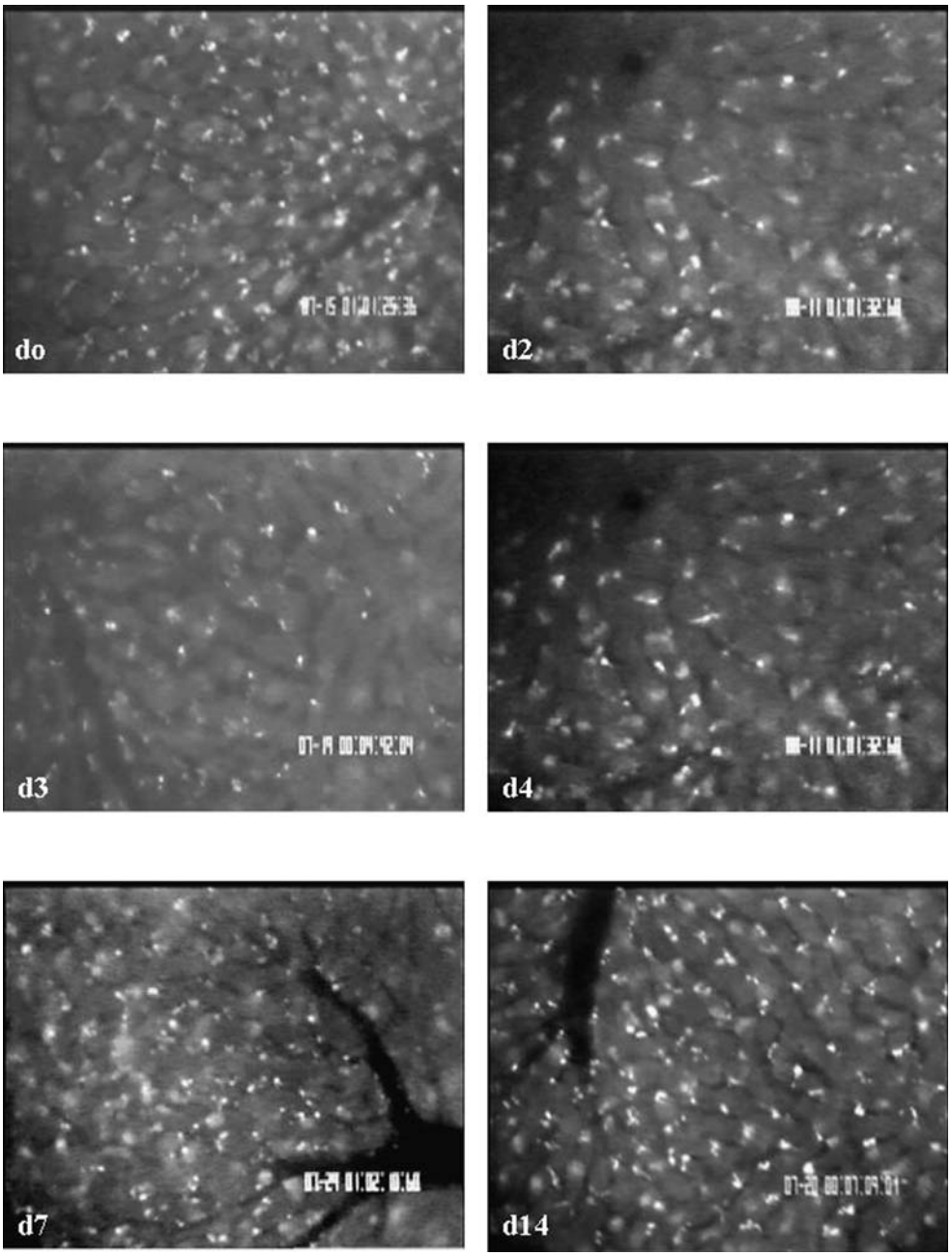

Fig. 5 Intravital microscopical image of hepatic stellate cells at days 0, 2, 3, 4, 7, and 14. The number of autofluorescent HSCs decreased to a minimum on day 3, with a strong drop between the second and third day. In contrast to day 0, HSCs were arranged in clusters on the third day. From day 4 onwards, the number of HSCs continuously increased reaching their original number on day 7. HSCs were identified using their inherent vitamin A autofluorescence (*white spots*, HSCs). *d* Day after induction of liver regeneration by partial hepatectomy

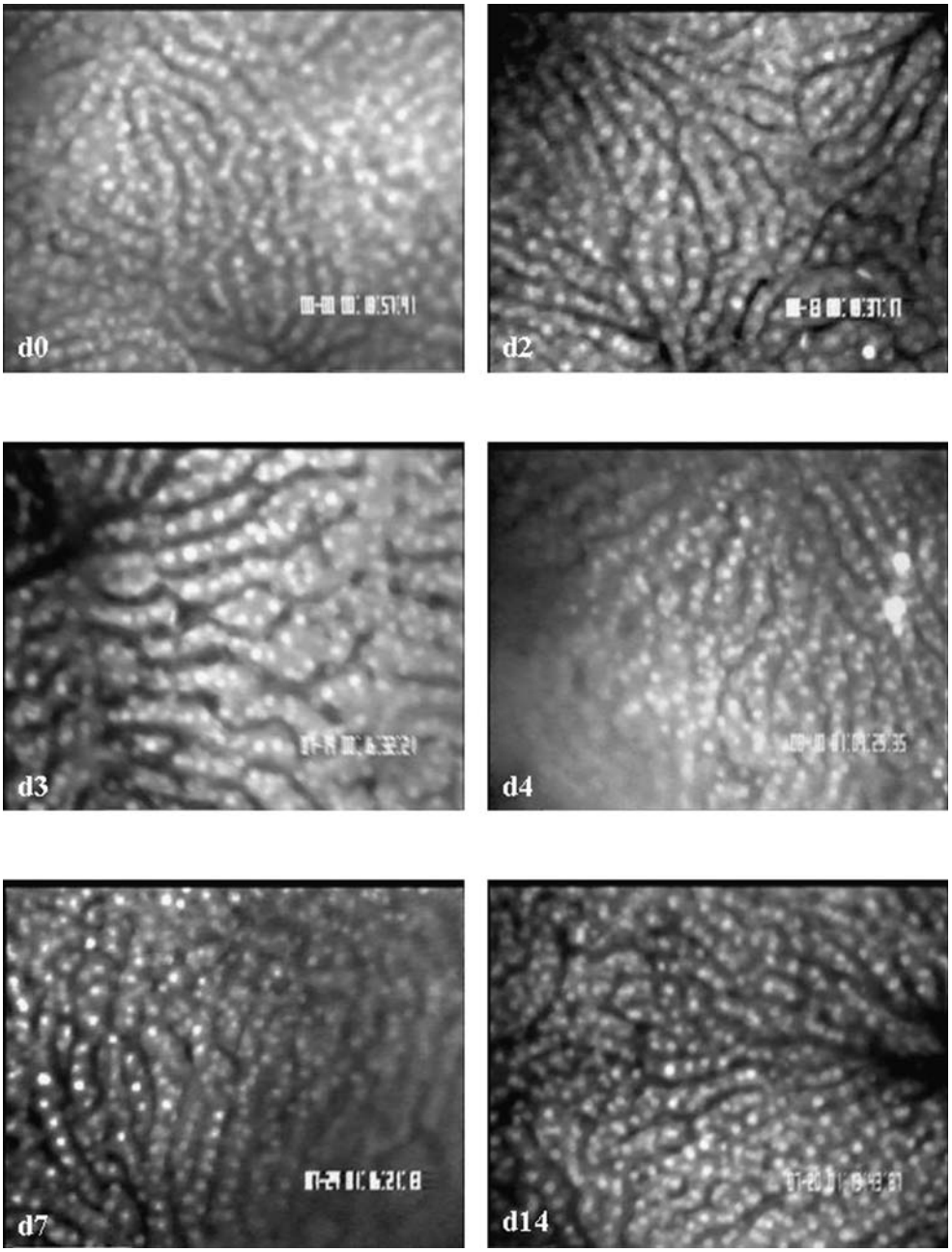

Fig. 6 Intravital microscopical image of hepatocyte at days 0, 2, 3, 4, 7, and 14. The number of hepatocytes decreased to a minimum on day 3. The number of hepatocytes continuously increased from day 4 reaching their original number on day 7 (*white spots*, hepatocytes). *d* Day after induction of liver regeneration by partial hepatectomy

contact to hepatocytes. HSC activation was followed by an increased expression of VEGF and its receptor and sinusoid density. After 7 days, angiogenesis was not completed as shown by solitary hepatocyte clusters, increased VEGF and VEGFR expression, and activated HSCs. After 14 days, liver architecture and ultrastructure was restored and HSCs were deactivated showing decreased α-SMA expression as well as increased apoptosis and no more direct cell contact to hepatocytes (Fig. 8).

Intravital microscopy represents an innovative tool for simultaneous and serial analysis of morphological features in the regenerating liver, particularly for the investigation of HSCs characteristics [9]. In healthy livers, HSCs represent around 5–8% of all liver cells. With the help of the intravital microscopy, HSCs can be specifically visualized because of their unique capability to store vitamin A, which possesses the characteristic of autofluorescence [2, 5, 18]. Precisely, only nonactivated HSCs are able to store vitamin A. Zhang et al. [18] and Bauer et al. [2] demonstrated in their studies that the number of visible vitamin A particles corresponds to the number of existing HSCs. By means of the intravital microscopy, it is therefore possible to investigate the chronological course of HSC-storing vitamin A during liver regeneration after partial hepatectomy in correlation to the angiogenesis represented by the sinusoid density. Activated HSCs could furthermore be immunohistologically identified by detection of α-SMA.

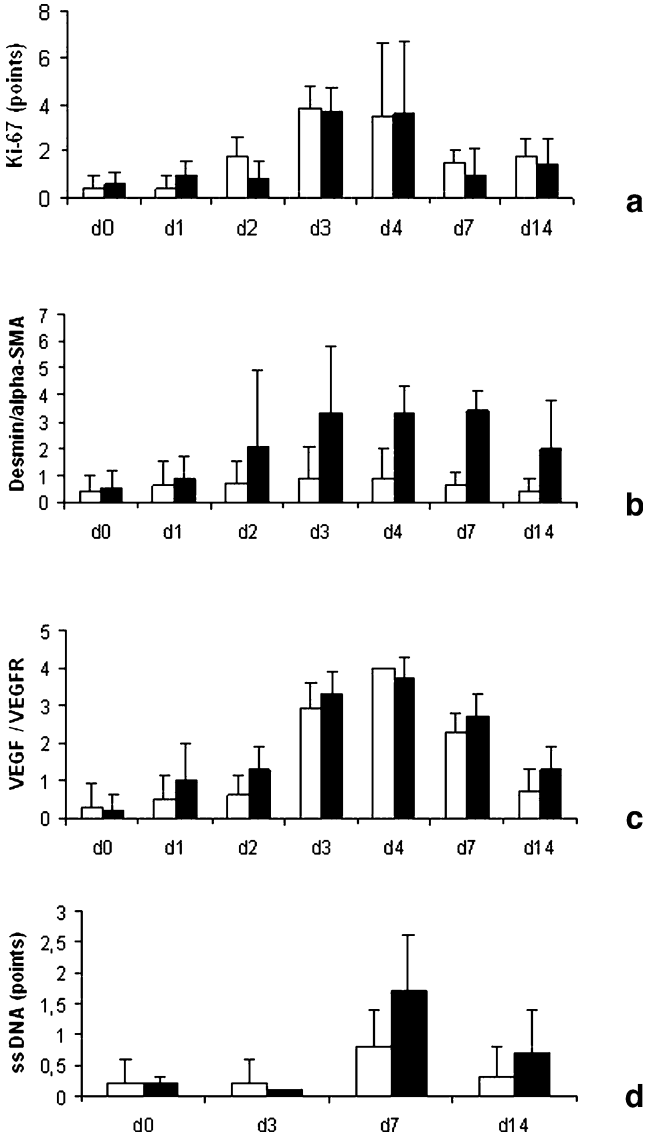

Fig. 7 Immunohistological features of liver regeneration. **a** Proliferation rate by Ki-67 expression of hepatocytes (*white box*) and nonparenchymal cells (*black box*); **b** HSC function by desmin (*white box*) and aSMA (*black box*) expression; **c** VEGF (*white box*) and VEGFR (*black box*) expression as markers for the angiogenesis; **d** ssDNA detection as an apoptosis index for hepatocytes (*white box*) and nonparenchymal cells (*black box*). *d* Day after induction of liver regeneration by partial hepatectomy

In this study we observed a discrepancy between intravital microscopy and immunohistology concerning the number of hepatocytes on the third day which was significantly lower by intravital microscopy while already increased by Ki-67 staining. Although at this time the total number of hepatocytes—as detected by bisbenzamide staining using intravital microscopy—was the lowest during the

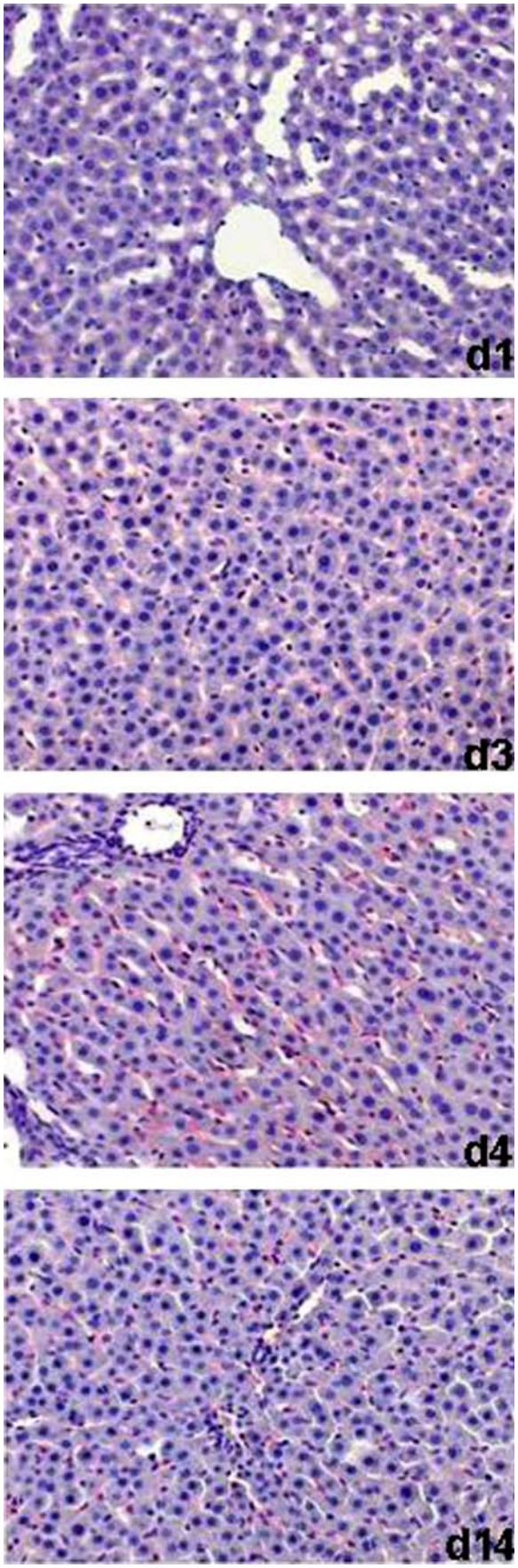

Fig. 8 VEGF expression in the regenerating liver. After liver resection, ▶ animals showed an increasing expression of VEGF between days 3 and 4 that dropped at day 7 reaching almost preoperative levels at day 14. *d* Day after induction of liver regeneration by partial hepatectomy

Table 1 Key steps of liver regeneration seen in light, electron, and intravital microscopy

Day	Light microscopy	Electron microscopy	Intravital microscopy
0	Regularly liver histology	Regularly ultrastructure of liver	Normal
1	Swelling and vesicular steatosis	Vesicular steatosis of hepatocytes and damage of the endothelial membrane	Increasing hepatic cord width
2	Mitotic figures in hepatocytes and nonparenchymal cells	No cell–cell contact between hepatocytes and HSCs, some mitotic figures in hepatocytes and nonparenchymal cells	Begin of drop of hepatocytes
3	Clusters of hepatocytes	Increased cell–cell contact between hepatocyte and HSCs	Drop of hepatocytes and HSCs, minimum sinusoid density
4	Hepatocyte clusters surrounded by endothelial cells	Signs of cluster vascularization, increased cell–cell contact between hepatocytes and HSCs	Increasing hepatocyte number and normalization of HSC number
7	Normally arranged liver histology with enlarged lobules	Normal hepatic ultrastructure, no damage of endothelial cells, no increased cell–cell contact between hepatocyte and HSCs	Increasing sinusoid density
14	Regularly liver histology	Regular ultrastructure of liver	Normal

whole course of liver regeneration, the proportion of hepatocytes which have already entered the G1-phase—as detected by Ki-67 staining—has been already increased. Additionally, we observed a compensatory swelling of hepatocytes between the first and third day after induction of liver regeneration as represented by the increased hepatic cord width which might also explain the decreased number of hepatocytes at the third day.

There is a growing body of evidence that HSCs represents the key cells in the regenerating liver [1, 4, 5, 12]. HSCs have several important functions like retinoid storage and homeostasis, regulating the width of the sinusoid lumen by contraction and dilatation in response to endothelin, angiotensin, thromboxane, and prostaglandins, remodeling of extracellular matrix by production of collagens, matrix metalloproteinases (MMPs), growth factors, and cytokines [8, 15]. During liver regeneration nonparenchymal cells like HSCs and SECs enter the DNA synthesis 24 h later than hepatocytes and proliferate and migrate into the hepatocyte clusters at the fourth day before the normal sinusoidal architecture has been restored [4, 17]. In this study, the ratio between HSCs and hepatocytes was about 0.78 corresponding to other studies [9]. Regeneration led to a shift of the ratio in favor of HSCs on the third day with a ratio of 1, loss of vitamin A particles, and increase in α-SMA, normalizing in the later course [5, 9]. Parallel to the increase in HSC/hepatocyte ratio, angiogenesis started which was accompanied by an increased VEGF expression in hepatocytes showing the interdependence between the activation of HSCs and VEGF expression. Furthermore, activated HSCs modulated the extracellular matrix. Most activated HSCs have been found near the hepatic clusters and possibly contributed to synthesis and deposition of ECM into the avascular islands to aid infiltration of sinusoid endothelial cells [10].

The initiation for HSC activation is still unclear [6, 8, 15]. In this study we showed by electron microscopy that a cell-to-cell contact between hepatocytes and HSCs represents the precondition for HSC activation. A direct cell-to-cell contact between hepatocytes and HSCs has been found on day 3 and 4, whereas under normal conditions, HSCs have a close contact with SEC through incomplete basement membrane components but only a loose contact to hepatoctyes by interstitial collagen fibers and nerve fibers run through the space of Disse. The above-named temporal interdependence between HSC activation and VEGF expression demonstrated in this study could be explained by the release of several growth, matrix, and chemotactic factors, e.g., TGF-β1 and PDGF, which are known to have potent fibrogenic and proliferative properties as well as increase VEGF expression [3, 7]. Relating to the direct cell-to-cell contact, several studies provided evidence that HSCs require the basement membrane components for their normal, nonactivated function, and remodeling of the ECM by MMPs, like MPP-1, MPP-2, MPP-3, MPP-9, MPP-11 and TIMP-1 and TIMP-2 which can also be released by hepatocytes [10]. This could also explain the release of MMPs and TIMPs in the first hours after liver resection, when HSCs are not yet activated. Furthermore, it has not yet been resolved how activation of HSCs will end, e.g., by apoptosis or simple conversion in a

deactivated state [6]. In this study we showed by intravital microscopy that the number of nonactivated HSCs—identified by their autofluorescence—has already normalized at day 4 which was accompanied by an increased apoptosis rate and α-SMA expression. Additionally, electron microscopy revealed that from day 7 on the HSCs reobtain a loose contact to hepatocytes suggesting that several pathways for HSC deactivation may exist.

In summary, the synthesis of intravital, light, and electron microscopic analysis of the regenerating rat liver demonstrated the central role of HSCs in governing angiogenesis and ECM remodeling. Due to the recent identification of several receptors at the surface of activated HSCs, e.g., endothelin-A-receptor, angiotensin-1-receptor, and PDGF receptor, new treatment approaches for modulating HSC function in the regenerating liver are available that warrant more extensive preclinical studies [6].

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ORIGINAL ARTICLE

Association of progressive structural changes in the bronchial epithelium with subepithelial fibrous remodeling: A potential role for hypoxia

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Abstract In airway remodeling that occurs in association with chronic obstructive pulmonary disease (COPD), the relationship between the subepithelium and structural changes of the bronchial epithelium is not well defined. To investigate whether the subepithelium and epithelium undergo remodeling as an integrated unit, we performed morphological examination of 55 bronchial biopsy specimens obtained from explanted or resected lungs from tobacco smokers with COPD. Our results indicate that reticular basement membrane (RBM) thickness is increased and the subepithelial microvascular bed is reduced in association with progression from the normal epithelium to squamous metaplasia. Subsequent bronchial epithelial transformation to dysplasia is characterized by differential subepithelial remodeling with normalization of RBM thickness and subepithelial blood vessel density. Because fibrous remodeling of the subepithelium could limit delivery of nutrients and oxygen to the epithelium, we assessed expression of hypoxia-inducible factor-1α (HIF-1α) and carbonic anhydrase IX (CA IX) as markers of cellular hypoxia. The number of HIF-1α-positive epithelial cells increased with progression of epithelial structural changes, RBM thickness, and reduction in blood vessels in the subepithelium. These findings suggest that the HIF-1α pathway is activated in response to subepithelial remodeling and contributes to progressive premalignant epithelial lesions in the airways of tobacco smokers with chronic airway inflammation.

Keywords Chronic obstructive pulmonary disease · Bronchial epithelium · Premalignant lesions · Cellular hypoxia · Hypoxia inducible factor

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Introduction

Airway remodeling comprises a spectrum of structural changes in conducting airways such as smooth muscle hypertrophy and hyperplasia, thickening and fibrosis of

subepithelial reticular basement membrane (RBM), enlargement of submucosal bronchial glands, and structural alterations of the bronchial epithelium [21, 39]. Together, these processes cause significant thickening of airway walls (especially respiratory bronchioles) with narrowing and/or distortion of the airway lumen [19]. While subepithelial and epithelial structural changes have been identified in airways of patients with chronic obstructive pulmonary disease (COPD), it is not clear whether these changes occur in concert as an integrated unit or represent separate, localized responses to injurious stimuli or ongoing inflammation. Understanding the relationship between progressive structural changes of the airway epithelium and subepithelial remodeling in tobacco smokers could shed light on factors that regulate progression of airway inflammation and remodeling and predispose to premalignant transformation of the bronchial epithelium.

According to the World Health Organization grading, neoplasia of the bronchial epithelium develops sequentially from the normal epithelium to basal cell hyperplasia, squamous metaplasia, and dysplasia to carcinoma in situ and microinvasive squamous cell cancer [4, 13]. The development of the squamous phenotype and accumulation of mutations in key cell regulatory genes are two major factors contributing to airway epithelial malignant transformation [5, 36]. Squamous metaplasia and dysplasia are considered as preneoplastic lesions, and their development increases the risk of epithelial malignancy [4, 13], and both are found in airways of patients with COPD [37, 38]. Therefore, factors that control epithelial structural changes could directly impact progression toward malignant transformation. In this study, we hypothesized that in airways of patients with COPD, the epithelium and subepithelium form an integrated unit that undergoes remodeling in a coordinated fashion in response to chronic exposure to tobacco smoke. Further, subepithelial fibrous remodeling contributes to progressive epithelial changes by altering the local microenvironment. To evaluate this hypothesis, we undertook histological, morphometric, and immunohistochemical (IHC) investigations of bronchial biopsy specimens from 55 current or former tobacco smokers with COPD and chronic bronchitis. Our initial findings indicated that subepithelial fibrous remodeling and capillary loss correlated with progressive structural changes in the overlying epithelium. These findings suggested that remodeling of inflamed subepithelium could reduce delivery of oxygen and nutrients to the bronchial epithelium, contributing to progressive epithelial structural changes. Prior studies have found that cellular hypoxia can increase genetic instability and lead to overexpression of key growth factors and their receptors [3, 7]; therefore, identification of hypoxic responses in the bronchial epithelium could help clarify mechanisms linking airway remodeling in COPD with epithelial structural changes and development of lung cancer. For assessment of hypoxic signaling in bronchial specimens, we identified the ubiquitously expressed oxygen-sensitive transcriptional regulator, hypoxia-inducible factor-1α (HIF-1α) [2, 9, 43]. HIF-1α is unstable in normal oxygen tension but becomes stabilized under hypoxic conditions and translocates to the nucleus to function as a transcriptional regulator. HIF-1α is responsible for the regulation of a broad array of hypoxia-responsive genes, whose products are involved in promotion of angiogenesis [10, 32] and regulation of energy metabolism [14, 24]. We also evaluated another widely used marker of cellular hypoxia, carbonic anhydrase IX (CA IX), a transmembrane protein that catalyzes the reversible hydration of carbon dioxide to carbonic acid, thus regulating pH in hypoxic cells [45, 47]. Using IHC techniques, we found upregulation of these markers in the altered bronchial epithelium, suggesting a mechanistic link between microenvironmental changes in the subepithelium and progressive structural abnormalities in the bronchial epithelium.

Materials and methods

Clinical material Surgical bronchial biopsy specimens from segmental or subsegmental bronchi from 55 current or former smokers with COPD were selected retrospectively from the pathology tissue banks of Vanderbilt University Medical Center (Nashville, TN, USA) and Novosibirsk City Thoracic-Surgical Hospital (Novosibirsk, Russia). In 18 cases, bronchial biopsy specimens were received from explanted lungs of patients with COPD undergoing lung transplantation. In 37 cases, bronchial biopsy specimens were obtained from patients who underwent lobectomy or pneumonectomy for lung cancer (15 squamous cell carcinoma, five adenocarcinoma), chronic lung abscess (ten), or

Table 1 The demographic and clinical characteristics of COPD patients

Clinical groups	Age (years)[a]	Number (male/female)	FEV_1% of predicted[a]	Smoking history (pack-years[a])
COPD and lung transplantation	55 (43–63)	18 (9/9)	22 (12–30)	51 (30–105)
COPD and lung cancer	65 (47–77)	20 (11/9)	71 (36–93)	59 (26–90)
COPD and bronchiectasis	50 (41–63)	7 (5/2)	78 (62–96)	51 (32–80)
COPD and chronic lung abscess	46 (37–58)	10 (8/2)	71 (58–85)	48 (22–72)

[a] Numbers shown are mean followed by range in parentheses.

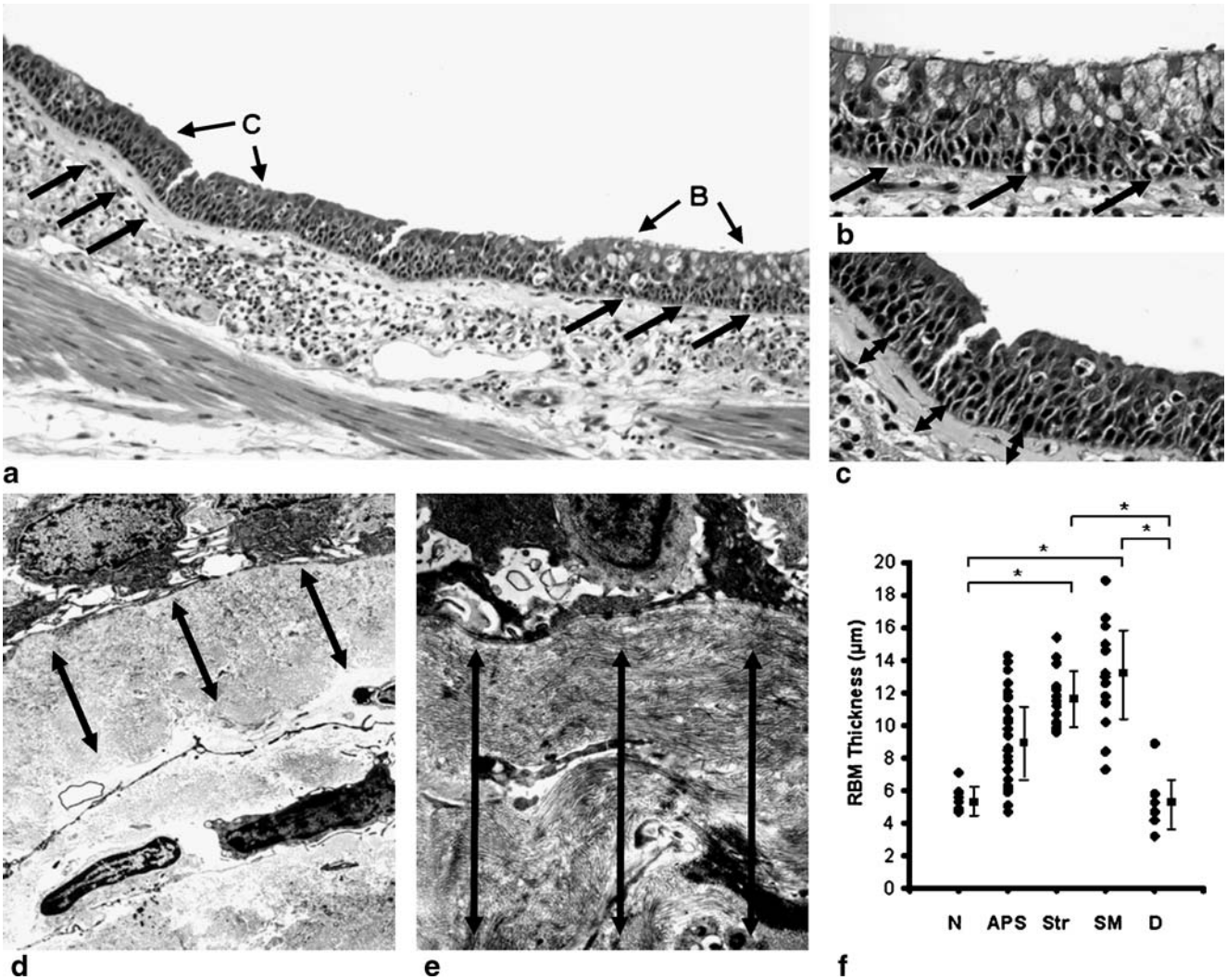


Fig. 1 Subepithelial fibrous remodeling of airways in patients with COPD correlates with extent of epithelial structural change. **a** H&E-stained paraffin section showing reticular basement membrane (RBM) thickness varying within the same airway depending on the overlying epithelium (see *arrows*; magnification, 100×). **b**, **c** *Insets* with higher magnification (300×) demonstrating differential thickness of RBM beneath altered pseudostratified bronchial epithelium (**b**) compared with stratified bronchial epithelium (**c**). The RBM is identified by *arrows*. **d**, **e** Electron micrographs showing the fine structural organization of normal RBM (**d**) and fibrous remodeling of the RBM with rough bundles of collagen fibers (**e**; see *arrows*). **f** Graph showing relationship of RBM thickness with increasing severity of structural alterations of airway epithelium. *Points* represent the average RBM thickness associated with a particular epithelial phenotype in each specimen. Mean±SD is indicated for each structural variant, *asterisk*, $p<0.001$. *N* Normal pseudostratified structure, *APS* altered pseudostratified structure, *Str* stratification, *SM* squamous metaplasia, *D* dysplasia

bronchiectasis (seven). In cases of lung cancer, tissue specimens were obtained as remote from the tumor as possible. All subjects had smoked greater than 10 years and had a clinical diagnosis of COPD or symptoms of chronic bronchitis. Table 1 summarizes demographic and clinical characteristics of these patients. This study was approved by the institutional review board of Vanderbilt University School of Medicine and the ethical committee of Novosibirsk City Thoracic-Surgical Hospital.

Light microscopic examination Tissue specimens were fixed in 10% solution of neutral-buffered formalin for 24 h and after dehydration were embedded in paraffin. For primary light microscopy, tissue sections (4–5 μm thickness) were stained with hematoxylin and eosin (H&E) and the periodic acid-Schiff (PAS) reaction for detection of mucin.

Morphometry Thickness of the RBM, epithelial height (L_{ep}), blood vessel density (total number of blood vessels per 1 mm^2, N_{cap}), and blood vessel volume density (VV_{cap}) of the lamina propria, the space between RBM and smooth muscle layer, were estimated. VV_{cap} was defined as the percentage of the lamina propria area occupied by blood vessels. RBM thickness and L_{ep} were quantified by measuring 100 sites (spaced 20 μm apart) on ten subse-

quent tissue fields along the length of the epithelium in each tissue section with measurements in a line perpendicular to the epithelial layer. For each field, the bronchial epithelial structure was determined (normal structure, altered pseudostratified, stratified, squamous metaplasia, dysplasia), and mean RBM thickness associated with each structural variant was recorded. N_{cap} and VV_{cap} were measured on ten nonoverlapping tissue fields of lamina propria for each tissue specimen. As above, bronchial epithelial structure was determined for each field, and N_{cap} and VV_{cap} associated with each structural variant of the overlying epithelium was recorded. All measurements were made by calibrated image analysis using Image-Pro Express software (Media Cybernetics, Silver Springs, MD). Thickening of RBM and L_{ep} were measured in H&E-stained tissue sections in 400× magnification, whereas N_{cap} and VV_{cap} were measured in PAS-stained tissue sections at 200× magnification, which gave better contrast to endothelial cells.

Electron microscopic analysis Electron microscopy (EM) was performed according to a previously described protocol [38]. A small component of each of 24 biopsy specimens selected for EM was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), then in a 1% solution of OsO_4 in PBS, and, after dehydration, embedded in a mixture of Epon and Araldite (Electron Microscopy Sciences, Fort Washington, PA). Semithin sections were stained with Toluidine blue and analyzed by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate.

Immunohistochemical analysis Of the 55 biopsies, tissue was available for additional unstained sections on 38. IHC studies were performed on these 38 tissue specimens using techniques as previously described [28, 29]. IHC was performed on two serial tissue sections for detection of active HIF-1α or CA IX using polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) followed by a standard immunoperoxidase/avidin–biotin complex protocol (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). Immunostained tissue sections were analyzed in a blind fashion. The percentage of HIF-1α-positive cells was calculated on ten sequential, nonoverlapping tissue fields using 400× magnification, which represented the score for the specimen.

Statistical analysis Statistical analysis was performed with GraphPad Instat (GraphPad Software, San Diego, CA) using a Kruskal–Wallis test (nonparametric analysis of variance) with a Dunn's multiple comparisons test for individual variables ($p<0.05$ considered significant). Correlations were calculated using Spearman's rank test.

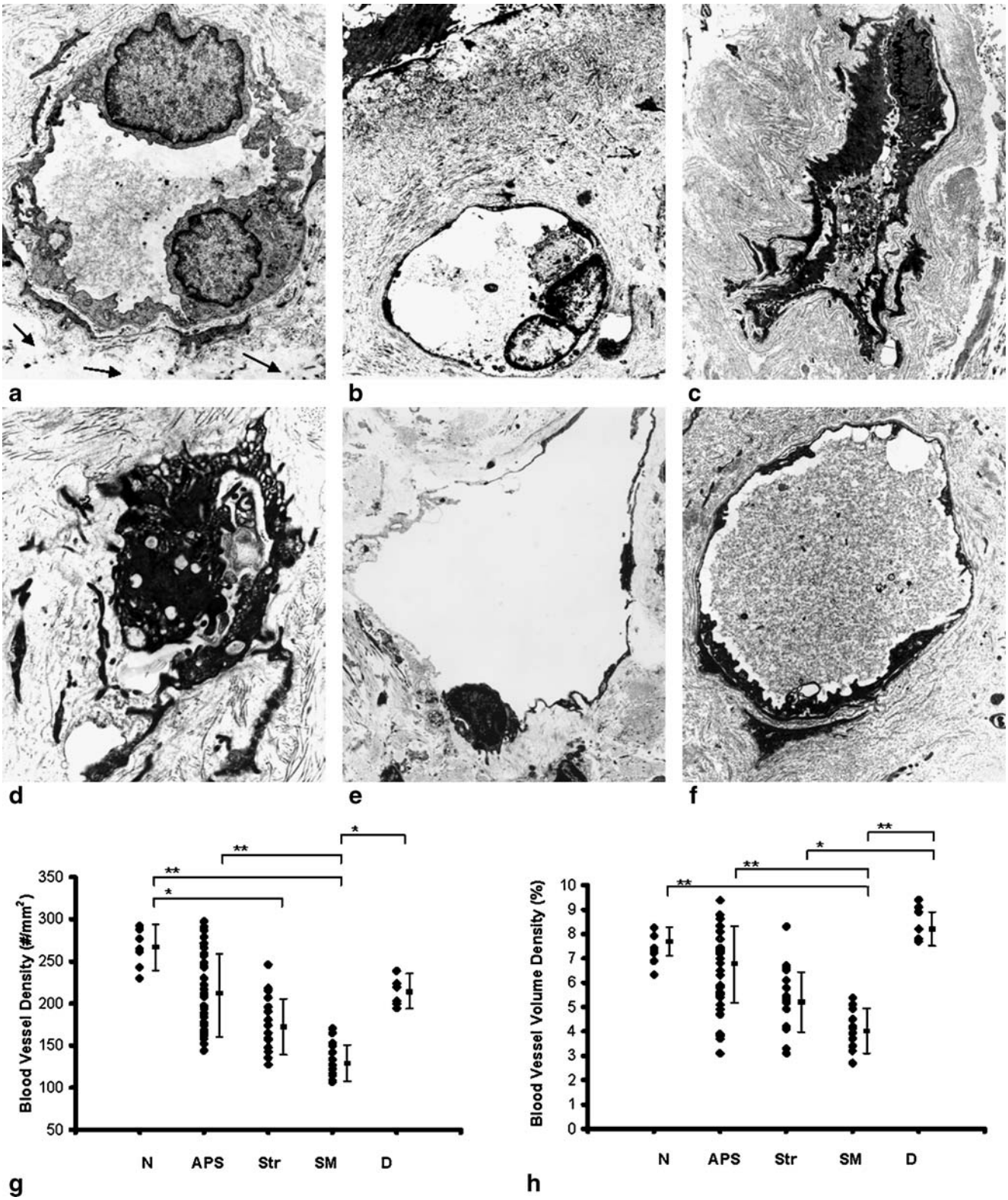
Fig. 2 Microvascular disorders in the subepithelium correlate with epithelial structural alterations. **a–f** Electron micrographs (3,000×) showing a spectrum of microvascular changes in the subepithelium of airways with epithelial pathology. **a** Normal ultrastructural organization of blood capillary with signs of pericapillary edema, and local dissociation of extracellular matrix are seen (see *arrows*). **b** Marked thinning of endothelial cells and destruction of nuclei. **c** Massive pericapillar fibrosis; rough bundles of collagen fibers with circular orientation are seen. **d** Destruction of blood capillary. **e** Dilated lymphatic capillary. **f** Lymphatic capillary with signs of lymphostasis; lumen of capillary is filled with low-electron-dense proteinaceous-rich lymph. **g**, **h** Graphs showing relationships between subepithelial blood vessel number (**g**) and blood vessel volume density (**h**) with variants of bronchial epithelium structure. *Points* represent the average N_{cap} and VV_{cap} associated with a particular epithelial phenotype in each specimen. Mean±SD is indicated for each structural variant, *asterisk*, $p<0.01$; *double asterisk*, $p<0.001$. *N* Normal pseudostratified structure, *APS* altered pseudostratified structure, *Str* stratification, *SM* squamous metaplasia, *D* dysplasia

Results

Comparison of bronchial epithelial structural alterations with parameters of subepithelial remodeling

To characterize structural changes of the bronchial epithelium, we used a previously described classification that includes the bronchial epithelium with normal-appearing structure and four basic pathological states: altered pseudostratified epithelium (or hyperplasia of basal and/or goblet epithelial cells), stratified epithelium (or immature squamous metaplasia), squamous metaplasia, and dysplasia [4, 23, 38].

The distribution of pathological variants of epithelial remodeling was mosaic, and more than one pattern of epithelial structural change was frequently observed in the same bronchial cross-section (Fig. 1a–c). Epithelial height varied widely, and both hyperplasia of basal cells and enlargement of epithelial cells contributed to increased epithelial height. Of the 55 bronchial biopsy specimens analyzed, areas of normal bronchial structure were identified in only seven, underscoring the widespread nature of epithelial structural alterations in this patient population. Other epithelial states were characterized by variable manifestations of basal and/or goblet cell hyperplasia, incomplete differentiation, or epidermoid metaplasia/dysplasia. An altered pseudostratified epithelium was identified in 39 specimens. Areas of stratified epithelium were identified in 18 specimens. In those areas, hyperplasia of basal epithelial cells was characterized by focal formation of at least four epithelial cell layers, consisting of proliferating basal epithelial cells and uniform surface cells without clear differentiation into ciliated or goblet cells. Surface cells of the stratified epithelium lose contact with basement membrane but do not undergo flattening as in squamous metaplasia [38]. Areas of squamous metaplasia or dysplasia were found in 14 and 6 biopsies,



respectively. Of the six biopsies with dysplasia, four cases were mild, and two were moderate in severity.

In the lamina propria, prominent fibrosis of the bronchial wall was present. RBM alterations included collagen deposition and increased thickness. RBM thickness was variable and could be significantly different within the same tissue specimen (Fig. 1a–c). In areas without significant subepithelial remodeling, the RBM had a structural organi-

zation consisting of finely organized collagen fibers, with RBM thickness usually less than 5–7 μm (Fig. 1d). In contrast, areas with prominent subepithelial fibrosis showed abundant collagen deposition within the RBM and increased RBM thickness, usually more than 10–12 μm. Collagen fibers were packed in rough bundles with longitudinal orientation (Fig. 1e). Intermediate variants were characterized by accumulation of fibroblasts and freshly synthesized bundles of collagen fibers in the RBM.

To characterize the association between structural alterations of the bronchial epithelium and subepithelial fibrosis, we performed morphometric analysis of RBM thickness and correlated this parameter with the state of the overlying epithelium. As shown in Fig. 1f, RBM thickness increased with severity of epithelial structural lesions from normal structure to basal cell hyperplasia, stratification, and squamous metaplasia. However, RBM thickness returned towards normal in areas of dysplasia, suggesting that additional matrix remodeling accompanies progression from squamous metaplasia to dysplasia. This normalized RBM thickness was observed only in areas with epithelial dysplasia, whereas areas with less prominent epithelial changes in the same bronchial biopsy specimens always had increased thickness of RBM.

Microvascular disorders were observed in most specimens and included structural alterations of blood vessels and fibrous remodeling of perivascular connective tissue. In normal-appearing blood capillaries, no inflammatory cells or proteinaceous aggregates were observed, and collagen accumulation was not observed in the pericapillary connective tissue (Fig. 2a). However, most capillaries showed significant ultrastructural alterations with damage to endothelial cells. Such capillaries, especially those localized subjacent to the epithelium, showed evidence of structural damage, including damage to endothelial cells with clarification of nuclear matrix (Fig. 2b). Capillaries localized deeper in the subepithelium were characterized by variable structural alterations ranging from insignificant changes to total destruction. The lumen of most capillaries was packed with fragments of destroyed cells, clarified red cells, or proteinaceous aggregates. In areas of prominent subepithelial fibrosis, rough bundles of collagen fibers with circular orientation were found around most capillaries and postcapillary venules (Fig. 2c). Some capillaries appeared to be destroyed and unlikely to participate in the microcirculation (Fig. 2d). The overwhelming majority of lymphatic capillaries were distended, forming thin-walled cavities (Fig. 2e), whereas some showed signs of lymphostasis (Fig. 2f), a finding that increased in parallel with fibrous remodeling.

The described ultrastructural manifestations of blood vessel damage increased with fibrous remodeling of the subepithelium, and we investigated whether these manifestations correlated with the severity of bronchial epithelium alterations. Morphometric analysis of N_{cap} and VV_{cap} in subepithelial connective tissue showed that these parameters inversely correlated with progressive structural alterations of the bronchial epithelium from normal to squamous metaplasia (Fig. 2g,h). These findings indicate that in addition to subepithelial fibrosis and RBM thickness, microcirculatory changes in the subepithelium correspond to epithelial structural alterations. As with RBM thickness, N_{cap} and VV_{cap} normalized in areas of dysplasia, suggesting that a differential remodeling process is activated in the progression from squamous metaplasia to dysplasia. However, most of the blood vessels localized beneath foci of epithelial dysplasia were structurally abnormal. The endothelial cells lining these blood vessels usually had significant structural damage, and the basement membrane was interrupted or totally absent.

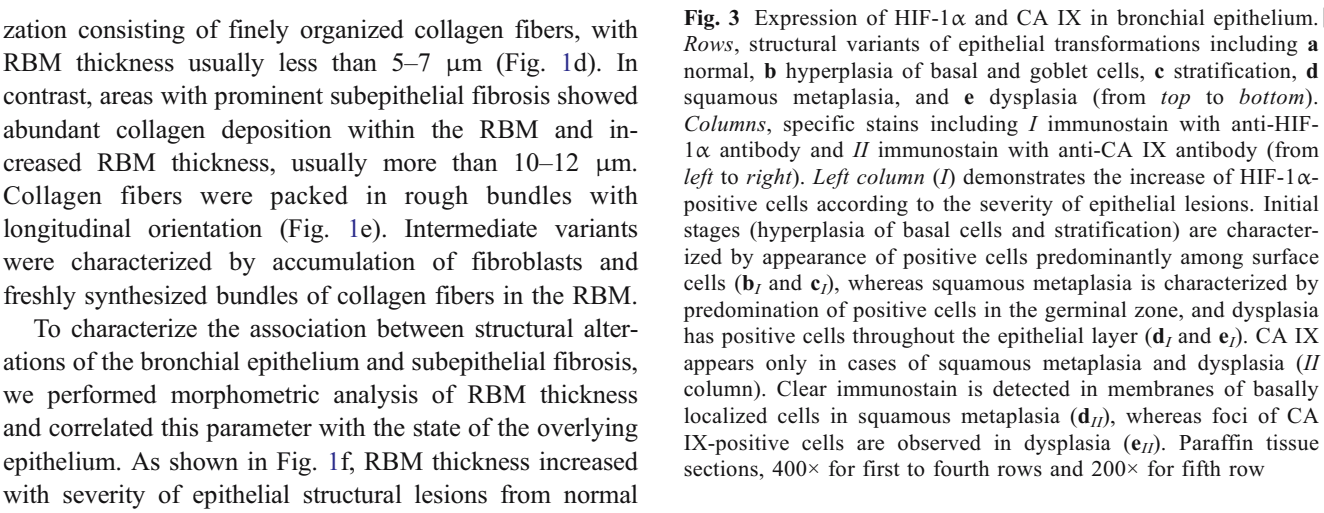
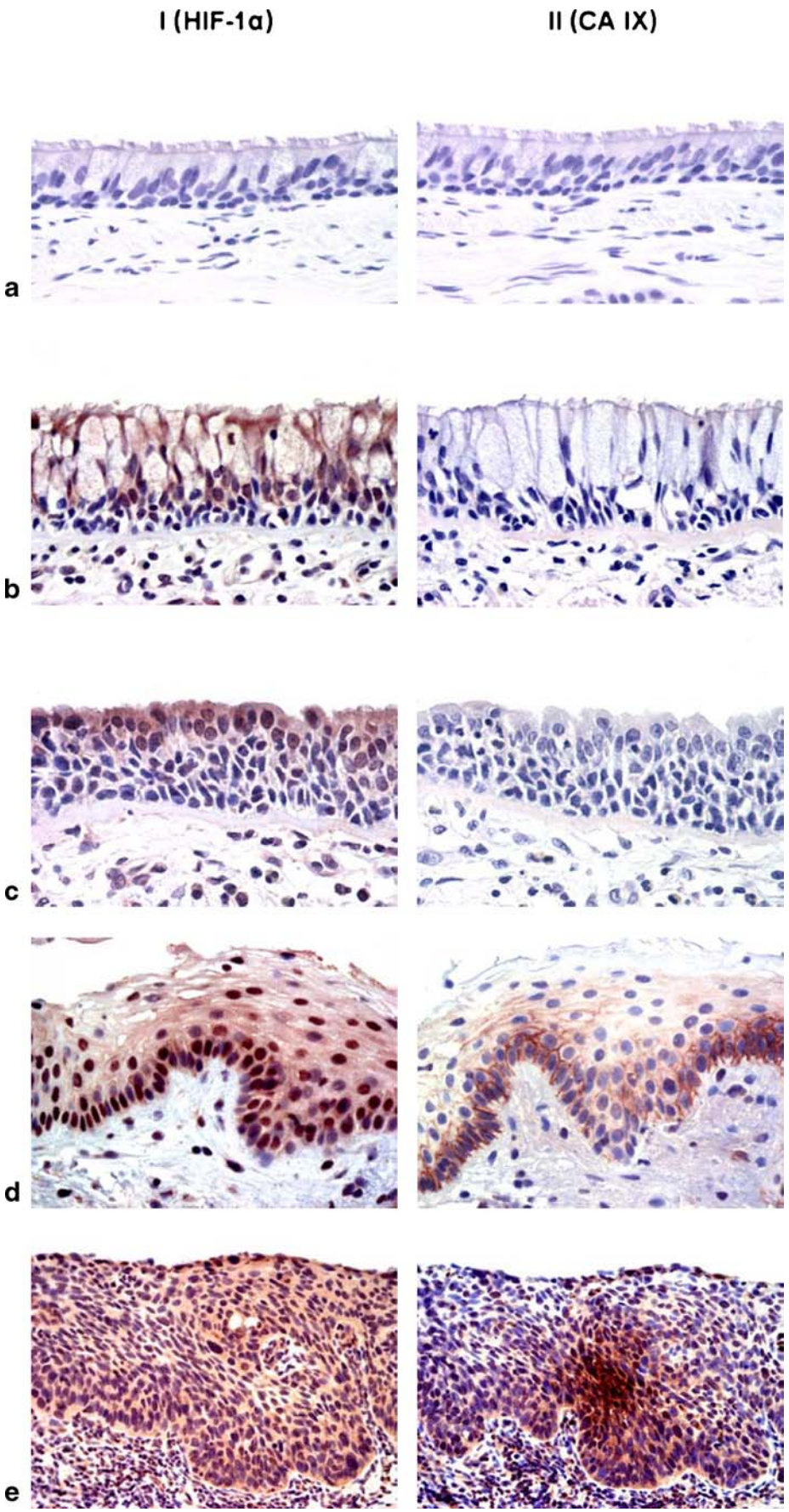


Fig. 3 Expression of HIF-1α and CA IX in bronchial epithelium. *Rows*, structural variants of epithelial transformations including **a** normal, **b** hyperplasia of basal and goblet cells, **c** stratification, **d** squamous metaplasia, and **e** dysplasia (from *top* to *bottom*). *Columns*, specific stains including *I* immunostain with anti-HIF-1α antibody and *II* immunostain with anti-CA IX antibody (from *left* to *right*). *Left column* (*I*) demonstrates the increase of HIF-1α-positive cells according to the severity of epithelial lesions. Initial stages (hyperplasia of basal cells and stratification) are characterized by appearance of positive cells predominantly among surface cells ($\mathbf{b}_I$ and $\mathbf{c}_I$), whereas squamous metaplasia is characterized by predomination of positive cells in the germinal zone, and dysplasia has positive cells throughout the epithelial layer ($\mathbf{d}_I$ and $\mathbf{e}_I$). CA IX appears only in cases of squamous metaplasia and dysplasia (*II* column). Clear immunostain is detected in membranes of basally localized cells in squamous metaplasia ($\mathbf{d}_{II}$), whereas foci of CA IX-positive cells are observed in dysplasia ($\mathbf{e}_{II}$). Paraffin tissue sections, 400× for first to fourth rows and 200× for fifth row

Identification of markers of cellular hypoxia in structurally altered bronchial epithelium

In this part of the study, we hypothesized that loss of functional subepithelial capillaries with fibrosis and increasing RBM thickness could lead to reduced delivery of oxygen and nutrients to the epithelium, contributing to progressive epithelial structural changes. We performed IHC on bronchial biopsy specimens to identify induction of HIF-1α. In these studies, HIF-1α was not expressed in pseudostratified epithelium with normal structure (Fig. $3a_I$). All pathological states of the bronchial epithelium were accompanied by increased HIF-1α expression in both cytoplasm and nuclei of epithelial cells with specific patterns of localization. In the altered pseudostratified epithelium and stratified epithelium, HIF-1α expression was mostly observed in intermediate and surface cells (Fig. $3b_I$,c_I). In contrast, the epithelium with squamous metaplasia was characterized by a predominance of HIF-1α-positive cells in the germinal zone


I (HIF-1α)
II (CA IX)
a
b
c
d
e

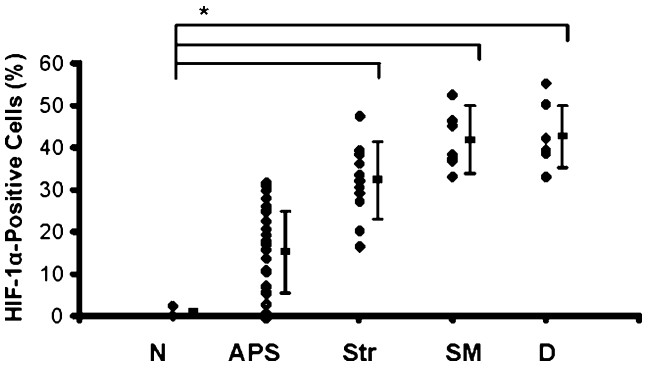


Fig. 4 Graph showing percentage of HIF-1α-positive cells in bronchial epithelium along the spectrum from normal to dysplasia. *Points* represent the average HIF-1α-positive cells associated with a particular epithelial phenotype in each specimen. Mean±SD is indicated for each structural variant. *Asterisk*, $p<0.001$. *N* Normal pseudostratified structure, *APS* altered pseudostratified structure, *Str* stratification, *SM* squamous metaplasia, *D* dysplasia

of the epithelial layer (Fig. 3d_I). When IHC was performed for CA IX, this marker was detected in bronchial epithelial cells in areas of squamous metaplasia and dysplasia (Fig. 3, column II). In squamous metaplasia, CA IX was expressed in membranes of most basal epithelial cells (Fig. 3d_{II}). In both mild and moderate dysplasia, HIF-1α-positive cells were observed throughout the epithelial layer (Fig. 3e_I), and CA IX was detected not only in cell membranes but also in nuclei and cytoplasm (Fig. 3e_{II}). Foci of extensive immunostained cells were also seen.

Quantification of HIF-1α immunostaining showed that the percentage of HIF-1α nuclear-positive cells increased with severity of epithelial alterations (Fig. 4). As seen in Figs. 1f and 2g,h, RBM thickness, blood vessel density, and blood vessel volume density were altered along the spectrum from normal to squamous metaplasia. Analyzing these epithelial states, correlations between HIF-1α expression and morphometric parameters reflecting subepithelial fibrous remodeling showed that the percentage of HIF-1α-positive epithelial cells significantly increased with thickness of the RBM (Fig. 5a), with reduction in blood vessel number (Fig. 5b) and with increased epithelial height (Fig. 5c).

Discussion

Our data indicate that chronic airway inflammation in tobacco smokers with chronic bronchitis and/or COPD is accompanied by structural changes of bronchial epithelium and subepithelial stroma. Specifically, we detected fibrosis and thickening of the RBM and disorders of the subepithelial microvasculature, including reduction in blood vessel density, severe perivascular fibrosis, and structural damage to endothelial cells. Microvascular disorders were identified in both blood and lymphatic microcirculatory systems. Quantitative parameters of subepithelial remodeling, including RBM thickness and blood vessel density, correlate with severity of bronchial epithelial structural lesions. These findings suggest that the epithelium and subepithelium are remodeled as an integrated functional unit. Fibrous remodeling of the subepithelium may alter the physiological balance between the epithelium and underlying stroma

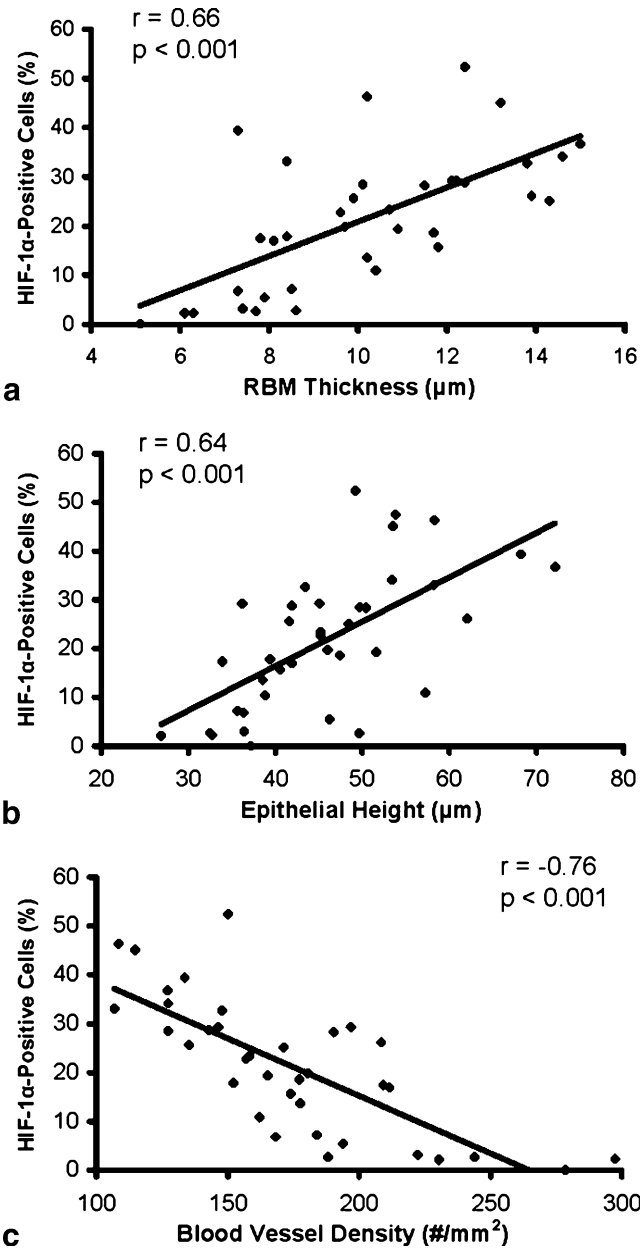


Fig. 5 Correlation of HIF-1α-positive cells with structural changes in airway epithelium and parameters of subepithelial remodeling. **a** Graph showing association between number of HIF-1α-positive cells and RBM thickness. **b** Graph showing association between number of HIF-1α-positive cells and blood vessel density in the lamina propria. **c** Graph showing association between number of HIF-1α-positive cells and epithelial height

and create favorable conditions for development of squamous metaplasia/dysplasia, which are considered as early premalignant lesions. Detection of increased expression of HIF-1α and CA IX in the bronchial epithelium indicates that hypoxic signaling is induced in association with epithelial and subepithelial structural changes.

Prior studies have shown a significant association between tissue hypoxia measured by oxygen microelectrodes and expression of HIF-1α and CA IX [16, 48]; therefore, IHC detection of HIF-1α with nuclear localization and CA IX within cellular membranes in airway epithelial cells indicates the presence of hypoxia. The principal mechanism of HIF-1α regulation is its post-translational modification and subsequent degradation under normoxia or stabilization under hypoxia [2, 9, 43]. Both HIF subunits, HIF-α and HIF-β, are constitutively transcribed and translated; however, the α subunit has a very short lifespan under normoxic conditions and is usually undetectable. Under hypoxic conditions, HIF-1α rapidly stabilizes then translocates to the nucleus and acts as a transcriptional factor [2, 9, 43]. While hypoxia is a major stimulus governing the regulation of HIF activity, several other factors can also stimulate low-level HIF activity including growth factors (such as epidermal growth factor and platelet-derived growth factor) and cytokines (such as tumor necrosis factor-α and interleukin-1β) that can increase HIF-1α expression [15, 50]. However, the amplitude of HIF induction is much lower than hypoxic activation, and only hypoxia leads to HIF-1α stabilization [1]. CA IX is a transmembrane protein, and its expression is closely associated with hypoxia development [45, 47]. Thus, both HIF-1α and CA IX may be considered as endogenous markers of cellular hypoxia.

Although somewhat surprising, given the proximity of airway epithelium to the relatively high oxygen tension of the airway, our results suggest that subepithelial fibrotic remodeling and increased epithelial thickness result in induction of HIF-1α signaling in the airway epithelium. Airway fibrous remodeling with RBM thickening and reduction in effective microvasculature could impair oxygen delivery via the bronchial circulation. In addition, increased epithelial height in areas of structural change could increase diffusion length from the airway lumen. A combination of these factors could lead to insufficient oxygen supply to portions of the bronchial epithelium. Therefore, despite contact between the luminal surface of the bronchial epithelium and inhaled air, our findings suggest that hypoxia of bronchial epithelial cells occurs in areas of airway remodeling.

We observed HIF-1α expression more often than CA IX, which was expressed only in germinal zones of the epithelium with squamous metaplasia or dysplasia. These differences in expression of HIF-1α and CA IX may be associated with the degree of cellular hypoxia that is necessary for HIF-1α stabilization and CA IX expression. The degree of hypoxia required to induce HIF-1α is lower than that required to induce expression of CA IX [25, 45]. Thus, colocalization of both HIF-1α and CA IX in germinal zones of the epithelium with squamous metaplasia or dysplasia may represent bronchial epithelium foci with the greatest degree and/or duration of hypoxia.

Hypoxia has been proposed to be a key microenvironmental factor involved in the development of genetic instability, enhanced mutagenesis, and functional impairment of deoxyribonucleic acid repair pathways [3]. The consequences of hypoxia-induced or hypoxia-repressed gene expression have important implications in disease progression, especially in chronic inflammation and tumor development. While HIF-1α plays a major role in controlling the transcriptional response to hypoxia [2, 9, 43], a number of other transcriptional factors may also be activated either directly or indirectly. Some of the most important pathways involve activation of nuclear factor kappa-B, the cyclic adenosine monophosphate response element-binding protein, and activating protein-1 [7], resulting in the mediation of the global cellular response to hypoxia. Thus, epithelial cellular hypoxia may be an important factor in driving progressive bronchial structural changes and ultimately the development of premalignant and malignant transformations of the bronchial epithelium.

It has been shown that overexpression of the epidermal growth factor receptor and its ligands can develop because of hypoxia-dependent activation of HIF-1α [44, 46], and in turn, this overexpression may lead to epithelial hyperplasia and squamous metaplasia [8]. Thus, the development of epithelial cellular hypoxia might initiate this pathway leading to epidermoid metaplasia. Our data show that squamous metaplasia is associated with maximal fibrous remodeling of underlying subepithelium, reduction in subepithelial microvasculature, and substantial HIF-1α activation. At the same time, established cellular hypoxia in basal layers of epithelial cells in squamous metaplasia may initiate other hypoxia-dependent signaling pathways that can be critical for secondary remodeling in the subepithelium. As noted in this study, RBM thickness decreases and subepithelial vascularity increases within areas of dysplasia, while cellular hypoxia remains prevalent based on HIF-1α and CA IX immunostaining. We speculate that this increased subepithelial vascularity in dysplasia is associated with initiation of angiogenesis via the vascular endothelial growth factor (VEGF) pathway [10, 27, 32] while the reduction in RBM thickness is due to extracellular matrix degradation via matrix metalloproteinase pathways [12, 35]. However, because of severe abnormalities of these newly formed blood vessels and damage to their lining endothelial cells, this secondary stromal remodeling does not restore normal microcirculation.

Our working hypothesis for progressive bronchial epithelial transformation is that epithelial injury initiates airway inflammation and results in fibroblast and myofibroblast activation, leading to fibrous remodeling of the subepithelium [26, 41]. Progressively increased remodeling of the subepithelial connective tissue in turn affects the bronchial epithelium microenvironment and creates conditions for progression to squamous metaplasia. The structurally altered epithelium is unable to sustain the normal functions of the pseudostratified epithelium such as mucociliary clearance, mucosal immune host defense, and modulation of bronchial smooth muscle tone. As a result, airways with altered epithelial structure lose important functional abilities, contributing to subsequent progression of airway inflammation [26, 38]. The formation of this pathological cycle results in progression of airway inflammation and remodeling. The development of epithelial cellular hypoxia and activation of hypoxia-dependent signaling pathways within this pathological cycle may play central roles in carcinogenesis, predisposing to the development of a squamous phenotype of bronchial epithelium and enhancing genetic instability.

The association between inflammation and cancer has been illustrated in many epidemiologic and clinical studies [6, 30, 36, 42]. The specific cellular and molecular pathways that link inflammation to lung cancer are associated with recruitment and activation of inflammatory cells and the subsequent respiratory burst that releases free radicals. Free radicals peroxidate lipids and induce genetic mutations, thus contributing to malignant transformation [6, 18, 22, 30]. The accumulation of mutations in key cell regulatory genes eventually leads to malignant transformation, whereas active proliferation of injured epithelial cells contributes to selection of genetic mutations specific for preneoplastic and neoplastic transformations of the bronchial epithelium [11, 17, 20, 31, 34]. An inflammatory microenvironment enriched with multiple cytokines and growth factors creates good conditions for tumor growth and progression [40, 49]. This pathway is generally assumed as a working hypothesis, whereas our study reveals an alternative pathobiological mechanism that also may contribute to epithelial neoplasia because of changes in the epithelial cell microenvironment and formation of local foci of epithelial cellular hypoxia.

In this study, we demonstrate a close relationship between subepithelial remodeling and structural changes in the bronchial epithelium. We also suggest that the activation of HIF-1α signaling may be an important part of the bidirectional epithelial and mesenchymal interactions that drive airway remodeling. Our findings create a basis for future studies to better define interactions between the airway epithelium and underlying stroma that impact ongoing remodeling. Combined with the results of prior studies showing that VEGF, which is induced by HIF-1α, is increased in bronchial dysplasia [27, 33], our results suggest that hypoxic signaling is a potential therapeutic target to prevent progression of epithelial structural abnormalities, neoangiogenesis, and ultimately the development of squamous cell lung cancer in high-risk individuals with chronic bronchitis and COPD.

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ORIGINAL ARTICLE

Changes in the profile of simple mucin-type *O*-glycans and polypeptide GalNAc-transferases in human testis and testicular neoplasms are associated with germ cell maturation and tumour differentiation

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Abstract Testicular germ cell tumours (TGCT) exhibit remarkable ability to differentiate into virtually all somatic tissue types. In this study, we investigated changes in mucin-type *O*-glycosylation, which have been associated with somatic cell differentiation and cancer. Expression profile of simple mucin-type *O*-glycans (Tn, sialyl-Tn, T), histo-blood group H and A variants and six polypeptide GalNAc-transferases (T1–4, T6, T11) that control the site and density of *O*-glycosylation were analysed by immunohistochemistry during human testis development and in TGCT. Normal testis showed a restricted pattern; gonocytes expressed abundant sialyl-Tn and sialyl-T, and adult spermatogonia were devoid of any glycans, whereas spermatocytes and spermatids expressed exclusively glycans Tn and T and the GalNAc-T3 isoform. A subset of mature ejaculated spermatozoa expressed an additional glycan sialyl-T. The pattern found in testicular neoplasms recapitulated the developmental order: Pre-invasive carcinoma in situ (CIS) cells and seminoma expressed fetal type sialylated glycans in keeping with their gonocyte-like phenotype. Neither simple mucin-type *O*-glycans nor GalNAc-transferase isoforms were found in undifferentiated nonseminoma, i.e. embryonal carcinoma, whereas teratomas expressed them all to some extent but in a disorganized manner. We concluded that simple mucin-type *O*-glycans and their transferases are developmentally regulated in the human testis, with profound changes associated with neoplasia. The restricted *O*-glycosylation pattern in haploid germ cells suggests a role in their maturation or egg recognition/fertilization warranting further studies in male infertility, whereas the findings in TGCT provide new diagnostic tools and support our hypothesis that testicular cancer is a developmental disease of germ cell differentiation.

Keywords Testicular neoplasm · Glycosyltransferases · GalNAc-transferases · Mucin-type *O*-linked glycosylation · Carcinoma in situ testis

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Introduction

Testicular germ cell tumours (TGCT) are a good model for studying cell differentiation because of the unusual features, such as embryonic pluripotency and phenotypic heterogeneity. TGCT occur predominantly in young adults and

originate from a common precursor, carcinoma in situ (CIS) cell [38]. CIS cells morphologically resemble primordial germ cells/gonocytes (fetal pre-spermatogonia), and both cell types share expression of a number of genes; therefore, we have proposed that CIS cells are transformed fetal germ cells that have been arrested in their differentiation [23, 32, 33, 39]. More recent studies have demonstrated that CIS cells express several genes that are necessary for maintaining pluripotency in embryonic stem cells, e.g. *POU-5F1* (OCT-3/4) and *NANOG* [1, 18, 20, 28, 34]. After puberty, CIS cells transform into overt tumours, which can be divided into two main histological types, seminoma or nonseminoma. Seminomas retain the CIS cell morphology (germ cell-like), whereas nonseminomas can exhibit a broad spectrum of phenotypes from undifferentiated embryonal carcinoma (EC) to highly differentiated teratomas, which may contain all types of somatic tissues. In contrast to TGCT, somatic cell tumours, e.g. Leydig cell-derived, are very rare in the testis.

Mucin-type *O*-glycans are found in high density on mucins as well as in glycoproteins, and they play numerous important biological roles, including modulation of protein structure and protease resistance, intracellular sorting of glycoproteins, cell–cell adhesion, microbial interactions and cell maturation. The initial step in mucin-type *O*-glycosylation involves the transfer of GalNAc from UDP-GalNAc to selected serine and threonine residues in proteins. A large family of homologous UDP-GalNAc, polypeptide *N*-acetylgalactosaminyltransferase isoforms (GalNAc-transferases), catalyse the transfer, and these isoforms are differentially expressed in cells and have different specificities for acceptor peptide sequences [12, 19]. GalNAc *O*-glycans are normally subsequently further elongated by other glycosyltransferases forming a variety of structures usually terminated by sialic acid or fucose. The simple mucin-type *O*-glycans, designated Tn (GalNAcα1-*O*-Ser/Thr), sialyl-Tn (NeuAcα2-6 GalNAcα1-*O*-Ser/Thr) and T (Galβ1–3GalNAcα1-*O*-Ser/Thr) antigens (Fig. 1), represent the most immature *O*-glycans in the *O*-glycosylation pathway. These are not generally expressed by normal cells but are abundant in many types of neoplasia and are considered pan-carcinoma markers [41]. The changes in glycosylation during neoplastic transformation are interesting, viewed both from a biological and therapeutical perspective, as the resulting immature carbohydrate structures frequently reflect developmental phenotypes and may be recognised as antigens by immunological defence mechanisms as well as be considered targets for immunological drug design [14, 16].

A large number of studies have investigated 'histo-blood group' antigens and glycolipids in testicular tumours [3, 4, 13, 27, 31, 45]. By contrast, very little is known about the expression of simple mucin-type *O*-glycans Tn, T and sialyl-Tn and sialyl-T as well as GalNAc-transferases that initiate *O*-linked glycosylation and form the Tn antigen

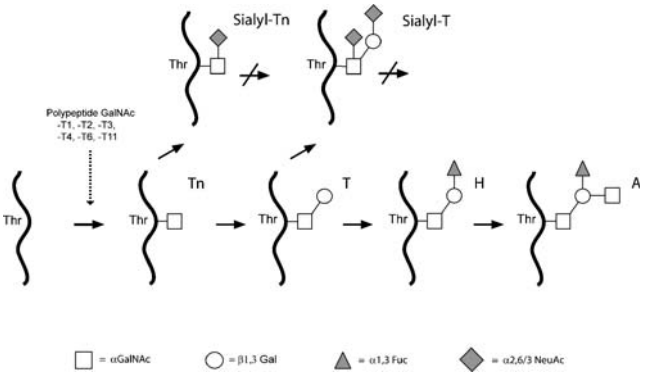

Fig. 1 Schematic depiction of simple mucin-type *O*-glycosylation. GalNAc-transferase enzymes and glycan structures investigated in the present study are indicated. The most immature *O*-glycans Tn, sialyl-Tn and T are considered pan-carcinoma markers

within the testis, and even less is known about their function. *O*-glycans have been implicated as involved in sperm maturation [11]. In addition, a recent study indicated that polyunsaturated, fucosylated glycosphingolipids are essential for spermatogenesis [35]. The mechanism by which spermatozoon binds the egg has not yet been completely elucidated, but there is a consensus that this interaction is carbohydrate-mediated and initiated by glycan-binding molecules (reviewed in [44]).

It has not yet been established to what extent *O*-glycosylation patterns change during germ cell differentiation and maturation in the human testis, nor is it known whether any changes of this pattern occur during malignant progression of CIS to TGCT. Identification of these changes, in particular, possible differences between seminomas and nonseminomas, would shed some light on the pathogenesis and malignant progression in germ cells. Therefore, in this study, we analysed for the first time the expression of cancer-associated mucin-type *O*-glycans Tn, sialyl-Tn and sialyl-T, as well as GalNAc-transferase isoforms that are responsible for *O*-glycosylation during development of normal human testis and in a large series of testicular neoplasms. We demonstrate that simple mucin-type *O*-glycans and their transferases are developmentally regulated. The expression profile in pre-invasive CIS and seminomas mirrored the phenotype of fetal gonocytes, while differentiated nonseminomas had a pattern only partially similar to that in somatic cells, with important pathogenetic and diagnostic implications.

Materials and methods

Tissue and cell samples

The use of human tissue samples for the study was approved by a local Medical Research Ethics Committee.

A series of 94 samples of TGCT and adjacent testicular tissues (which in most cases contained CIS) were obtained from patients who had undergone orchidectomy in three collaborating hospitals. After gross pathological evaluation, small tissue fragments were either frozen or fixed in Stieve's fluid or formalin and paraffin-embedded. The diagnosis of TGCT was confirmed by experienced pathologists after histological evaluation of all samples. The specimens selected for this study included a representative spectrum of histological types, classified as normal testis parenchyma with ongoing spermatogenesis, carcinoma in situ (CIS), seminoma, EC and immature or mature teratomas with variable somatic tissue components. Among specimens containing CIS (n=24), eight samples were adjacent to a seminoma, eight samples in vicinity to nonseminomas, five to combined tumours (containing both a seminoma and a nonseminoma in one testicle), and three specimens were true pre-invasive CIS, without the presence of an overt tumour. All samples with CIS were examined for the expression of sialyl-Tn because the antibody (3F1/TKH2) was suitable for all types of fixatives in paraffin-embedded samples, while a representative selection was stained with all other antibodies. In addition to TGCT, two testicular tumours derived from somatic cells—one Leydig cell tumour and one B cell lymphoma—were also included. A few paraffin-embedded tissue blocks with anonymous fetal tissues left from a previous study of CIS markers [23] were included to study the glycosylation profile during early testis development. The samples were from fetuses autopsied after abortions at the gestational age of 12–20 weeks when there are still numerous gonocytes present. We had no access to frozen fetal tissues. The fetal samples thus were only studied for the glycan profile, and not for the transferases, because antibodies for the latter were not suitable for paraffin-embedded specimens.

In addition, a few anonymised fresh semen samples (N=5) were investigated. These samples were obtained with informed consent from young healthy volunteers who were enrolled into a concurrent prospective study of reproductive health in the Danish population [24]. The staining was performed on leftover smears collected for the assessment of sperm morphology in these subjects.

Finally, a human teratocarcinoma-derived cell line, NTERA2 (NT2/D1) was examined before and after retinoic-acid-stimulated differentiation, as first described by Andrews [2]. Retinoic acid (RA, Sigma) was added to the medium (final concentration of 10^{-5} M) for 3 weeks until cells have differentiated. Immunostaining was performed on formalin-fixed cytospins of untreated and RA-treated cells, as described below.

Antibodies

A panel of well-characterized mouse monoclonal antibodies to simple mucin-type *O*-glycans and human polypeptide GalNAc transferases were used in this study. We have previously raised most of these antibodies and validated their specificity [6–8, 29, 30, 37]. Table 1 below lists all antibodies, their targets and the tissue preparation method.

Immunohistochemistry

For most antibodies, except 3F1, TKH2 and HH8 (after neuraminidase pre-treatment), frozen tissues or cultured

Table 1 The list of antibodies (all murine monoclonal) used in this study

Target antigen, protein	Antibody name/symbol	Tissue preparation recognised	Source
Simple mucin-type *O*-glycans			
Tn	5F4	Paraffin-embedded	Copenhagen University
Sialyl-Tn	3F1 or TKH2	Paraffin-embedded	Copenhagen University
T	HH8	Cryo-preserved	Copenhagen University
Sialyl-T	Neuraminidase +HH8	Paraffin-embedded	Copenhagen University
Histo-blood group variants			
H-type 3	HH14	Cryo-preserved	Copenhagen University
A-type 3	HH5	Cryo-preserved	Copenhagen University
GalNAc-transferases			
GalNAc-T1	UH3 (4D8)	Cryo-preserved	Copenhagen University
GalNAc-T2	UH4 (4C4)	Cryo-preserved	Copenhagen University
GalNAc-T3	UH5 (2D10)	Cryo-preserved	Copenhagen University
GalNAc-T4	UH6 (4G2)	Cryo-preserved	Copenhagen University
GalNAc-T6	UH7 (2F3)	Cryo-preserved	Copenhagen University
GalNAc-T11	1B2	cryo-preserved	Copenhagen University
Testicular germ cell tumour (TGCT) markers			
Placental-like alkaline phosphatase	PLAP	Paraffin /cryo-preserved	DAKO-Cytomation
TRA-1-60 embryonic antigen	TRA-1-60	Paraffin /cryo-preserved	Dr. P. W. Andrews

cells were used. Frozen tissues were cut into 6-μm-thick sections. Cultured cells were dispersed on microscopic slides by centrifugation (Cytospin, Shandon, Pittsburgh, PA, USA). Subsequently, the sections or cytospins were fixed in 4% buffered formalin (15 min at 4°C) and rinsed in Tris-buffered saline (TBS). Paraffin sections were cut into 4-μm-thick sections, dewaxed in light petroleum and rehydrated in a series of ethanol solutions. For detection of sialyl-T, the sections were pre-treated for 2 h in 0.1 U/ml type VI neuraminidase in acetate buffer (Sigma, USA) to remove sialic acid and subsequently stained with HH8 antibody (which without this pre-treatment recognises T). If T was not present, but a sample appeared positive with HH8 antibody after neuraminidase treatment, we could conclude that sialyl-T was expressed. Endogenous peroxidase activity was blocked by 30-min pre-incubation with 1% H_2O_2. Pre-diluted non-immune goat serum (Zymed Laboratories, S. San Francisco, CA, USA) was used to decrease unspecific staining. The incubation with primary antibodies was carried out overnight at 4°C. For all our own monoclonal antibodies, we used 'neat' hybridoma supernatants, without any further dilution. Incubations with the second (link) antibody (biotinylated goat-anti-mouse IgG) and the colour development (a streptavidin-peroxidase conjugate followed by aminoethyl carbazol, AEC, or diaminobenzidine, DAB, and H_2O_2) were performed at room temperature, according to the instructions of the manufacturer (Zymed, Histostain System). The positive reaction manifested as a red (AEC) or brown (DAB) stain. The sections were counterstained in Mayer's haematoxylin. A serial section from each specimen was processed with a dilution buffer substituted for the primary antibody, as a negative control. For positive controls, all specimens were stained for two established germ cell tumour markers, PLAP [22] or TRA-1-60 [3]. The stainings were evaluated by two primary evaluators (ERM and SP), with additional examiners consulting (AG, UM), if needed. Entire specimens of substantial size (approximate average size was 5× 5 mm) were examined; in many cases, two to three sections from the same tissue sample were examined, often in separate staining experiments. Because of heterogeneity of staining in some cases, additional semi-quantitative estimation was added, as indicated in the footnotes to Table 2.

For some tissue sections and cell cytospins, especially spermatozoa, immunofluorescence was used. The protocol was essentially the same as described above for immuno-

Table 2 Summary of the expression of simple mucin-type *O*-glycans and GalNAc-transferases in the human testis and testicular neoplasms

Cell type	Simple mucin-type *O*-glycans				GalNAc-transferases					
	Tn	Sialyl-Tn	T	Sialyl-T	T1	T2	T3	T4	T6	T11
Normal adult germ cells										
Spermatogonia	–	–	–	–	–	–	–	–	–	–
Spermatocytes	–	–	13/17[a]	n.d.	–	–	16/17[b]	–	–	–
Spermatids	2/2[a]	–	17/17[b]	n.d.	–	–	15/17[a]	–	–	–
Spermatozoa (ejaculated)	5/5[b]	–	5/5[a]	5/5[a]	–	–	5/5[a]	–	–	–
Somatic cells (adult testis)										
Leydig cells	3/17[a]	17/17[b]	–	–	16/17[a]	17/17[b]	–	9/17[a]	–[c]	–[c]
Sertoli cells	–	–	–	–	–	–	–	–	–	–
Peritubular cells	–	6/17[a]	–	–	–	–	–	–	–	–
Endothelial cells	–	17/17[b]	–	10/10[b]	–	–	–	–	–	–
Fetal gonocytes	–	7/9[b]	–	2/2[b]	n.a.	n.a.	n.a	n.a.	n.a.	n.a.
Testicular neoplasms										
CIS	–	22/24[a]	–	14/15[a]	1/13[a]	8/13[a]	–	1/13	–	–
Seminoma	–	12/13[b]	–	13/13[b,d]	4/10[a]	11/11[a]	–	4/10[a]	–	–
Nonseminoma										
EC component	2/7[a]	–	–	–	–	–	1/7[a]	–	–	–
Teratomatous somatic components	4/9[a]	8/9[a]	3/7[a]	7/8[a]	7/10[a]	10/10[a]	8/9[a]	1/9[a]	3/9[a]	8/9[a]
EC (NT2/D1) cells[e] −/+RA	+−/+−	−/−	+−/+	−/−	+/+−	−/+−	−/−	−/−	−/−	−/−
Leydig tumour	–	1/1[a,d]	–	–	–	1/1[b]	–	–	–	–
B cell lymphoma (testis)	–	–[d]	–	1/1[b,d]	1/1[b]	1/1[a]	–	–	1/1[a]	–

The positive results are shown as the number of positive samples per total number of samples examined. The negative marked as '−', the impossible to determine marked as 'n.d.' and not available marked as 'n.a.'.

[a] Heterogeneous staining (ranging from negative to clearly positive in a variable number of cells but <50%)

[b] Strong staining in >50% cells

[c] Single positive cells (resembling plasma cells) seen in the interstitial compartment in some cases

[d] Positive cells observed in stromal cells in some cases

[e] Results denoted as + (positive), +− (heterogeneous), − negative

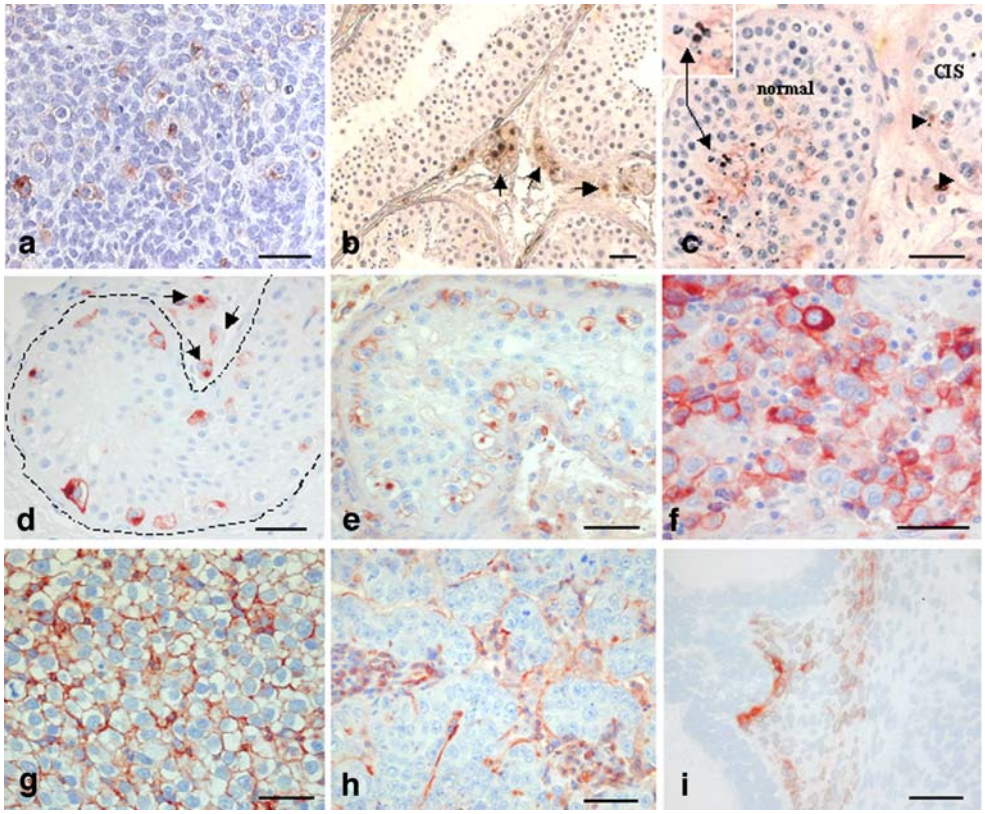

Fig. 2 Examples of immunoexpression of simple mucin-type *O*-glycans in normal human testis (**a**, **b**, **c**), pre-invasive CIS (**c**, **d**, **e**) and overt tumours (seminomas, **f** and **g**, and nonseminomas, **h** and **i**). Sialyl-T antigen is present in fetal gonocytes (**a**), absent in spermatogonia of the adult testis, but present in Golgi apparatus in spermatocytes and acrosomal structures of spermatids (**c**, magnified in the inset; see also Fig. 4) as well as in CIS cells (**c**, marked by *arrowheads*; **e**) and seminoma (**g**) but not in the undifferentiated EC component of teratomas (**h**) where is also present in vascular endothelium. Sialyl-Tn is completely absent from the normal adult seminiferous epithelium (**b**), but it is expressed in Leydig cells (**b** and **d**: *arrows*), heterogeneously in CIS cells (**d**, red-stained cells inside an outlined tubule) and seminoma cells (**f**), while in nonseminomas, it is not present in undifferentiated components but only in rare somatic components of teratomas. Only some somatic elements of non-seminomas express Tn (**i**). Magnification bar=50 μm

peroxidase, except that at the end, instead of a streptavidin-peroxidase conjugate, an FITC conjugate (rabbit anti-mouse IgG, F-261, DAKO-Cytomation or goat anti-mouse IgG1; Southern Biotech) giving a bright light-green signal or an Alexa fluor 594 conjugate (goat anti-mouse IgM; Molecular Probes) giving a light-red signal was used. The sections were counterstained with DAPI and subsequently visualized under a fluorescent microscope.

Results

Normal adult testis

We first established the pattern of expression of simple mucin-type *O*-glycans and GalNAc-transferases in the normal testicular tissue. A summary of the results is given in Table 2, and representative examples are shown in Figs. 2 and 3. The studied glycans were absent from spermatogonia and appeared first in spermatocytes, i.e. T was seen in spermatocytes and spermatids, whilst Tn was first found in spermatids. Ejaculated spermatozoa expressed Tn in >50% of the cells in all cases and T and sialyl-T in a subset of cells (Fig. 4). Sialyl-Tn was not detected in the normal adult germ cells. The A variant was seen in spermatocytes and spermatids only in a subset of samples (presumably in individuals with A blood type), and the H variant was negative in all samples (not shown). As presented in Fig. 3b, germ cells (except spermatogonia) expressed exclusively GalNAc-T3, which appeared first in spermatocytes (>50% in 16 of 17 cases) and was most intense in round and late spermatids. The GalNAc-T3 isoform was also the only GalNAc-transferase that was expressed in ejaculated spermatozoa (>50% in five of five cases; Fig. 4d). The sub-cellular pattern of expression of Tn, T and GalNAc-T3 was very characteristic: Spermatocytes had staining localised to a perinuclear rounded organelle (Figs. 3b and 4d, inset), whereas ejaculated round spermatids and mature spermatozoa displayed a ring-shaped equatorial staining consistent with the edge of acrosome (Fig. 4a, b, d). Sialyl-T, on the other hand,

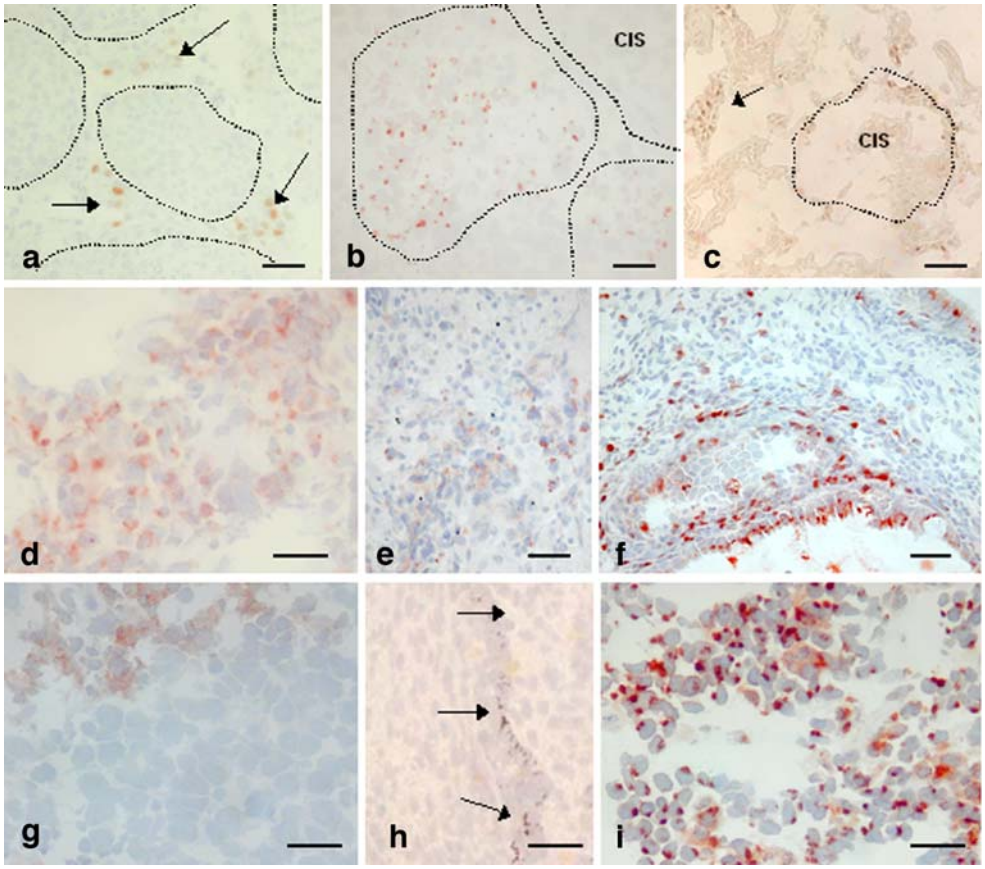

Fig. 3 Immunoexpression of GalNAc-transferases in human testis and testicular neoplasms. Note that all images show frozen tissue samples, where tissue morphology is inferior to paraffin-embedded specimens; thus, seminiferous tubules need to be outlined (**a**–**c**) to delineate their boundaries. GalNAc-T2 is not present in normal adult germ cells, but it is detectable in normal Leydig cells (**a** and **c**, marked with *arrows*), whereas only a very weak staining is seen in a subpopulation of CIS cells in some samples (**c**). GalNAc-T2 is also detectable in seminomas (**d**) and in some tissue element of teratomas and teratocarcinomas (**e**). GalNAc-T3 in normal adult testis appears in spermatocytes and round- and late-spermatids (**b**), but it is absent in pre-meiotic spermatogonia and CIS cells (**b**), while some epithelium-like structures are focally stained in teratomas (**h**, *arrows*). Other GalNAc-transferases shown are: GalNAc-T4 in some components of teratomas (**f**), GalNAc-T6 in the inflammatory infiltrate but not in seminoma cells (**g**) and GalNAc-T1, which is strongly positive in Golgi-like structures of B cell testicular lymphoma cells (**i**). Magnification bar=50 μm

localized to the whole spermatozoa including both the acrosome area and the tail (Fig. 4c).

Leydig cells preferentially expressed sialyl-Tn and GalNAc-T2 (Figs. 2b and 3a) and weakly expressed Tn, GalNAc-T1 and GalNAc-T4. Sialyl-Tn was also present in the endothelium of the blood vessels and present in some of the peritubular myoid cells. In addition, sialyl-T was strongly expressed in endothelial cells of blood/lymphatic vessels.

Expression pattern in testicular neoplasms

We then investigated the expression pattern of simple mucin-type *O*-glycans and GalNAc-transferases in testicular tumours, with special emphasis on the germ-cell-derived neoplasms. We found a completely different pattern of expression in malignant germ cells (Table 2). CIS and especially seminomas expressed sialyl-Tn and sialyl-T, in contrast to Tn and T that characterized the normal testis (Fig. 2). CIS cells demonstrated a weak and heterogeneous expression of GalNAc-T2 in 10 of 13 cases (Fig. 3c) and rarely GalNAc-T1 and GalNAc-T4. The observed pattern was the same in all samples of CIS, regardless of the type of the adjacent tumour. GalNAc-T3, GalNAc-T6 and GalNAc-T11 were not detected in CIS at all (Fig. 3b). This pattern was mirrored by seminomas, i.e. a large number of the seminoma cells expressed GalNAc-T2 (11 of 11; Fig. 3d), GalNAc-T1 and GalNAc-T4 in only 4 of 10 cases, and GalNAc-T6 and GalNAc-T11 were not expressed. GalNAc-T6 was present in a subset of lymphocytic infiltrates associated with seminomas (Fig. 3g). The undifferentiated form of nonseminoma, EC, was practically devoid of any staining, except for a trace of GalNAc-T3 in one sample and focal expression of Tn in a few cells. Teratomas, however, which are differentiated forms of nonseminoma, displayed a focal expression of nearly all the simple mucin-type *O*-glycans and GalNAc-transferases in some epithelial and glandular elements (Table 2, Fig. 3e,f).

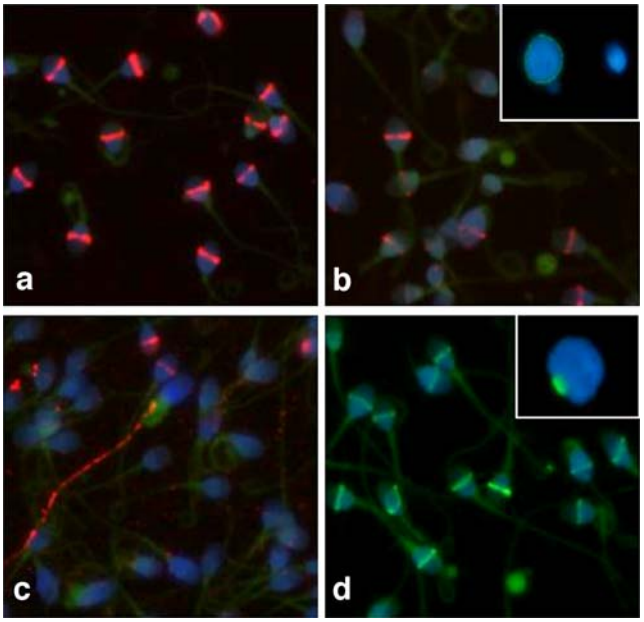

Fig. 4 Examples of immunofluorescence with monoclonal antibodies to simple mucin-type *O*-glycans **a** Tn (marked in *red*), **b** T marked in *red* or *green* (*inset*), **c** sialyl-T (*red*) and **d** GalNAc-T3 (*green*) on ejaculated spermatozoa. In all images, cell nuclei are counterstained with DAP (*blue*). Tn, T and GalNAc-T3 as a ring-shaped staining consistent with the beginning of acrosome formation (**a**, **b**, **d**). Note that Tn and GalNAc-T3 stained most cells and T only few cells. In the *inset* in **b**, T antigen is shown as a round halo in an ejaculated round spermatid, and no staining is seen in the adjacent spermatozoon. Sialyl-T (**c**) is visible on the surface of the tails of spermatozoa, while the heads most probably show the expression of T. GalNAc-T3 (**d**) is visible in the equatorial position of the spermatozoon's head; the GalNAc-T3 expression first begins in spermatocytes in the Golgi apparatus (*inset*)

To further explore the changes of expression during differentiation of nonseminomas, an EC cell line (NT2/D1) was examined for expression of simple mucin-type *O*-glycans, and GalNAc-transferases before and after differentiation triggered in vitro by retinoic acid [2]. Both undifferentiated and differentiated cells expressed Tn and T as well as only two of the GalNAc-transferase isoforms, i.e. GalNAc-T1 and GalNAc-T2. Sialyl-Tn, T and the A and H variants could not be detected.

Somatic cell tumours in the testis demonstrated again a completely different pattern of expression. In one Leydig cell tumour, we could only detect sialyl-Tn and also only one of the GalNAc-transferase isoforms, i.e. GalNAc-T2. A specimen of B cell lymphoma was characterised by very strong expression of sialyl-T and GalNAc-T1 (Fig. 3i), weak expression of GalNAc-T2 and GalNAc-T6 and no trace of other simple mucin-type *O*-glycans or GalNAc-T3 or GalNAc-T4.

Mucin-type antigens in fetal gonocytes

Having observed the marked difference in expression of simple mucin-type *O*-glycans between CIS/seminoma cells and normal adult germ cells, we investigated the pattern of expression of mucin-type *O*-glycans in fetal germ cells, which resemble CIS to a much greater extent than adult germ cells [33]. The majority of fetal samples demonstrated the presence of sialyl-Tn and sialyl-T in early fetal gonocytes (Fig. 2a), similar to CIS and seminoma cells and different from the normal adult testis and EC, which expressed Tn and T.

Discussion

Changes in the expression pattern of mucin-type *O*-glycans are associated with malignant transformation in cancer, and in the present study, we demonstrated marked changes in *O*-glycans and key enzymes involved in *O*-glycan biosynthesis in testicular cancer in comparison to the normal adult testis. We found also distinct changes during differentiation and maturation of normal germ cells. The pattern of expression in germ-cell-derived neoplasms recapitulated closely developmental changes in germ cells, with a notable exception of teratomas, which exhibited peculiar changes in *O*-glycan configuration and GalNAc-transferases, apparently caused by their partial somatic differentiation.

In the normal testis, we found a very restricted pattern of *O*-glycans, combined with expression of only one transferase (GalNAc-T3) in the maturing post-meiotic germ cells. The expression of this transferase was not detected in spermatogonia and was first present at the more mature spermatocyte stage, in a perinuclear area resembling Golgi apparatus. With cell maturation to round spermatids and later to spermatozoa, the protein formed a round equatorial ring at the base of the acrosome. This finding is consistent with our earlier studies that reported the presence of GalNAc-T3 in normal sperm [8, 29]. GalNAc-T3 thus appears to have a specific function in spermatozoa, which may be related to sperm maturation, oviduct attachment or to the adhesion of spermatozoa to the zona pellucida of an egg. GalNAc-T3 was postulated to initiate *O*-glycosylation of zonadhesin, a mucin molecule evolutionarily related to von Willebrand factor [17], as previously shown by in vitro studies of the substrate specificity [8]. Zonadhesin is a testis-specific protein, known to bind the zona pellucida; therefore, we hypothesise that GalNAc-T3 may play a role as an additional mediator of sperm–egg binding, such as, for example, recently identified mouse SED1 [15].

Loss of expression of GalNAc-T3 due to a mutation of the *GALNT3* gene was associated with familial tumoral calcinosis [21, 42, 43]. This rare disease manifests in childhood or early adolescence; therefore, it is presently unknown if familial tumoral calcinosis has implications for fertility. Recently, however, a family was reported with a nonsense mutation in GalNAc-T3, and one affected adolescent boy

exhibited testicular calcium deposits (microlithasis) and oligozoospermia [10]. We hypothesize that lack of GalNAc-T3 expression may impair maturation of spermatozoa and are currently investigating males with fertility problems.

In TGCT, the pattern of expression of simple mucin-type *O*-glycans and GalNAc-transferases was markedly different than in the normal testis. The most striking features were an abundance of sialyl-Tn and sialyl-T, a complete lack of GalNAc-T3 and appearance of GalNAc-T2 in CIS and seminoma. Sialyl-Tn was not detectable in normal adult germ cells, and we have thus shown that sialyl-Tn is a new marker for their neoplastic counterparts in adult men. On the other hand, the practical use of this marker in routine diagnosis is limited because of the presence of sialyl-Tn in Leydig cells and endothelial cells of vessels in the interstitial compartment of the testis.

Accumulation of Tn and sialyl-Tn antigens has been described in other cancers, e.g. breast cancer cells [9, 41], and sialyl-Tn has been implicated as a factor reducing cell interactions, thus promoting migratory and invasive phenotype of cells [25]. Of particular interest is that sialyl-Tn was also detected in fetal gonocytes. This may have something to do with the ability of gonocytes to move inside the tubules from the central parts of seminiferous cords toward the basal membrane. When gonocytes reach the membrane, they mature to spermatogonia, downregulate pluripotency markers and lose sialyl-Tn. The presence of sialyl-Tn (and sialyl-T) in gonocytes is also consistent with similarity between the antigenic phenotype of CIS cells and gonocytes and supports our hypothesis that gonocytes are the cell of origin for CIS [23, 33, 34, 39].

We noticed that, in the neoplastic specimens, sialyl-T displayed a pattern of staining strikingly similar to that of D2-40 antibody, which recognises a tumour-associated antigen, M2A, but does not require sialic acid residues [5]. M2A was recently identified as podoplanin/Aggrus [36, 40]; thus, we hypothesise that in immature testes and TGCT, sialyl-T is associated with podoplanin molecules, while in post-meiotic germ cells, this glycan is bound to another protein, perhaps specific for haploid cells. The reported presence of a sialylated *O*-glycan on the podoplanin/Aggrus molecule supports our hypothesis [26].

The current study focused on the analysis of germ cells. However, we noticed that sialyl-Tn and Tn as well as GalNAc-T1, GalNAc-T2 and GalNAc-T4 were present in Leydig cells. This localisation suggests a possible role in the synthesis or secretion of testicular hormones produced in Leydig cells. In addition, the ability of nonseminomas to somatic differentiation diverted also our attention from germ-cell-specific to somatic patterns of mucin-type *O*-glycosylation. Interestingly, we observed a marked difference between EC, an undifferentiated component of nonseminomas, and differentiated teratomas. Nearly all studied *O*-glycans and GalNAc-transferases could be found focally in small components resembling somatic cells within teratomas. The presence in teratomatous somatic components of simple mucin-type *O*-glycans, which are not usually detectable in normal cells, suggests that these elements still have abnormal pattern of glycosylation, which is one of the hallmarks of cancer cells. These findings could be confirmed only partially by the analysis of NT2/D1 cells differentiating in vitro after retinoic acid treatment. A likely explanation for the lack of expression of the entire panel of studied *O*-glycans and GalNAc-transferases in the differentiated NT2/D1 cells is that their phenotype is largely limited to two somatic cell types: neuron-like elongated cells and flat unclassified cells [2].

In summary, this study has established a complete profile of simple mucin-type *O*-glycans and GalNAc-transferases that control *O*-glycosylation in the human testis and testicular tumours, thus providing a solid reference for further studies. The observed restricted *O*-glycosylation pattern in post-meiotic male germ cells suggests a possible role in their maturation or egg recognition/fertilization and warrants further studies in male infertility. Our findings in testicular tumours demonstrate that malignant germ cells display specific changes in mucin-type *O*-glycosylation and in the initiators of *O*-glycosylation, the polypeptide GalNAc-transferases. Some of these changes reflect the immature phenotype of malignant germ cells, thus providing additional evidence that testicular cancer is a developmental disorder of cell differentiation. In addition, we detected specific changes in the expression pattern associated with differentiation of nonseminomas, which involves transformation from embryonic and partially germ-cell-like phenotype into phenotypes resembling variable somatic lineages. These changes may have an auxiliary diagnostic and prognostic value, as they allow to delineate the somatic-like lineages that retain partially transformed cancerous phenotype.

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ORIGINAL ARTICLE

Ligneous conjunctivitis: a clinicopathological, immunohistochemical, and genetic study including the treatment of two sisters with multiorgan involvement

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Abstract Ligneous conjunctivitis (LC) is a rare disease characterized by wood-like pseudomembranes developing on the ocular and extraocular mucosae secondary to plasminogen (PLG) deficiency. In this paper, we report two cases of LC in two sisters of 57 and 62 years of age that presented with recurrent, bilateral pseudomembranes on conjunctiva and a history of consanguinity and deafness. Pseudomembranes showed superficial and/or subepithelial deposits of eosinophilic amorphous hyaline, amyloid-like material with a variable proportion of granulation tissue, and inflammatory cells. The eosinophilic deposits were negative for Congo red stain, immunoreactive for fibrinogen, and consistently negative for amyloid A component, transthyretin, β_2-microglobulin, albumin, fibronectin, collagen type IV, vimentin, and cytokeratins. Among inflammatory cells, a percentage of positivity of roughly 60% for lymphocytes T (CD3+) and 40% for lymphocytes B (CD8+), with a relation of cytotoxic/helper (CD8/4) T cells of 3:2, was found. In one case, nasal polyps and recurrent gastric peptic ulcer were also characterized by the same subepithelial hyaline deposits. A novel homozygous point mutation c.1856 C>T was found in exon 15 of the *PLG* gene in both patients. Amniotic membrane transplantation was done in one case with promising results.

Keywords Ligneous conjunctivitis · Plasminogen gene · Nasal polyps · Gastric ulcer · Mutation analysis

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Introduction

The term ligneous conjunctivitis (LC) was coined in 1933 by Borel [4] to describe a rare form of chronic, bilateral, recurrent conjunctivitis characterized by the development of firm fibrin-rich, woody-like pseudomembranous lesions mainly on the upper tarsal conjunctivae, and less frequently in the lower eyelid and the bulbar conjunctive [38]. LC was first described by Bouisson [5] as early as 1847, and the microscopic appearance of pseudomembranes presented as inflammatory fungus-shaped masses first reported in 1924 [19]. Because similar lesions can occur on other mucous surfaces indicating a systemic disease, Mingers et al. [23] proposed the term pseudomembranous disease for this entity; the same group [22] also demonstrated severe inherited type I plasminogen (PLG) deficiency as the cause of this disease in 1994. Familial occurrence was also reported [4, 38], and distinct homozygous and compound-heterozygous mutations in the *PLG* gene have been demonstrated by Schuster et al. [35, 39] and other groups since 1997.

Extraocular lesions were reported occurring in the oral cavity (mainly gingiva) [14, 40], the upper gastrointestinal tract [38], the ear (including temporal bone) [7, 15], the nasopharynx, larynx, tracheobronchial tree, and lung [10,

27], the female genital tract (sometimes with infertility) [8, 17, 28], and the renal collecting system [37]. A new syndrome or a coincidental association of non-Hodgkin's lymphoma, auricular hypoplasia, and juvenile colloid milium with LC were recently noted [16]. In very rare cases, congenital occlusive hydrocephalus has been associated with the more severe forms of the disease and may even precede LC [1, 10, 36]. More recently, the report of a single patient with extensive woody deposits in her eyes, hydrocephalus, gingival hyperplasia, otitis media, sinusitis, and genital tract involvement exemplified the various manifestations of the pseudomembranous disease characteristic of severe PLG deficiency [21].

In 1998, it was shown that repeated infusions of PLG could resolve ligneous pseudomembranes [34], and effective treatment with topical PLG was reported in 2002 [47]. More recently, amniotic membrane transplantation has shown promising results in conjuntival reconstruction in difficult-to-manage cases of LC [2, 3].

In this paper, we report the clinicopathological, immunohistochemical, and genetic data of two sisters that presented with LC in adulthood, one of whom was treated with amniotic membrane transplantation.

Clinical history

Two sisters of 57 (Case 1) and 62 (Case 2) years of age were referred to the Clinical University Hospital with a diagnosis of bilateral epidemic conjunctivitis and unsatisfactory responses to different treatments. Consanguinity and moderate deafness were found in both patients. In Case 2, there had been a previous history of deafness at age 32, recurrent gastric peptic ulcer at age 57, and recurrent nasal polyps at age 60. No other additional relevant data were found in the clinical records of the two patients. The patients showed abundant mucopurulent secretions with wood-like pseudomembranes on the upper and lower tarsal conjunctiva, with *Staphilococcus epidermidis* in culture.

A putative diagnosis of LC was made based on recurrences during the previous 6 months to 1 year period (in both cases) and on the clinical appearance of the lesions (Fig. 1a and b). Surgical excision of pseudomembranes with a thorough cleaning of the surgical field using 5,000 IU/ml heparin eye drops was carried out. Pathological examination of the removed tissue supported a diagnosis of LC, and a PLG deficiency was confirmed with a level of 0.061 U/ml in Case 1 and of 0.045 U/ml in Case 2 (normal range, 0.80–1.20). After surgery, both patients underwent topic treatment with dexametasone at 0.1% every 4 h, cyclosporine A at 2% every 6 h, heparin 5% eye drops every 2 h, and ofloxacine every 6 h until complete epithelization was achieved. Unfortunately, topical PLG drops were unavailable. Six weeks later, both patients presented with a new recurrence of pseudomembranes, and similar surgical and medical treatment was undertaken. Twelve weeks later, the Case 1 patient presented again with serious lesions in tarsal and nasal conjunctiva of both eyes with no corneal involvement. Excision of all inflamed tissue

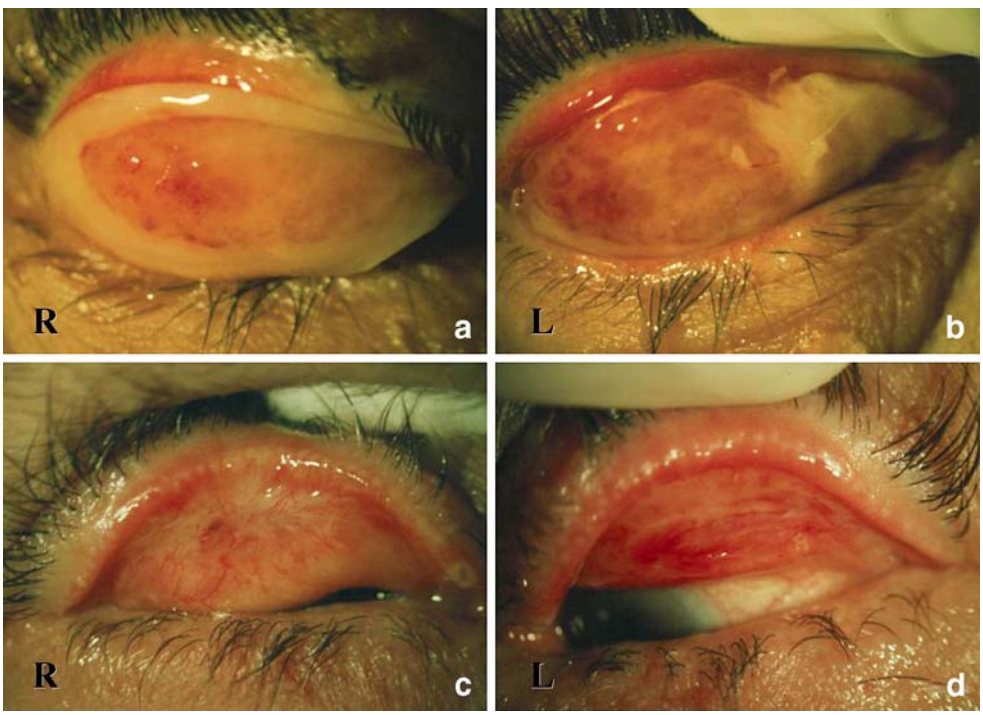

Fig. 1 LC. **a**–**b** A thick membrane covers the upper palpebral conjunctiva in both eyes (Case 1). **c**–**d** Postoperatively, amniotic membranes have disappeared, and there are no pseudomembranous lesions

together with amniotic membrane transplantation for conjunctival reconstruction under local anesthesia was decided upon. Previous to surgery, the ethics committee of our institution approved treatment, and the patient gave her written informed consent. The human amniotic membrane was prepared according to the method of Tseng et al. [46]. The corneal surface was covered by a therapeutic contact lens to prevent abrasion, and the amniotic membrane epithelium was secured up to the conjunctival edge with interrupted sutures. Perioperative topical treatment with intensive (every 30 to 60 min) heparin (5,000 U/ml) was carried out. The patient received dexamethasone and tobramicine eye drops four times daily for 6 weeks, heparin (5,000 U/ml) eye drops gradually tapered down from six times daily over 3 months, and topical cyclosporine A (2%) every 6 h daily for 6 weeks. Two months postoperatively, the amniotic membranes had dissolved, and the conjunctiva appeared free of pseudomembranous lesions (Fig. 1c and d). The patient has been examined monthly by the same ophthalmologist, and no recurrence of membranes has occurred in 39 months. Case 2 patient was lost on follow-up due to death caused by an ovarian poorly differentiated adenocarcinoma.

Materials and methods

Histological, histochemical, and immunohistochemical analyses

The surgical specimens were fixed in buffered formaldehyde and routinely embedded in paraffin. The 4-μm-thick sections were stained with hematoxylin and eosin and Congo red stain. Immunohistochemical studies were also performed on paraffin sections using a universal second antibody kit that utilized a peroxidase-conjugated labeled-dextran polymer (Dako EnVision Peroxidase/DAB; Dako, Glostrup, Denmark), to avoid misinterpreting endogenous biotin or biotin-like activity in tissues [9] as positive staining. The following antibodies were used: amyloid A component (clone mc1, dilution 1:50, Novocastra, Newcastle upon Tyne, UK), transthyretin (polyclonal, 1:1,000, antigen retrieval water bath, Dako, Glostrup, Denmark), β_2-microglobulin (polyclonal, 1:2,000, Dako), fibrinogen (polyclonal, 1:200, microwave, Dako), albumin (polyclonal, 1:2,000, BioGenex, San Ramon, CA, USA), fibronectin (polyclonal, 1:10,000, water bath, Dako), collagen type IV (CIV, 1:10, microwave oven and EDTA), vimentin (V9, 1:5,000, microwave oven, BioGenex), cytokeratins (AE1–AE3, 1:20, water bath and proteinase K, Dako), immunoglobulin A (IgA; polyclonal, 1:10,000, microwave oven, Dako), IgD (polyclonal, 1:1,000, water bath, Dako), IgG (polyclonal, 1:10,000, microwave oven, Dako), IgM (R1/69, 1:500, microwave oven, Dako), light chains kappa (polyclonal, 1:50,000, water bath, Dako), light chains lambda (polyclonal, 1:50,000, water bath, Dako), CD3 (SP7, 1:50, water bath and EDTA, Master Diagnóstico, Granada, Spain), CD4 (4B12, 1:4, water bath and EDTA, Master Diagnóstico), CD8 (4B11, 1:20, water bath and EDTA, Novocastra), and CD20 (L26, 1:50, water bath, Dako). Negative control samples (the primary antibody was replaced by nonimmune mouse serum) and positive tissue samples were included in each slide run.

Mutation analysis

Informed consent was obtained for this study, which was conducted according to the Spanish law including adherence to the Helsinki Principles of 1975, as revised in 1983. For Case 1, genomic DNA was extracted from peripheral white blood cells by standard methods. For both cases (Case 1 and Case 2), to confirm our results, genomic DNA was extracted from paraffin-embedded tissue using QIAamp DNA Kit and QIAamp DNA Blood Mini Kit (QIAGEN: www.qiagen.com), following the manufacturer's instructions. Thereafter, all coding regions and exon/intron boundaries of the *PLG* gene were amplified by polymerase chain reaction (PCR). To prevent the coamplification of homologous genes, we used primers and strategy described in Siboni et al. [41]. After the amplification, PCR products were purified. Both strands were subjected to cycle sequencing with the same PCR primers that covered the complete gene, both exons and introns, using the BigDye terminator kit and run in the 3730 xl DNA Analyser (Applied Biosystems, Foster City, CA, USA).

Results

Pathological and immunohistochemical findings

Histological examination revealed similar findings in both patients. Pseudomembranes showed superficial and/or subepithelial deposits of eosinophilic amorphous hyaline, amyloid-like material with a variable proportion of granulation tissue, and inflammatory cells, mainly plasma cells and lymphocytes (Fig. 2a). Ulceration and hyperplastic changes in the residual epithelium were observed. The eosinophilic deposits were negative for Congo red stain, immunoreactive for fibrinogen (Fig. 2b), and consistently negative for amyloid A component, transthyretin, β_2-microglobulin, albumin, fibronectin, collagen type IV, vimentin, and cytokeratins. A similar proportion of immu-

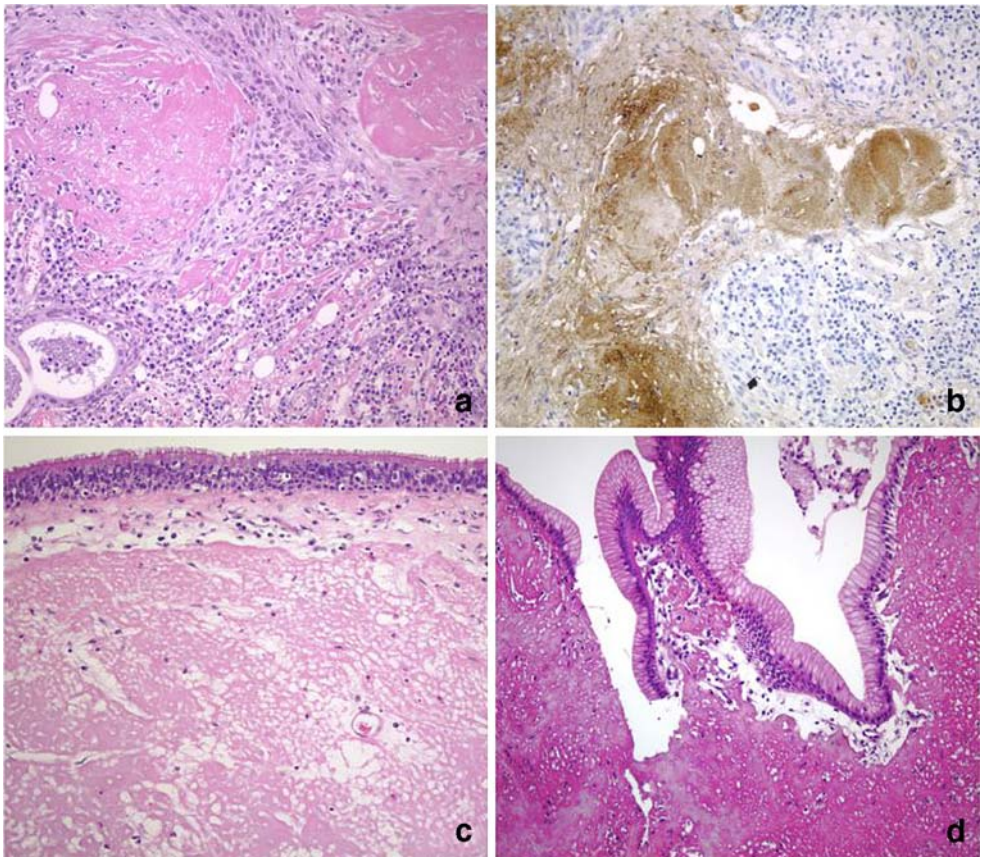

Fig. 2 Ligneous disease (**a**–**d**). An inflammatory cell component with amyloid-like material is noted in the histological section of the conjunctiva (**a**). Immunohistochemical analysis of the deposits demonstrates positive localization of fibrinogen (**b**). Subepithelial deposits of amorphous eosinophilic material in nasal polyp (**c**) and in gastric mucosa (**d**)

noreactivity for light chains was found in plasma cells, but no stain was found in the hyaline material. Among inflammatory cells, a percentage of positivity of roughly 60% for lymphocytes T (CD3+) and 40% for lymphocytes B (CD8+), with a relation of cytotoxic/helper (CD8/4) T cells of 3:2, was found.

Reevaluation of biopsies in Case 2 showed both exuberant granulation tissue and subepithelial fibrin deposits in gastric biopsies (Fig. 2d) and in metastatic vaginal lesions from the ovarian adenocarcinoma. The nasal polyps were also composed of subepithelial deposits of amorphous eosinophilic material instead of the loose mucoid stroma characteristic of nasal inflammatory polyps (Fig. 2c).

Genetic analysis

Direct sequencing of both single strands of PLG exon 15 from patient 1 showed a homozygous point mutation c.1856 C>T (using GenBank X05199 as reference sequence and starting with +1 at the A of the ATG translation initiation codon), which leads to an amino acid change ACT (Thr) to ATT (Ile) at position 600: p.Thr600Ile (the amino acid residues are numbered starting with the amino-terminal glutamic acid residue of the mature PLG as number +1) (Fig. 3). This mutation lies in the beta chain of PLG, 39 amino acids downstream of the Arg561-Val562 cleavage site that is necessary for the conversion of PLG to plasmin [29]. The same mutation was also present in homozygous state on the paraffin samples of the patient and her deceased sister.

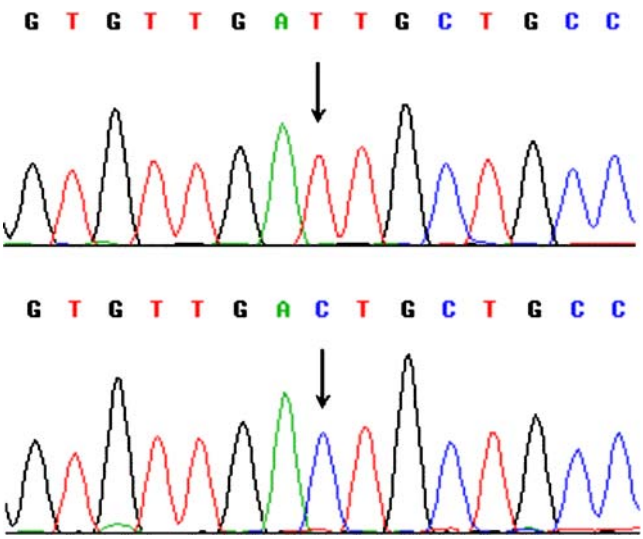

Fig. 3 Electropherogram of the direct sequence of exon 5 *PLG* gene. Homozygous novel germline mutation (c.1856 C>T) in DNA from patient's blood sample (*top*). Wild-type sequence corresponding to a healthy control DNA (*bottom*)

Discussion

According to the excellent revision of Shuster and Seregard [38], the diagnosis of LC is based on the clinical picture (pseudomembranous lesions), the typical histological findings, and eventually on a positive family history. All of these criteria were present in the two patients here presented, with some unusual features that deserve consideration.

The prevalence of LC may be roughly estimated in the range of 1.6 per 1 million people [38], at least in Europe, but in countries where consanguinity is more frequent, the prevalence of LC may be higher [10]. Although most cases of LC are sporadic, some familial cases have been reported with an autosomal-recessive pattern [4, 38]. In most cases, infants and children are affected, but late onset manifestation of LC may occur, as in our patients, ranging from birth to 85 years [18, 38]. Both eyes are affected in up to 51% of cases, and corneal involvement, a complication that may lead to blindness, occurs in 20–30% of cases [38]. Systemic signs and symptoms may precede or concomitantly occur with the ocular disease.

Histopathological findings of the ocular and extraocular lesions in LC are identical. The excessive depositions of fibrinogen in LC should be differentiated from very rare cases of amyloidosis of the eye [20]. While amyloid deposits are positive with the Congo red stain, fibrinogen is negative. It must be noted that, in some extraocular biopsies, the nature of the disease may be overlooked by the pathologist, particularly if there is no clinical suspicion of LC, as occurred in our Case 2. Immunohistochemical studies for fibrinogen can definitively confirm the nature of the eosinophilic material in LC as occurred in our biopsies.

PLG plays an important role in fibrinolysis and wound healing. In patients with LC, wound healing in mucous surfaces is impaired and seems arrested at the stage of granulation tissue [30, 38]. Histopathological and immunohistochemical findings indicate that fibrinogen is the major constituent of the membranes in LC. As was referred to in the literature, and as we confirmed in our patients, severe type I PLG deficiency (hypoplasminogenemia) is a major cause of LC [23, 24, 38]. The observation of cases of LC developed during treatment with tranexamic acid, an antifibrinolytic drug, also supports this idea [12].

Several mutations have been detected in the *PLG* gene of patients affected with LC [10, 33, 38, 43, 45]. PLG is the proenzyme of the serine protease plasmin and is a 791-amino-acid residue single-chain glycoprotein with a molecular weight of 93 kDa [26]. Its gene spans approximately 52.5 kb on chromosome 6q26–27 and consists of 19 exons [11]. The proteolytic activity of plasmin contributes to the digestion of the insoluble fibrin clot and thrombi by which normal recanalization and tissue repair can be accomplished [31]. The mutation p.Thr600Ile of the PLG gene is very close to His-603, one of the three amino acid residues that constitute the serine protease triad. Moreover, p.Thr600Ile is next to p.Ala601Thr, a mutation present in 2–4% of Japanese subjects that predisposes them to thrombophilia and is known as Plasminogen Tochigi [25]. But, as stated for ligneous conjunctivitis [35], the mutation found in our patients does not predispose them to thromboembolic episodes [6]. In these patients, however, the risk of thrombotic occlusions of implanted catheters may be markedly increased [21, 38]. To the best of our knowledge, this is the first report of this mutation described in patients with LC.

LC has also been reported in different animal species [38]; mice in which both PLG genes have been knocked out (PLG-deficient mice, $Plg^{-/-}$ mice) will develop conjunctival lesions indistinguishable from human lesions [13]. The clinical phenotype in $Plg^{-/-}$ was reported to differ from that in humans with LC, in that, in humans, gastric, colon, and rectal ulcers, rectal polyps, and liver involvement have not been reported so far. We believe, however, that our Case 2 patient is, as far as we know, the first description of recurrent gastric ulcer and LC.

Shuster and Seregard [38] and Tefs et al. [44] concluded that a potentially useful initial approach for LC is the topical application of a PLG concentrate, possibly in combination with a PLG activator (uPA or tPA), which may soften the pseudomembranes and facilitate removal. To prevent recurrence, manipulations of the conjunctiva should be kept to a minimum, and treatment with heparin and corticosteroids should be undertaken, sometimes combined with topical cyclosporine A [3, 38, 44]. Topical and subconjunctival fresh frozen plasma may also help in the prevention of membranes in susceptible PLG-deficient patients [42]. Therapy with intravenous infusion of lys-plasminogen was favorable in one case [34], but impossible as a long-term solution due to the short half-life of the preparation, the risk of intravascular fibrinolysis activation, and the high costs [3]. In rare cases, LC may resolve itself spontaneously [38]. Amniotic membrane transplantation is effective in some corneal and conjunctival lesions because of production of antiangiogenic and antiinflammatory proteins; Barabino and Rolando [2, 3] has proposed this as a promising therapy for LC. Although other experimented researchers [44] have not found this procedure adequate, we have had no recurrences of pseudomembranes in our patient for 39 months after amniotic membrane transplantation nor did any appear in the patient reported by Barabino and Rolando [3] for at least 3 years. Oral contraceptive therapy deserves attention as an alternative therapy in selected cases of LC [32].

In summary, the present paper, describing two cases of LC shows that LC is a rare disease characterized by wood-

like pseudomembranes developing on the ocular and extraocular mucosae secondary to PLG deficiency. Pseudomembranes are subepithelial deposits of amorphous eosinophilic material (fibrinogen) with a variable proportion of granulation tissue and inflammatory cells. The same subepithelial deposits occurred as nasal polyps and recurrent ulcers in gastric mucosa. A novel homozygous point mutation c.1856 C>T in exon 15 of the *PLG* gene was found in both patients, and amniotic membrane transplantation was done in one case with promising results.

Acknowledgment We thank Ms. Elena Couso Folgueiro for her technical assistance.

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ORIGINAL ARTICLE

Cellular and tissue localization of globotriaosylceramide in Fabry disease

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Abstract The pathogenesis of Fabry disease is poorly understood. We used a variety of immunohistological techniques to localize globotriaosylceramide, the main glycolipid that accumulates in Fabry disease. Globotriaosylceramide immunoreactivity in a heterogenous pattern was present in all organs examined of a patient on long-term enzyme replacement therapy. In the brain, immmunopositivity was found only in the parahippocampal region. Globotriaosylceramide immunostaining was present in the cell membrane and cytoplasm of endothelial cells, even in the absence of lysosomal inclusions. In kidney tissue, globotriaosylceramide colocalized with lysosomal, endoplasmic reticulum, and nuclear markers. Pre- and post-embedding immunogold electron microscopy of skin biopsies and untreated patient cultured skin fibroblasts confirmed the presence of globotriaosylceramide in the cell membrane, in various cytoplasmic structures, and in the nucleus. Control organ tissues and cultured fibroblasts from five unaffected subjects were uniformly negative for globotriaosylceramide by immunohistochemistry and immunogold electron microscopy. We conclude that a substantial amount of lysosomal and extralysosomal globotriaosylceramide immunoreactivity remains in cells and tissues even after years of enzyme replacement therapy in Fabry disease. These findings are crucial for the understanding of the disease mechanism and suggest the usefulness of immunostaining for globotriaosylceramide as a means to assess response to novel, specific therapies.

Keywords Glycolipids · Immunohistochemistry · Electron microscopy · Lysosomal disorder · Lysosome

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Introduction

Fabry disease, a systemic disorder caused by a deficiency of the lysosomal enzyme α-galactosidase A, results in a failure to catabolize α-D-galactosyl moieties and a systemic deposition of glycosphingolipids particularly globotriaosyl-

ceramide (Gb_3) [5]. The accumulation of Gb_3 in virtually all cell types causes a progressive disorder that consists of a painful small fiber neuropathy, cardiac disease, chronic renal insufficiency, and a high propensity for cerebrovascular strokes [6]. Gb_3 is assumed to be the main offending metabolite, although the mechanism by which its accumulation leads to the multisystem disorder of Fabry disease is not known. It has traditionally been stated that Gb_3 is stored only in lysosomes and that mechanically or by some other process these lipid-laden lysosomes cause disease [8, 15]. However, studies of the mechanisms of the vasculopathy of Fabry disease suggested increased production of reactive oxygen species invoking dysfunction of other cellular components such as endothelial nitric oxide synthase, which is associated with the cell membrane [1, 27]. Research into other lysosomal disorders pointed toward interaction of the accumulating glycosphingolipid with distinct ion channels or transporters that are mostly located in the endoplasmic reticulum (ER) [22, 34, 35, 44]. Similar interactions may occur for the accumulating substrate in Fabry disease. To gain insight into potential abnormal interactions between Gb_3 and molecular components, we studied the cellular and tissue localization of Gb_3 in patient cell culture, skin biopsies, and autopsy samples in the context of long-term enzyme replacement therapy. We hypothesized that Gb_3 is present in extralysosomal cellular compartments in Fabry disease.

Materials and methods

The study materials consisted of autopsy tissue sampled from a previously published 47-year-old patient with the classic form of the disease who died of a myocardial infarction after 2.5 years on agalsidase beta treatment (patient 1) [39]. This patient demonstrated the virtual absence of lysosomal inclusions in vascular endothelial cells. Non-Fabry autopsy control tissues from three patients who died from an acute myocardial infarction and a fourth who died from a cholecystitis and sepsis (complicated by an acute myocardial infarction) were used for immunohistochemical studies. Skin biopsies were obtained from a 28-year-old patient with the classic form of Fabry disease (patient 2) who has been treated with agalsidase alpha for 7 years at a dose of 0.2 mg/kg every 2 weeks and from a healthy control volunteer subject, and analyzed by immumohistochemisty and immunogold electron microscopy. Immunohistochemistry of cultured skin fibroblasts from two patients with classical Fabry disease (patients 2 and 3) and healthy controls were also studied. All subjects who had skin biopsies gave their written informed consent on a study protocol approved by the institutional review board of the National Institute of Neurological Disorders and Stroke.

Light microscopy

Formalin-fixed sections (5 μm thick) were stained with hematoxylin and eosin, Luxol fast blue, and toluidine blue. A pair of slides were selected from each organ for Gb_3 immunohistochemistry. Slides were deparaffinized, rehydrated, and then quenched at room temperature for 30 min in 0.03% hydrogen peroxide. All slides were then washed for 5 min in phosphate-buffered saline (PBS), immersed in 2.5% Horse serum (Vector Laboratories, Burlingame CA) for 20 min, incubated with anti-Gb_3 monoclonal antibody (1:400 in PBS, Seikagaku, Tokyo, Japan, clone BGR-23 [19]) washed for 5 min with PBS, and incubated with ready-to-use secondary antibody (Vectastain kit, Vector Laboratories). Adjacent sections were stained with the secondary antibody only. Slides labeled with the secondary antibody were washed with PBS for 5 min and incubated with avidin/biotinylated horseradish peroxidase (Elite Rabbit IgG Vectastain ABC kit,PK-6101,Vector Laboratories). 3,3′-Diaminobenzidine tetrahydrochloride was used as chromogen and hematoxylin was used as counterstain.

Immunostaining of cultured cells

Cultures of primary skin fibroblasts were derived from explant cultures of skin biopsies taken from patients affected with Fabry disease who have α-galactosidase A mutations K240X or R356W. The cells were maintained in antibiotic-free Alpha-Minimum Essential Medium culture medium (Invitrogen, Carlsbad, CA) supplemented with 10% cosmic calf serum (Hyclone, Logan, UT). Cells were used for staining before the tenth passage.

For immunolocalization studies by light microscopy, fibroblasts were seeded on 12-mm glass coverslips in growth medium and cultured until confluent (7 days). Cells were fixed with 2% paraformaldehyde for 20 min, incubated with blocking buffer (2% bovine serum albumin [BSA] in PBS) for 60 min at room temperature, and then incubated overnight at 4°C with anti-Gb3 mouse monoclonal antibody (Seikagaku America, Falmouth, MA; 1:200 in blocking buffer). After extensive washing, antibody binding was visualized by incubating the coverslips with Alexa 594-labeled goat anti-mouse IgG antibody (1:200 in blocking buffer) for 30 min at room temperature. The coverslips were washed and mounted on glass slides with FluorSave mounting medium (Calbiochem, San Diego, CA). Samples were imaged with a 40× objective and using a rhodamine filter set (excitation 546/emission 590) on a Zeiss Axioplan fluorescent microscope equipped with a Spot RT Slider digital camera.

For immunostaining and immuno-electron microscopy, Fabry or normal fibroblasts were seeded on glass 12-mm-

diameter glass coverslips in the growth medium and incubated for 3 days without additional feeding.

Immunogold electron microscopy

Pre-embedding and post-embedding techniques were used on different samples from the same subjects.

Pre-embedding technique Skin biopsy or cell culture material was immersion fixed in freshly made 4% paraformaldehyde in PBS for 1–12 h. Then, biopsies were cut with a vibratom (100-μm-thick slices) and fixed with 4% glutaraldehyde in 0.1 N sodium cacodylate buffer overnight for electron microscopic examination or processed for pre-embedding immunogold labeling. The immuno-labeling procedure was done as previously described [43] with steps carried out at room temperature unless otherwise indicated. Briefly, vibratomed slices were washed in PBS, blocked, permeablized with saponin (0.1% in PBS with 5% normal goat serum) for 1 h, treated with mouse anti-Gb_3 monoclonal antibody (1:400; Seikagaku America) for 1–2 h, washed, treated with secondary antibody conjugated with 5-nm gold particles (Nanogold from Nanoprobes, 1:250, Yaphand, NY) for 1 h, and silver enhanced for 10–20 min with the HQ kit from Nanoprobes. Slices were then treated with 0.2% OsO_4 in phosphate buffer for 30 min, mordanted en bloc with 0.25% uranyl acetate overnight at 4°C, dehydrated with ethanol, and flat embedded in epoxy resin. Thin sections were counterstained with uranyl acetate and lead citrate.

Post-embedding technique Each skin biopsy (Fabry and control) was fixed in glutaraldehyde (2.5% in PBS 0.1 M pH 7.3, overnight), dehydrated in a graded series of increasing ethanol concentrations, and embedded in Spurr low-viscosity epoxy using flat molds at 70°C for 18 h. Semi-thin sections (0.5 μm) were stained with 1% toluidine blue and then sectioned (500–700 Å) and placed on square mesh nickel (300 mesh), thin-bar, high-definition grids for the post-embedding immunogold reaction [3, 30, 41]. For antigen retrieval, grids were immersed in sodium citrate (0.1 M, pH 6.0, 95°C, 10 min), washed, and incubated with blocking solution (1% BSA in 0.1 M PBS, with 0.05% Tween 20) for 30 min at room temperature [42]. Sections were incubated with mouse anti-Gb_3 monoclonal antibody (1:200 in PBS) for 90 min at room temperature, washed, and incubated with a secondary goat anti-mouse antibody labeled with 15-nm gold particles or protein A polygold 15 nm (1:25 dilution of blocking solution Polysciences, Warrington, PA) for 1 h at room temperature. After an additional wash, sections were stained with uranyl acetate in distilled water for 20 min. Samples incubated with PBS instead of the first antibody (mouse anti-Gb_3) were used as controls. Samples were analyzed using a Philips CM100 EM electron microscope at 80 kV from the Biomedical Instrumentation Center, Uniformed Services University of the Health Sciences.

Immunofluorescence staining

Paraffin sections were melted (60°C), hydrated, and then put in Tris–ethylenediamine tetraacetic acid (EDTA) buffer PT Module™ Buffer 4 (100× Tris–EDTA Buffer, pH 9.0) TA-050-PM4X (Lab Vision, Fremont CA) for antigen retrieval. Sections were then rinsed once in 1× Tris-buffered saline (TBS) Tween (Lab Vision) for 5 min and incubated in peroxidase block (Biocare Medical, Concord, CA) for 15 min followed by a rinse in TBS Tween for 5 min. Sections were blocked using Background Sniper (Biocare Medical, Newport Beach, CA) for 15 min followed by incubation with the primary monoclonal antibody marker of lysosomes (anti-lysosome-associated membrane protein 1, BD Biosciences, San Jose CA) diluted in DaVinci Green (Biocare Medical) for 2 h at room temperature or overnight

Table 1 Results of immunohistochemical staining with anti-Gb_3 antibody viewed by light microscopy

Tissue	Region	Gb_3 detected	
		Fabry	Normal
Heart	Myocardium	Yes	No
	Endocardium	Yes	No
	Coronary arteries	Yes	No
	Intracardiac nerves	Yes	No
	Vasanervorum	Yes	No
Kidney	Distal tubules	Yes	No
	Proximal tubules	Yes	No
	Glomeruli	Yes	No
	Renal blood vessels	Yes	No
Brain	Cortex in general	No	No
	Meningeal blood	Yes	No
	Cerebral blood	Yes	No
	Parahippocampal gyrus	Yes	No
Adrenal	Vasculature of capsule	Yes	ND
	Glomerulosa	Yes	ND
	Reticularis	Yes	ND
	Fasciculata of cortex	No	ND
	Medulla	Yes	ND

ND Not done

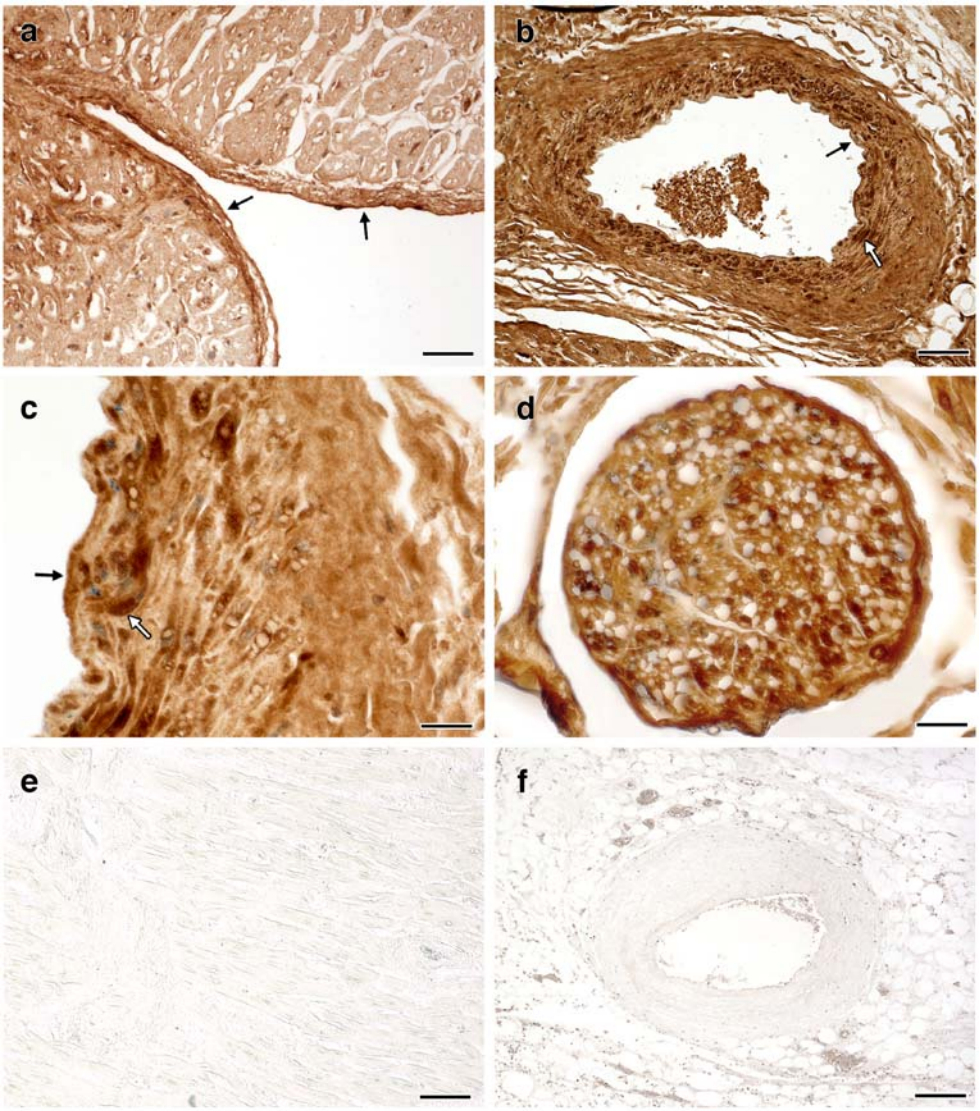

Fig. 1 Gb_3 immunohistochemistry in the heart of Fabry patient 1 shows extensive staining of the coronary artery, myocardium, and endocardium as well as intravascular and intracardiac nerves but no staining in normal tissue. **a** Staining of the myocardium and endocardium (*arrows*, original magnification 20×, *scale bar*=50 μm). **b** Coronary artery showing endothelial (*black arrow*) and vessel wall staining (*white arrow*, original magnification 10×, *scale bar*=100 μm). **c** Higher magnification of the same coronary vessel wall (original magnification 40×, *scale bar*=25 μm). **d** Staining of an intracardiac nerve showing Gb_3 in Schwann cells and endoneurium (original magnification 40×, *scale bar*=25 μm). **e** Staining or Gb_3 staining in a non-Fabry patient's heart with a myocardial infarction shows no reactivity (original magnification 10×, *scale bar*=100 μm). **f** Absence of staining of a coronary artery in a non-Fabry patient (original magnification 5×, *scale bar*=200 μm)

at 4°C. After three 5-min rinses in TBS Tween, slides were incubated in Envision secondary anti-mouse/rabbit (Perkin Elmer, Wellesley, MA) for 1 h at room temperature and then treated with Cy3 tyramide (1:50 in amplification buffer, Perkin Elmer) for 10 min. Subsequently, this procedure was repeated with a cocktail of primary antibodies for Gb_3 (Seikagaku Corp, 1:400) and anti-calreticulin (Stressgen, San Diego, CA) in DaVinci Green and incubated for 2 h at room temperature or overnight at 4°C. Slides were then incubated in Alexa Fluor secondary antibody (Molecular Probes, Carlsbad, CA) diluted in Envision mouse/rabbit (Perkin Elmer) for 1 h in the dark treated with Cy5 tyramide (1:50 in amplification buffer) for 10 min, mounted with Prolong anti-fade with diamidinophenylindole (Invitrogen), and allowed to dry overnight.

Results

Immunohistochemistry for Gb_3 in normal and Fabry patient 1 tissues

Results are summarized in Table 1. The strongest staining for Gb_3 was present in the heart and the kidney of the Fabry patient (Figs. 1 and 2). A diffuse pattern of Gb_3 staining was observed in the myocardium and endocardium, even in the absence of visible inclusions (Fig. 1a). Coronary arteries showed a strong endothelium staining pattern with virtually every cell in the vessel wall staining positive for Gb_3 (Fig. 1b,c). Intracardiac nerves and vasa-nervorum fibers stained positive in a heterogeneous pattern (Fig. 1d). No Gb_3 staining was seen in the myocardium

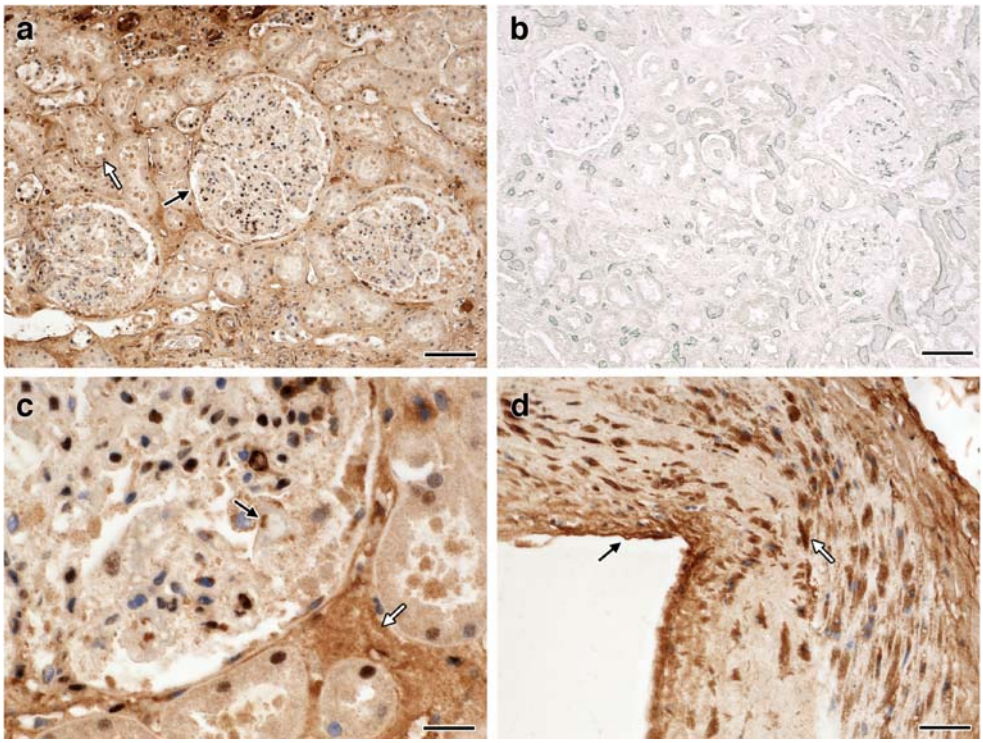

Fig. 2 Gb_3 staining in the kidney of Fabry and non-Fabry subjects showing variable immunopositivity in virtually all cell types (patient 1). **a** A glomerulus (*black arrow*) surrounded by tubules (*white arrow*, original magnification 10×, *scale bar*=100 μm). **b** No positive immunostaining for Gb_3 seen in a kidney of a non-Fabry control patient (original magnification 10×, *scale bar*= 100 μm). **c** A higher magnification of one glomerulus in the same field showing staining in a capillary endothelial cell or podocyte (*black arrow*) and interstitium (*white arrow*, original magnification 40×, *scale bar*= 25 μm). **d** A small renal artery showing Gb_3 immunostaining in endothelial cells (*black arrow*) and smooth muscle cells (*white arrow*, original magnification 20×, *scale bar*=50 μm)

(Fig. 1e) and coronary arteries (Fig. 1f) of a non-Fabry control. In non-Fabry controls, there was no difference in staining between sections with anti-Gb_3 and those when the secondary antibody only was used (data not shown). Gb_3 staining was present throughout the kidney of the Fabry patient but to a variable degree. Renal tubuli and most or all glomerular cells were immunopositive (Fig. 2a, c), although staining was relatively weak, while no Gb_3 could be detected in the normal control kidney (Fig. 2b). Interstitial kidney tissue (Fig. 2c) and renal blood vessels, including the endothelial cells, also stained positive for Gb_3 (Fig. 2d),

The areas of the brain we studied were largely negative for Gb_3. Staining was positive almost exclusively in the meningeal, meningothelial, and cerebral blood vessels (Fig. 3a–c). Neuronal staining was detected only in the parahippocampal gyrus (Fig. 3e) while no Gb_3 immunostaining was present in the non-Fabry brain (Fig. 3f).

In the adrenal gland, immunostaining for Gb_3 was present in the vasculature of the adrenal gland capsule (Fig. 4a), glomerulosa (Fig. 4c,e,f), and reticularis (Fig. 4h), but almost no staining was observed in the fasciculata region of the adrenal gland cortex (Fig. 4g). In the glomerulosa, Gb_3 stained distinct round structures that resemble lipid vacuoles (Fig. 4e,f). Heterogeneous staining was present in the adrenal medulla of the Fabry patient (Fig. 4i,j). No staining was seen using the secondary antibody only (Fig. 4b,d).

Subcellular localization of Gb_3

To identify the subcellular localization of Gb_3 in kidney cells from Fabry subjects, Gb_3 was colocalized with a combination of markers specific for the ER, lysosomes, and nucleus in a section of kidney tissue from patient 1 (Fig. 5a–j). We found that Gb_3 partially colocalizes with lysosomal, ER, and nuclear markers in renal cells of the Fabry patient (Fig. 5a–j). Staining for Gb_3 was much stronger in the patient compared to normal non-Fabry control (Fig. 5k,l).

We further studied Gb_3 localization in the skin. Using light microscopy; immunopositivity for Gb_3 was found mainly in the dermal blood vessels of patient 2 (Fig. 6a–c). Typical lysosomal inclusions were only detected in a small number of skin blood vessels (Fig. 6d), as detected by toluidine blue staining. However, pre- and postembedding immunogold electron microscopy showed Gb_3 labeling in a number of cellular areas, including lysosomal and non-lysosomal membrane structures, seemingly not membrane associated and in the nucleus (Fig. 6e–g). No labeling was present in normal control skin (Fig. 6h). Gb_3 immunogold

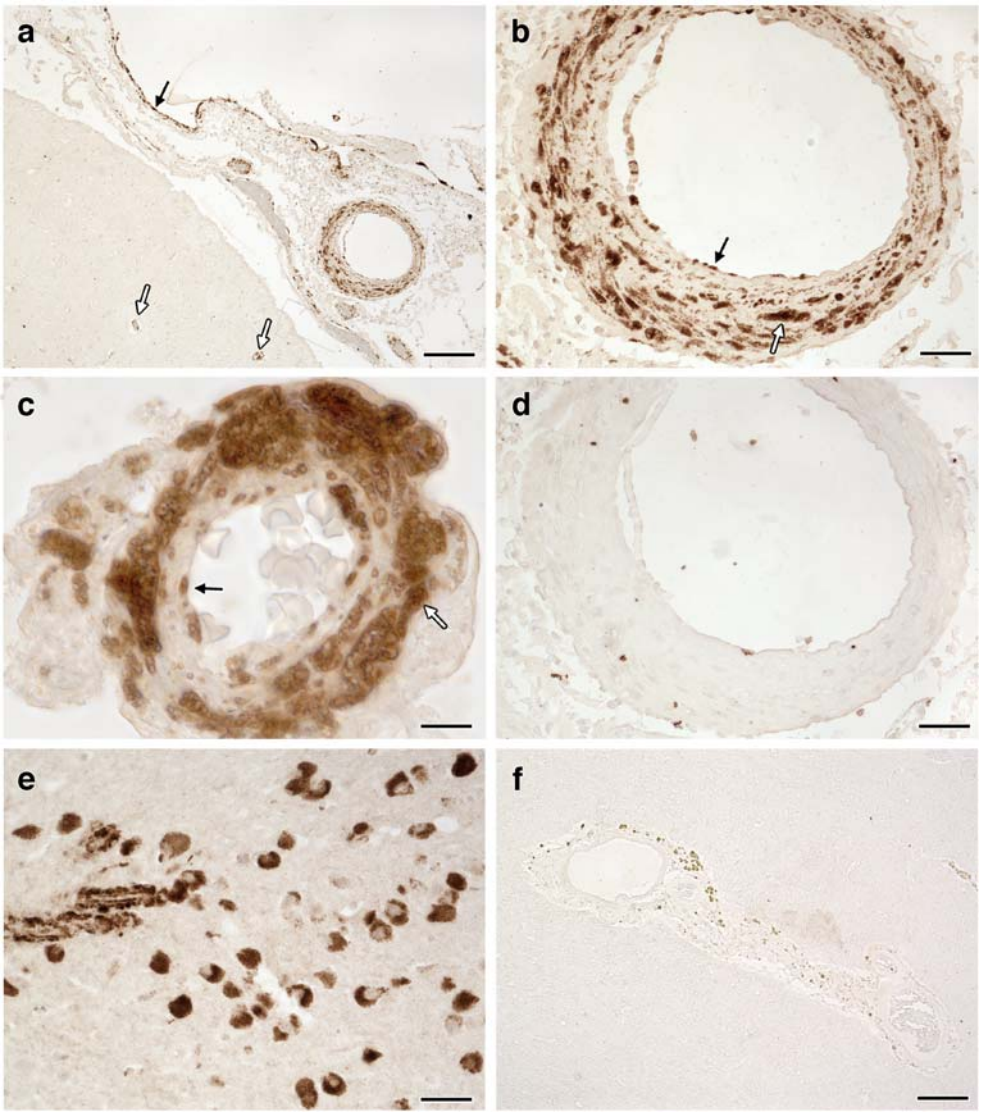

Fig. 3 Gb_3 staining of the brain of Fabry patient 1. **a** Brain and meninges of the Fabry patient includes staining of leptomeningeal membrane (*black arrow*) and parenchymal capillaries (*white arrows*, original magnification 5×, *scale bar*=200 μm) **b** A higher magnification of a meningeal artery showing endothelial (*black arrow*) and smooth muscle cell staining (*white arrow*, original magnification 20×, *scale bar*=50 μm). **c** A high magnification of a small intracerebral blood vessel showing staining in endothelial cells (*black arrow*) and smooth muscle cells (*white arrow*, original magnification 100×, *scale bar*= 10 μm). **d** Secondary antibody only of the image in **b** (*scale bar*=50 μm). **e** Parahippocampal gyrus showing Gb_3 neurons (original magnification 20×, *scale bar*=50 μm). **f** Normal brain immunostained for Gb_3; only hemosiderin pigments are seen around a blood vessel (original magnification 5×, *scale bar*=200 μm)

labeling was present even in capillaries devoid of lysosomal inclusions with toluidine blue staining (Fig. 6i).

Cultured skin fibroblasts showed variable staining by light microscopy (Fig. 7a) and also showed immunogold labeling of Gb_3 in cytoplasmic vacuoles, lamellar bodies, plasma membrane, and within the nucleus (Figs. 6f and 7b–d). Again, normal cultured fibroblasts from a non-Fabry subject showed no Gb_3 labeling (Fig. 7e).

Discussion

Using various forms of immunodetection, this study demonstrates that in Fabry disease, Gb_3 is not only present in lysosomes but is rather widely distributed in other cellular structures including the ER, cell membrane, and nucleus. Such distribution of Gb_3 was observed in cultured skin fibroblasts from patients with the classic form of the disease as well as in organs of patients on long-term enzyme replacement therapy, even in cells that were free of classic lysosomal inclusions [39]. Enzyme replacement therapy in Fabry disease has been shown to slow the progression of kidney disease and improve peripheral nerve function and sweating [2, 38, 40]. Another remarkable feature is the heterogeneity of Gb_3 distribution and accumulation at the organ, cellular, and subcellular levels. Although immunohistochemistry is not typically quantitative, the most intense staining was observed in the heart and kidney, organs that are known to accumulate the greatest amounts of Gb_3 [25, 37]. Among specific organs/tissues

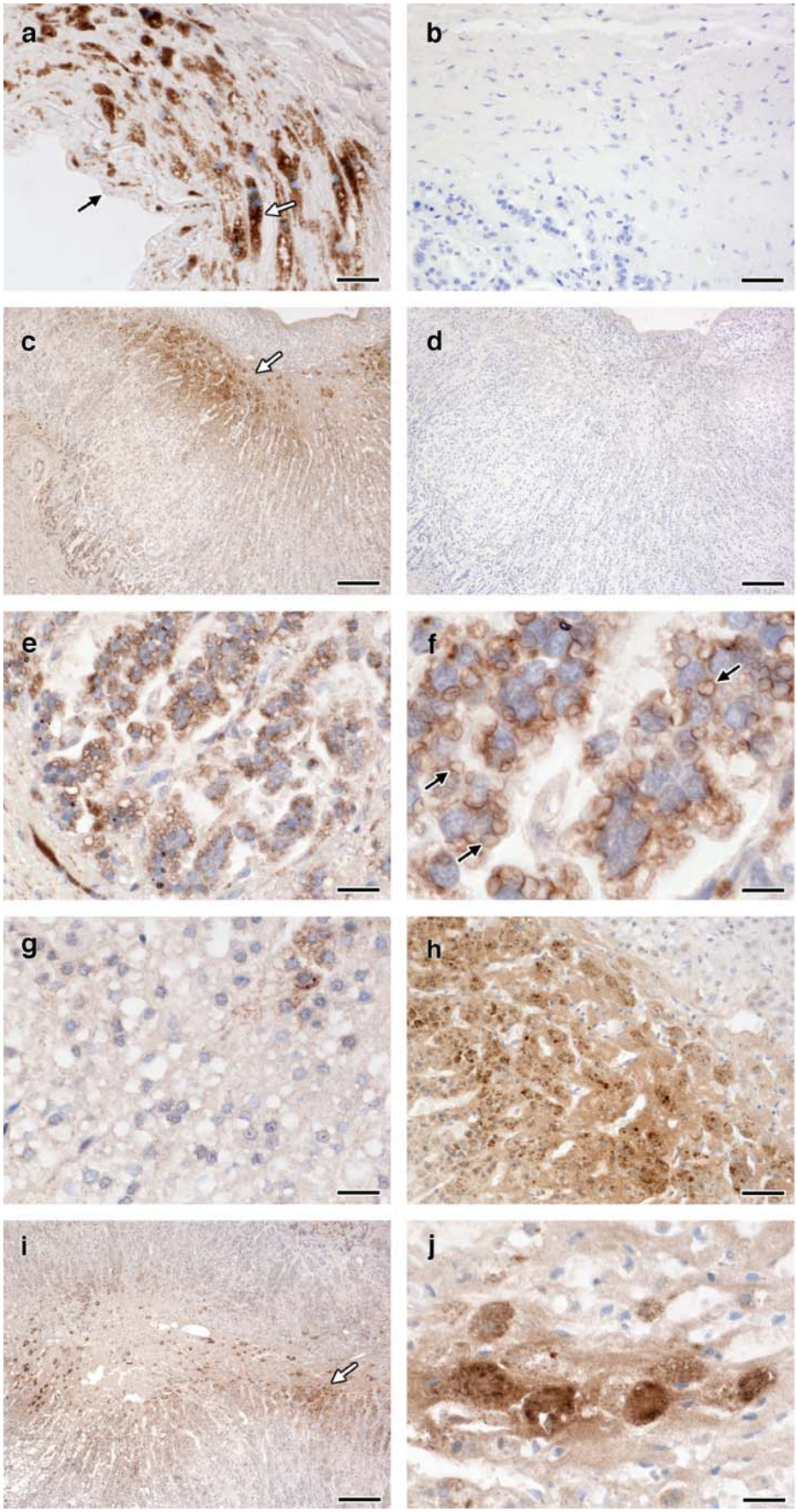

Fig. 4 Staining for Gb_3 in the adrenal gland of patient 1 on ERT for 2.5 years and a non-Fabry control. **a** Capsular artery showing endothelial (*black arrow*) and smooth muscle staining (*white arrow*, original magnification 40×, *scale bar*= 25 μm). **b** Adrenal gland capsule with secondary antibody only (original magnification 20×, *scale bar*=50 μm). **c** Overview of the capsule and the glomerulosa region (*arrow*) of the adrenal gland cortex (original magnification 5×, *scale bar*=200 μm). **d** Adrenal cortex with secondary antibody only (original magnification 5×, *scale bar*=200 μm). **e** Glomerulosa region of adrenal cortex (original magnification 40×, *scale bar*=25 μm). **f** Higher magnification of glomerulosa region with staining of round intracellular structures (*arrows*, original magnification 100×, *scale bar*= 10 μm). **g** Fasciculata adrenal cortical region (*arrows*, original magnification 40×, *scale bar*= 25 μm). **h** Adrenal reticularis showing greater Gb_3 staining than the fasciculate adrenal cortical region (original magnification 20×, *scale bar*=50 μm). **i** Overview of adrenal medulla (*arrow*) showing greater Gb_3 than surrounding areas (original magnification 5×, *scale bar*= 200 μm). **j** Higher magnification of medulla (original magnification 40×, *scale bar*=25 μm)

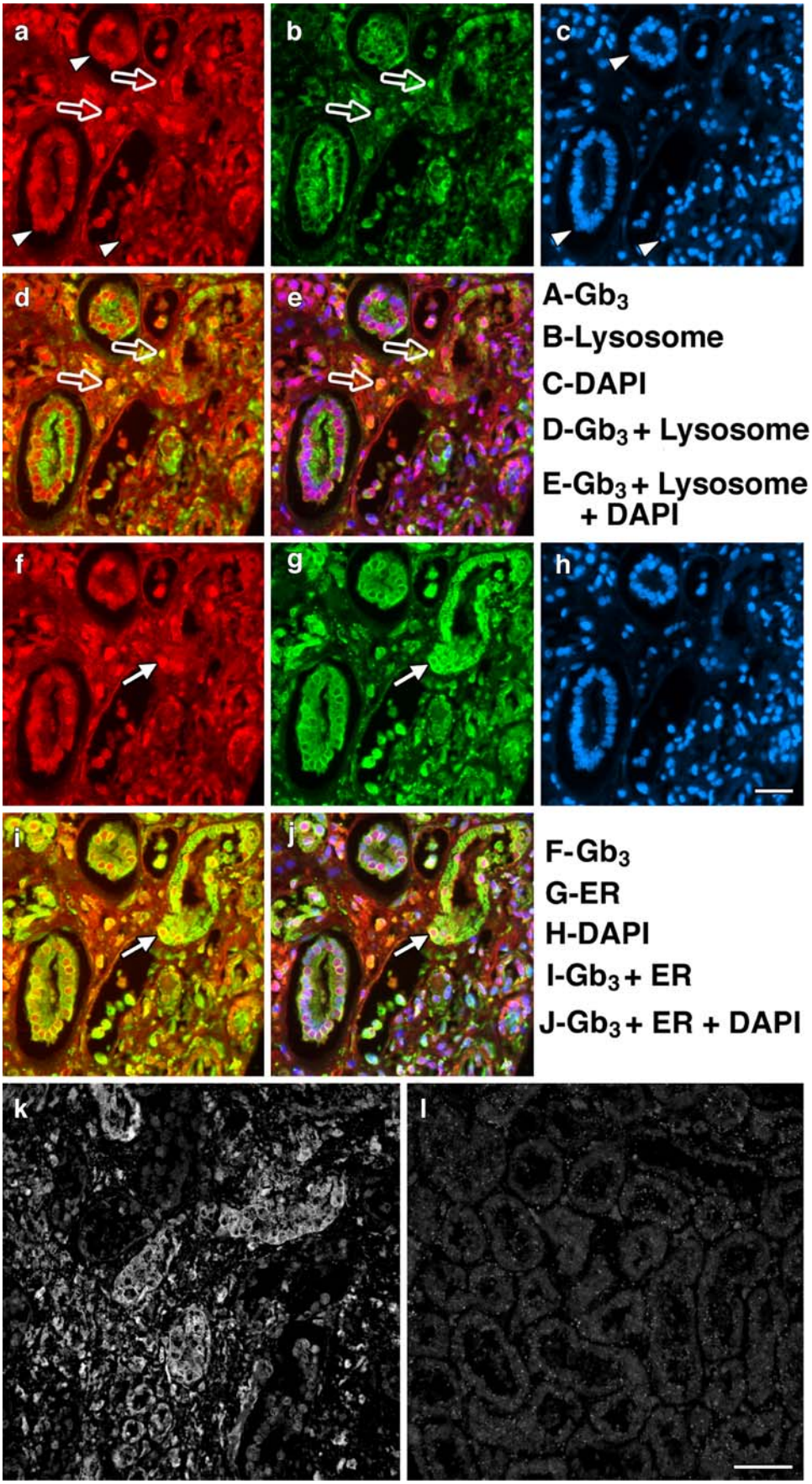

Fig. 5 **a**–**j** Immunofluorescent localization of kidney Gb_3 in the ER, lysosomes, and nucleus in a patient with Fabry disease. *Arrows* point to examples of colocalization of Gb_3 with other cellular markers in renal tubular structures. *Bottom panels* show the relative content of Gb_3 in Fabry kidney and a healthy control (**k**, **l**). These two images were obtained using the same conditions. The images were acquired at 40× using the PM-2000™ and AQUA® technology

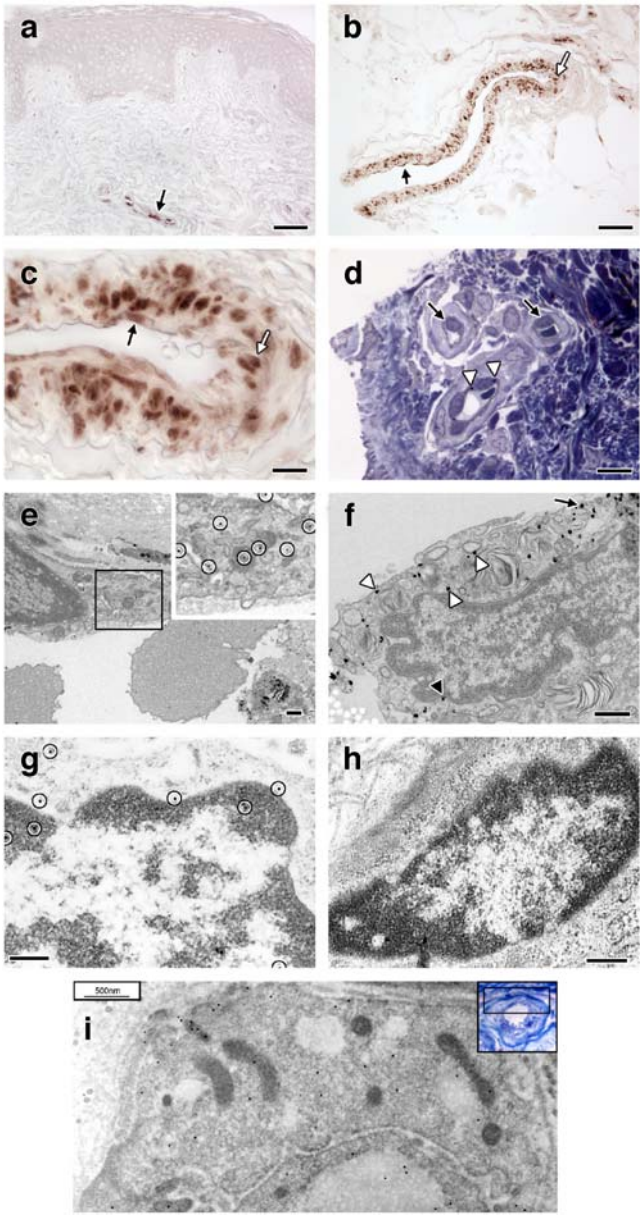

Fig. 6 Gb_3 immunolocalization in the skin of Fabry (patient 2) and non-Fabry subjects. **a** Overview of skin biopsy showing staining of dermal blood vessels (*arrow*, original magnification 20×, *scale bar*= 50 μm). **b** A dermal arteriole with endothelial (*black arrow*) and smooth muscle staining (*white arrow*, original magnification 20*arrow*50 μm). **c** Details of the same arteriole (original magnification 100×, *scale bar*=10 μm). **d** 1-μm section of skin stained with toluidine blue showing dermal vessels. Two capillaries have no lysosomal inclusions (*arrows*) while another capillary has two inclusions (*arrowheads*; original magnification 100×, *scale bar*= 10 μm). **e**, **f** Pre-embedding immunogold electron microscopy for Gb_3. **e** Labeling of a dermal blood vessel using 5-nm gold particles (19,000×, *scale bar*=500 nm). *Digital insert*: *black circles* surround gold particles. **f** A dermal fibroblast showing labeling of the cell membrane (*arrow*), cytoplasmic structures (*white arrowhead*), and probably the nuclear membrane (*black arrowhead*, 38,000×, *scale bar*=500 nm). **g**–**i** Post-embedding immunogold labeling for Gb_3. **g** Fibroblast showing labeling of the nucleus, associated preferentially with heterochromatin fibrils (39,000×, *scale bar*=266 nm). **h** Normal control skin fibroblast showing no labeling (17,000×, *scale bar*= 500 nm). **i** Dermal capillary endothelial cell with no lysosomal inclusions on toluidine blue staining (*insert*) is labeled for Gb_3 throughout the cell (21,000×)

such as arteries or the adrenal gland, staining patterns were especially heterogeneous. Staining of vascular endothelial cells varied from cell to cell. In the adrenal gland cortex, staining of cells within the glomerulosa region produced a specific intracellular vacuolar-like pattern while Gb_3 immunoreactivity was diffuse in reticulata cells. This heterogeneity suggests that the various cellular metabolic needs, including possibly the cell cycle stage, interact differently with the α-galactosidase A-deficient state. The finding of Gb_3 immunoreactivity only in the parahippocampal region

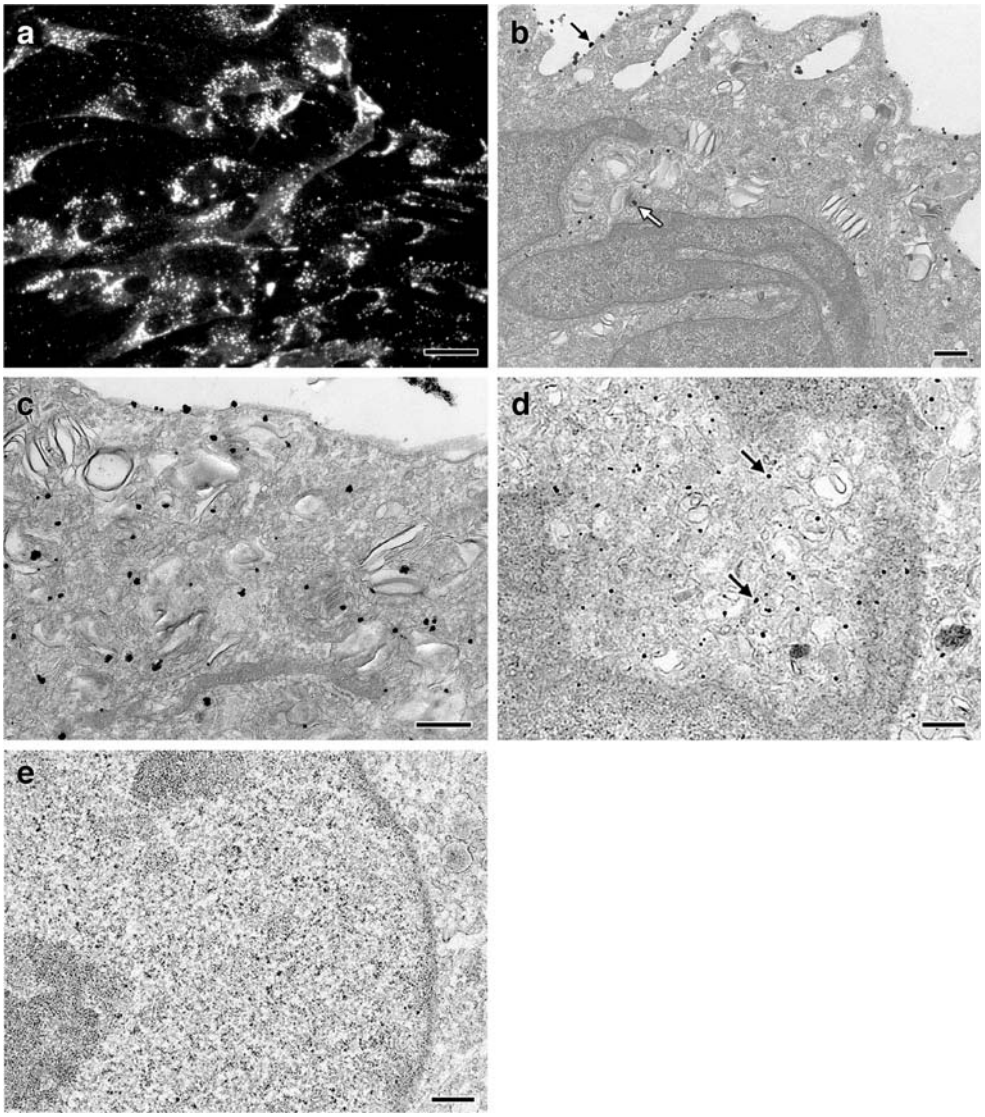

Fig. 7 Gb_3 immunolocalization in the skin of Fabry and non-Fabry subjects. **a** Light microscopic image of cultured fibroblasts from patient 3 showing cell-to-cell variation in immunoreactivity (40×, *scale bar*= 25 μm). Immunogold electron microscopy using the pre-embedding technique of cultured cells of patient 2. **b** A cell showing membranous (*black arrow*) and cytoplasmic labeling (*white arrow*, original magnification 4,000×, *scale bar*= 500 nm). **c** A higher magnification showing labeling of the cell membrane and various cytoplasmic structures (10,000×, *scale bar*=500 nm). **d** Membranous cytoplasmic (*arrows*) and nuclear labeling in another fibroblast (6,000×, *scale bar*= 500 nm). **e** Nucleus of a normal cultured fibroblast showing no Gb_3 labeling (6,000×, *scale bar*=500 nm)

fits well with previous quantitative analysis of this glycolipid in various brain regions showing marked regional heterogeneity [17].

In this study, we used a monoclonal antibody against Gb_3 that has been extensively characterized and used in a number of publications [9, 13, 16, 19, 36]. While this may be the first detailed study into the extralysosomal localization for Gb_3 in Fabry patients, examination of previously reported electron microscopy photographs support our current findings [16]. Although a difference in technique can account for the different findings, examination of previously reported immuno-electron microscopy photographs suggests extralysosomal labeling. Similarly, inspection of images published in an ultrastructural lectin histochemical analysis shows rather extensive labeling of Gb_3 in a number of cellular structures including the mitochondria [15]. Gb_3 has also been detected on the surface of vascular endothelial cells [33] and erythrocytes and has been described as a P^k blood group antigen [24]. Gb_3 is present in normal human cells [29]; therefore, the absence of immunopositivity in non-Fabry tissue likely reflects the relative insensitivity of the anti-Gb_3 antibody we used. For example, another possibly more sensitive but also somewhat less specific monoclonal antibody has been shown to identify Gb_3 in a variety of normal tissues including the kidney, stomach, and vascular endothelial cells [31].

The propensity of Gb_3 to localize in the cell membrane and nucleus in Fabry disease may be related to its physiologic function [21] or to unknown biological functions. The subcellular pattern of Gb_3 localization in cells from patients with Fabry disease could be a result of

retrograde transport from the lysosome to the ER, plasma membrane, and nucleus [20]. Studies with verotoxin have demonstrated that Gb_3 can be distributed throughout the cell via retrograde transport. Gb_3 is a receptor (also known as CD77) for verotoxin at the cell surface of certain B cells present in the germinal center of lymph nodes [12]. In such CD77+ lymphocytes, Gb_3 appears to be critical for both the receptor-mediated endocytosis and the transport to the nucleus of the CD19 protein, as well as for its ability to induce increased production of reactive oxygen species and apoptosis [18, 45]. Gb_3 is also an antigenic target of natural killer cells in antimicrobial defense, as an alternative to lipopolysaccharide for innate recognition [23].

It is likely that Gb_3 disrupts biological interactions by interacting with other lipids or proteins or by altering the composition of lipid rafts (or microdomains) and endocytic trafficking [32]. For example, the enhanced atherosclerosis in apoE knockout mouse is associated with a marked increase in Gb_3 [11]. This atherosclerotic process is further enhanced when α-galactosidase A is also deficient [4]. In cells from Gaucher, GM1 gangliosidosis, and GM2 gangliosidosis, it has been demonstrated that accumulation of glycosphingolipids in the ER disrupt specific Ca^{2+} homeostatic mechanisms [34, 35, 44], further supporting the existence of glycosphingolipid trafficking between the organelles of the endosomal pathways and the ER [10]. Increasing evidence suggests that ganglioside accumulation is a stress inducer resulting in ER stress and mitochondria-elicited cell death pathways [7]. Gangliosides or other glycoshingolipids were immunolocalized in cellular regions outside of the primary storage material in the mucopolysaccharidoses suggesting that they are extralysosomal in nature [47]. Additionally, GM2 ganglioside has been shown to be associated with the presence of ectopic dendrites in the cerebral cortex [46]. In Fabry disease, there is a suggestion that the clinical abnormalities are caused by perturbations of extralysosomal functions. Excess amounts of Gb_3 in the cell membrane may interfere with the function of caveolae/rafts that are known to contain glycosphingolipids and a number of important signal transduction proteins such as endothelial nitric oxide synthase (eNOS) [26]. There is evidence of increased reactive oxygen species in Fabry disease, possibly in relation to eNOS [1, 4, 27], as well as an abnormality of the fibrinolytic system [28].

This study has demonstrated that Gb_3 accumulates in nonlysosomal cellular compartments and membranes and that further studies are needed to investigate the natural functions of Gb_3 as well as the mechanisms of toxicity associated with its accumulation. In addition to helping us understand the pathogenesis of Fabry disease, the cellular localization of Gb_3 may be a clinical outcome measure to assess the effect of novel therapies that are designed to further reduce the amount of Gb_3 in Fabry disease [14].

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CASE REPORT

Primary epithelioid sarcoma of the oesophagus

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Abstract Epithelioid sarcoma is a rare soft tissue tumour presenting two main variants: the 'classical' distal type and the more recently described proximal type. The latter is distinguished from the former by occurrence in elderly patients, more axial and deep location, prominent atypical and pleomorphic appearance and eventually aggressive clinical behaviour with poor outcome. To date, only few perivisceral epithelioid sarcomas have been described, and they are mainly related to the colon and bladder in pelvic and perineal sites. We report a hitherto undescribed epithelioid sarcoma of the distal oesophagus and discuss the relevance of molecular cytogenetics.

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Keywords Epithelioid sarcoma · Extra-renal rhabdoid tumour · Oesophagus · Cytogenetics · FISH

Introduction

Epithelioid sarcoma is an uncommon mesenchymal tumour of unknown histogenesis with a predominant epithelial differentiation, showing reactivity for both epithelial and mesenchymal markers, such as cytokeratin, epithelial membrane antigen (EMA), vimentin and CD34. The 'classical' epithelioid sarcoma, first described as a distinct clinicopathological entity by Enzinger in 1970 [2], usually occurs in the distal extremities of young adults, mainly in the hand and around the wrist and presenting as a slowly progressive, solitary or multinodular tumour with a superficial or subcutaneous location and frequently associated with ulceration of the overlying skin. Three morphological variants are recognized: the more common granuloma-like, a fibroma-like and an angiomatoid-like variant. In 1992, a clinically more aggressive entity, the large-cell 'proximal type' epithelioid sarcoma has been described by Guillou et al. [3], which differs from the 'classical type' by a multinodular growth pattern, a more proximal/axial distribution and a deep seated location, mainly but not exclusively involving the pelvic, genital and perineal area in elderly patients [4]. It is not well known if the reduced survival of this type is more related to the histotype by itself or to the lesser resectability because of the deeper location, the proximal distribution and/or the bigger size at the diagnosis. Microscopically, this proximal type consists of a proliferation of large epithelioid cells with copious eccentric cytoplasm, arranged in multiple nodules. Tumoural necrosis is a common finding but usually without the granuloma-like aspect.

We report a hitherto undescribed case of epithelioid sarcoma arising at the gastro-oesophageal junction and infiltrating the visceral wall, with multiple local lymph node metastases. Because visceral locations have not been reported, the diagnostic value of molecular cytogenetics is highlighted.

Clinical features

A 64-year-old man presented with a painful, ulcerating, not-obstructive lesion of the gastro-oesophageal junction, measuring 5×3 cm at endoscopic examination. Magnetic resonance imaging showed enlarged lymph nodes at the hepatophrenic, hepatogastric and oesophagophrenic ligaments. A staging laparoscopy was performed, and one of the oesophagophrenic lymph nodes was removed for histological examination. In addition, a partial oesophagogastrectomy with local and regional lymph node dissection was performed.

Materials and methods

A part of the samples was snap frozen in liquid nitrogen-cooled isopentane, while the largest part was fixed in 4% formalin followed by conventional processing and embedding in paraffin wax. Sections were cut and stained with haematoxylin and eosin. Immunohistochemical stainings were performed with a panel of antibodies using the avidin–biotin peroxidase complex technique. The antibodies used include anti-epithelial membrane antigen (EMA; DAKO, Glostrup, Denmark, dilution 1:50), Prekeratin (Serotec, Oxford, UK, dilution 1:200), Keratin AE1/AE3 (Forlab, Brussel, Belgium, dilution 1:100), carcinoembryonic antigen (CEA; DAKO, dilution 1:1000), Chromogranin (DAKO, dilution 1:200), S-100 protein (DAKO, dilution 1:300), CD34 (Becton Dickinson, Franklin Lakes, NJ, dilution 1:10), CD31(DAKO, dilution 1:1000), CD30 (DAKO, dilution 1:20), CD45 (DAKO, dilution 1:500), HMB45 (DAKO, dilution 1:1000) and beta-catenin (Santa Cruz Biotecnology, Santa Cruz, CA, dilution 100).

Chromosome preparation of tumour cells was obtained after an overnight culture of the lymph node metastatic lesion. G-Banded chromosomes were evaluated and classified according to the International System for Human Cytogenetic Nomenclature (2005). Fluorescence in situ hybridization (FISH) utilizing either biotin- or digoxigenin-labelled BAC's RP11-742c19 and RP11-71G19 deoxyribonucleic acid probes, specific for the *PDGFB*/22q13 and *SMARC-B1*/22q11.2 genes, respectively, was performed on frozen sections of tumour specimen according to standard procedures.

Results

Gross pathology

At the level of the gastro-oesophageal junction, along the small curve, a nodular haemorrhagic and necrotic mass was observed. The cut surface showed a 5-cm greyish white nodule extensively infiltrating the muscularis propria, without evident serosal involvement. In addition, a 4-cm-large lymph node adhering to the stomach wall was reported (Fig. 1).

Light microscopy

Microscopically, the tumour was composed of a multinodular proliferation of large polygonal cells mainly arranged in discohesive sheets, merged with necrotic areas and inflammatory cells. The neoplasia infiltrated the mucosa, submucosa and muscularis propria and subserosa. Prominent perineural invasion was seen but no evidence of vascular invasion. The neoplastic cells showed an abundant, often eccentric eosinophilic cytoplasm, with well-defined cellular margins. The nuclei were atypical, vesicular and characterized by prominent, central nucleoli (Fig. 2a). Brisk mitotic activity and numerous apoptotic figures were easily observed (37 mitoses/10 high power fields). The residual mucosa did not display any signs of atypia (Fig. 2b). Immunohistochemical stainings showed positivity for EMA and beta-catenin and no reactivity for the other antibodies. The enlarged local lymph nodes

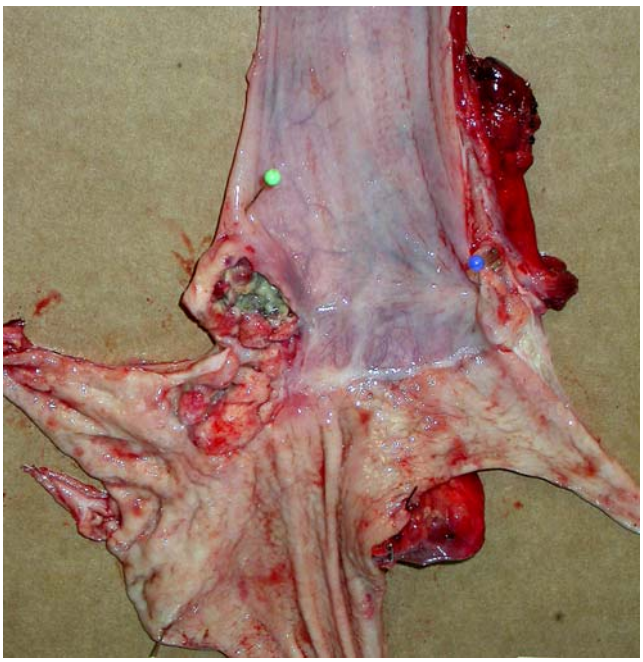

Fig. 1 Evidence of the tumour on the surgical specimen

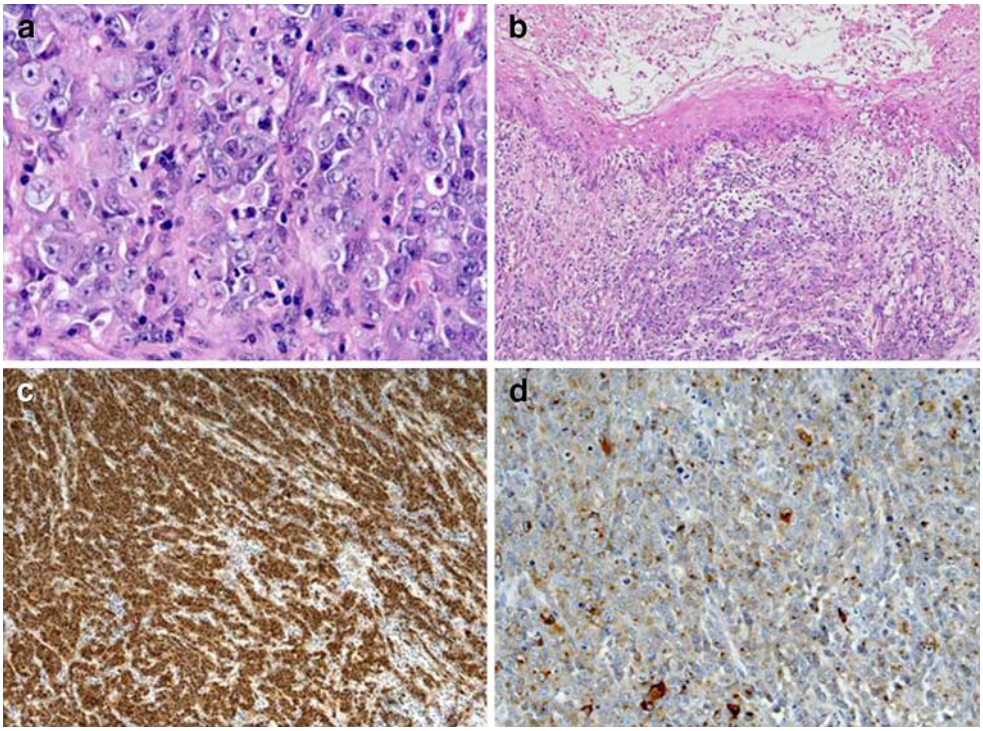

Fig. 2 **a** Neoplastic cells showing large vesicular nuclei, prominent nucleoli and brisk mitotic activity. **b** Tumour cells intermingled with inflammatory cells, infiltrating the mucosa, the epithelium of which shows no signs of atypia. **c** Diffuse positivity to beta-catenin immunostaining. **d** Focal positivity to EMA immunostaining

contained metastatic disease, whereas no peripheral nodal metastases were documented.

Cytogenetic analysis

Cytogenetic analysis revealed 45–46,XY,t(9;13)(p22;q34),-22[3][cp6]/46,XY[11] karyotype (data not shown). Dual-color interphase FISH analysis showed loss of *SMARC-B1*/22q11.2 in reference to control *PDGFB*/22q13 loci in 87% of nuclei (Fig. 3), with 31% cells showing nullisomy for the *SMARC-B1* gene.

Discussion

Epithelioid sarcoma is an uncommon malignant neoplasia of soft tissue with unknown lineage, characterized by a local recurrence rate of up to 70% and a median time to recurrence after surgery ranging from 6 to 82 months [10]. It often culminates in regional lymph nodes or lung metastases, occurring in up to 45% of patients [10]. Nevertheless, an overall 5-year survival up to 70% is reported.

The more common sites reported so far are distal extremities for the classical 'distal' type and proximal extremities or limb girdles for the 'proximal' variant. Chest wall, trunk, back and perineum have been reported as possible locations for the latter, more aggressive type, but no visceral primary tumour has been described so far.

The morphology of the reported lesion was in agreement with the features of epithelioid sarcoma, either the nodular pattern or the epithelioid/rhabdoid-like cells nests alternating with necrotic areas, but the gastrointestinal location associated with the lymph node involvement and the nodular appearance prompted more common diagnoses,

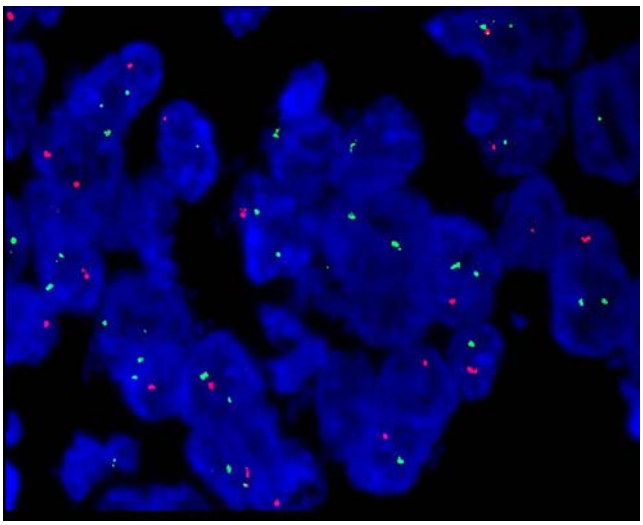

Fig. 3 Dual-color interphase FISH on frozen tumour sections using a biotin-labelled BAC RP11-742C19 (*green signals*) and digoxigenin-labelled RP11-71G19 (*red signals*) DNA probes, which map to *PDGFB*/22q13 and *SMARC-B1*/*INI1*/22q11.2 loci, respectively. Over-representation of green hybridization signals in reference to red signals indicates loss of *SMARC-B1* loci, with many cells being nullisomic for the gene. Nuclei are counterstained with DAPI

such as undifferentiated carcinoma, diffuse large B cell lymphoma on a pre-existing extra-nodal marginal zone B cell lymphoma and primary or metastatic melanoma. Nevertheless, the lack of reactivity for all the epithelial markers, except for EMA, lymphoid markers, S-100 protein and melanocytic markers, such as HMB-45 and Melan A, definitely excluded these hypotheses [6].

Epithelioid-appearing malignant mesenchymal tumours such as synovial sarcoma could be included in the differential diagnoses because the tendency for lymph node metastases is well known, and gastrointestinal locations have been reported in at least ten cases (nine of them in the oesophagus and one in the gastric antrum). The EMA positivity could be in line with this diagnosis but by cytogenetic analysis did not show the characteristic t(X;18) translocation. Two other entities presented a morphologic and immunohistochemical profile compatible with our case: extra-renal rhabdoid tumour and epitheliod sarcoma, whose features usually overlap. Extra-renal rhabdoid tumour is a highly aggressive neoplasia, typically occurring in infancy and histologically characterized by large "rhabdoid" cells, presenting juxtanuclear, globular and amphophilic inclusions and vesicular nuclei with prominent nucleoli [9]. The wide variety of topographic locations of this neoplasia includes the gastro-oesophageal tract. The most consistently expressed immunohistochemical markers are EMA and cytokeratin, but S100 and neuroectodermal antigens can be displayed as well. CD34, expressed in 50% of epithelioid sarcoma, is usually negative. An immunohistochemical study on adhesion molecules expression in epithelioid sarcoma and extra-renal rhabdoid tumour showed different expression of beta-catenin, with striking positivity in 11 of the 15 epithelioid sarcomas examined (73%) and no staining in all the six extra-renal rhabdoid tumours. Our case exhibited a diffuse membranous and cytoplasmatic staining for beta-catenin.

Previous studies revealed striking similarity between malignant rhabdoid tumour and proximal-type epithelioid sarcoma on the cytogenetic and molecular level, e.g. rearrangements or deletions of chromosome 22q11.2, which have been associated with a bi-allelic inactivation of a tumour suppressor gene implied in the oncogenesis [1, 5, 7, 8]. The later was identified as the *hSNF/INI1/SMARC-B1/BAF47* gene, a member of the SWI/SNF chromatin-remodelling complex [5]. However, the spectrum of somatic *SMARC-B1/INI1* mutations in epithelioid sarcomas seems to be restricted to deletions and not to include point mutations frequently observed in malignant rhabdoid tumours. Our oesophageal tumour presented the translocation t(9;13) (p22;q34), so far not associated with any specific mesenchymal neoplastic entity, and loss of chromosome 22 in a subset of cells. By FISH analysis, the latter was associated with the cryptic 22q11.2 microdeletion that resulted in homozygous loss of both *SMARC-B1/INI1* gene copies in 31% of the examined cells. This finding supports the diagnosis of epithelioid sarcoma rather than extra-renal rhabdoid tumour (although it does not exclude the latter completely). Furthermore, additional factors such as the age of the patient and the diffuse beta-catenin expression in tumour cells are in favour of an epithelioid sarcoma diagnosis. Altogether, this case again underscores the value of molecular genetics in establishing the correct diagnosis.

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CASE REPORT

Clear cell sarcoma of the ileum: report of a case and review of literature

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Abstract Clear cell sarcoma (CCS) is a high grade soft tissue sarcoma with a distinct molecular profile and with morphological features resembling those of melanoma. CCS has been rarely described in other locations other than the soft tissues, including the gastrointestinal tract. In this study, we report a case of CCS arising in the ileum of a 31-year-old woman. Histologically, the tumor involved the entire thickness of the intestinal wall. Tumor cells were polygonal or fusiform, with clear or eosinophilic cytoplasm, arranged in a uniform nested to fascicular growth pattern. Immunohistochemical studies revealed strong positivity for vimentin and S-100 protein. HMB-45, Melan-A, tyrosinase, cytokeratins, EMA, smooth muscle actin, CD34, CD31, CD117, CD99, synaptophysin, chromogranin A, CD56, and NSE were negative. Fluorescence in situ hybridization analysis demonstrated the presence of a t(12;22)(q13;q12) translocation, the diagnostic hallmark of CCS of soft parts. The present case, together with a detailed review of the literature on this topic, demonstrates that the gastrointestinal tract is a possible site of CCS of soft tissues and that making a reliable diagnosis of this tumor requires cytogenetic or molecular diagnostic investigations.

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Keywords Clear cell sarcoma · Gastrointestinal tract · Immunohistochemistry · FISH

Introduction

Clear cell sarcoma (CCS) (also known as malignant melanoma of soft parts) is a soft tissue sarcoma of adolescents and young adults with melanocytic differentiation typically involving tendons and aponeuroses. The distal extremities, in particular foot and ankle regions, are the main sites of involvement. The prognosis is poor, owing to a high percentage of regional and distant metastasis. The immunoprofile of CCS is characterized by the strong expression of S-100 protein and of more specific melanoma markers, mainly HMB-45 [8]. The presence of melanosomes has also been well documented by ultrastructural studies [8]. Although gene expression profiles have demonstrated CCS to be a subtype of melanoma [26], the two entities are also genetically distinct: CCS lacks BRAF mutations [21], so common in melanoma, and shows a distinct and recurrent chromosomal translocation, t(12;22)(q13;q12), in which the 5′ region of the EWS gene on chromosome region 22q12 recombines with the 3′ region of ATF1 on 12q13 [30].

Beyond the soft tissues, CCS has also been described in a wide range of other anatomical sites such as the ear, bone, penis, kidney, pleura, pancreas, and gastrointestinal tract [1–6, 9, 10, 13, 16, 17, 19, 22, 24, 25, 27–29]. This latter site is extremely rare. To date, 15 genetically confirmed cases of gastrointestinal CCS have been reported, of which seven where located in the ileum [1, 2, 4, 5, 9, 10, 13, 17, 22, 27–29]. In this study, we report an adjunctive case of CCS arising in the ileum and discuss the tumor diagnosis approach, the importance of molecular genetics in tumor

diagnostics, along with a review of the literature on the clinicopathological characters of this rare tumor.

Clinical history

A 31-year-old female was admitted for a weight loss of 8 kg in a 3-month period and symptoms of intestinal obstruction such as abdominal pain and vomiting. Her past medical history was unremarkable. No laboratory abnormalities were present. A computed tomography scan revealed a segmental thickening of the distal ileum. At laparotomy, a single stenotic lesion of the ileum, 3 cm in diameter, was found. No other tumor deposits in the abdominal cavity or liver metastasis could be observed. A partial resection (20 cm) of the small bowel was performed and regional mesenteric lymph nodes were removed.

Materials and methods

The specimen was fixed in neutral, phosphate-buffered, 10% formalin and routinely processed with tissue sections embedded in paraffin and stained with hematoxylin and eosin. Immunohistochemical studies were performed on representative sections of the tumor. Prediluted antibodies were used for AE1/AE3, CAM 5.2, CK20, EMA, HMB45, Melan-A, tyrosinase, vimentin, smooth muscle actin (clone 1A4), CD34, CD31, CD99, synaptophysin, chromogranin, CD56, and NSE (all from Ventana Medical Systems, Tucson, AZ). The other antibodies tested were S-100 protein (1:500; DAKO, Carpinteria, CA) and CD117 (1:500; DAKO). The reaction product was detected with 3,3-diaminobenzidine chromogen. Appropriate positive and negative controls were employed throughout.

Sections from the same paraffin-embedded blocks used for immunohistochemistry were processed for fluorescence in situ hybridization (FISH) analysis which was performed according to the manufacturer's instructions and laboratory protocol using the EWS (22q12) dual-color, break-apart probe (Vysis, Downer's Grove, IL, USA). A positive result was reported when greater than 10% of the tumor nuclei had evidence of an EWS rearrangement. Only tumor nuclei with all four signals present were evaluated, whereas overlapping cells with indistinguishable separate nuclei were excluded from the analysis. A total of 100 tumor nuclei were examined.

Results

Grossly, the resected segment of ileum was centered by a superficially ulcerated, well-defined firm, circumferential tumor involving the entire thickness of the intestinal wall extending into the subserosa. Cut section showed a gray-white mass measuring 2.8 cm in maximum diameter. On microscopic examination, the tumor was found to be based mainly in the muscularis propria and to have invaded the submucosa, the mucosa, and subserosa (Fig. 1). Tumor cells were arranged in a uniform nested to fascicular pattern of growth. Fibrous septa divided the fascicles and nests of tumor cells. The cells were polygonal or fusiform with abundant clear or eosinophilic cytoplasm. The nuclei were vesicular, round to oval with prominent nucleoli (Fig. 2). The mitotic activity was low (two mitosis per ten high-power fields; one high power field: 0.174 mm^2). Neither melanin pigment, nor multinucleated tumor cells, nor osteoclast-like multinucleated giant cells were present. Metastasis to 2 of 14 mesenteric lymph nodes were identified. The ileal resection margins were free of disease.

The tumor cells were strongly immunoreactive for vimentin and S-100 protein (Fig. 3). HMB-45, Melan-A, tyrosinase, cytokeratins, EMA, smooth muscle actin, CD34, CD31, CD117, CD99, synaptophysin, chromogranin A, CD56, and NSE were all negative.

Dual-color FISH analysis revealed hybridization signals in 95% of the nuclei. In 85 out of 100 nuclei examined, the applied probe yielded one juxtaposed red and green signal (one per nucleus), indicating the normal EWS allele in one chromosome, and split, single red and green signals, indicating the presence of an EWS (22q12) gene rearrangement, in the second chromosome, thus allowing a definite diagnosis of CSS.

Discussion

Originally described by Enzinger in 1965 [7], CCS is an unusual type of sarcoma that shows features of melanocytic

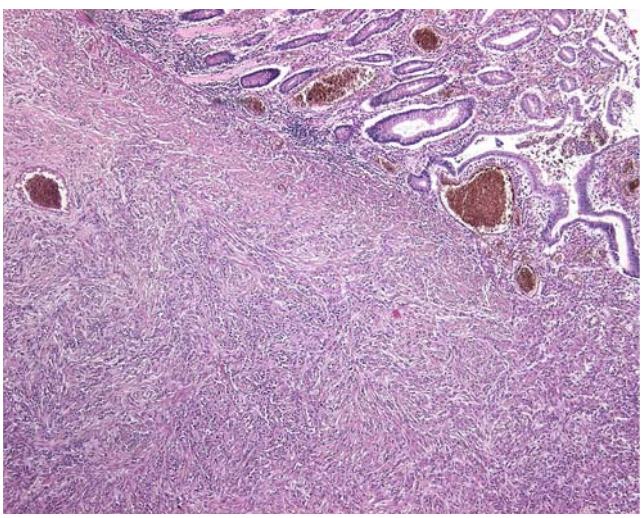

Fig. 1 Low power view of the tumor diffusely infiltrating the mucosa and submucosa of the ileum

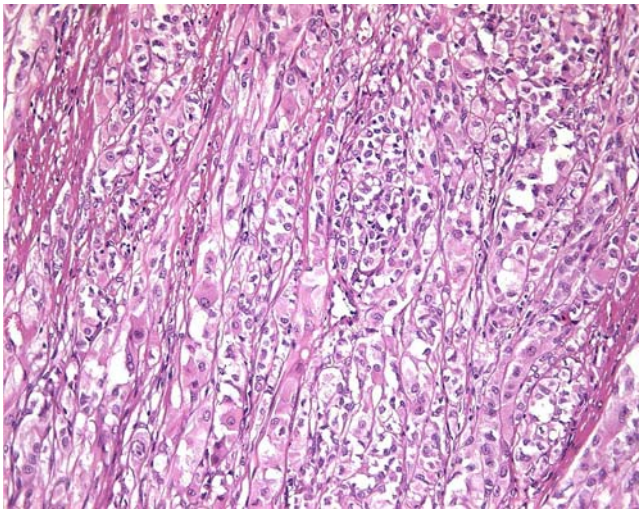

Fig. 2 Nests and fascicles of tumor cells separated by fibrous septa. The cells are polygonal or fusiform with abundant eosinophilic or clear cytoplasm. The nuclei are vescicular, round to oval, with prominent nucleoli

differentiation and that affects predominantly adolescents and young adults [8]. The neoplasm typically presents as a slow-growing tumor arising in the deep soft tissues of the lower extremities close to tendon, fascia, and aponeuroses. Recent data from the literature have shown that CCS may arise at a variety of other sites, including the viscera [1–6, 9, 10, 13, 16, 17, 19, 22, 24, 25, 27–29]. Gastrointestinal involvement is extremely rare [1, 2, 4–6, 9, 10, 13, 17, 22, 27–29]. Some of the most peculiar clinicopathological features of previously genetically confirmed reported cases, together with the present case, are shown in Table 1. As observed within soft tissue lesions, there is a slight female predominance. The median age is 39 years (range: 15–85 years) and the most common site within the gastrointestinal tract, appears to be the small bowel, in particular, the ileum. Most tumors are relatively small (median size: 5 cm; range: 1.8–8.7 cm), just like soft tissue lesions. The majority of the tumors are described as ulcerated masses involving all the layers of the intestinal or gastric wall, deep into the serosa. About half of the cases have already spread to the regional lymph nodes and/or to the mesentery at the time of diagnosis. The overall clinical behavior seems to be aggressive, with a high percentage of regional and distant metastasis. Most patients develop liver metastasis; intraperitoneal spread, pancreatic spread, lung, and mediastinal lymph node metastasis have been reported in few cases as well.

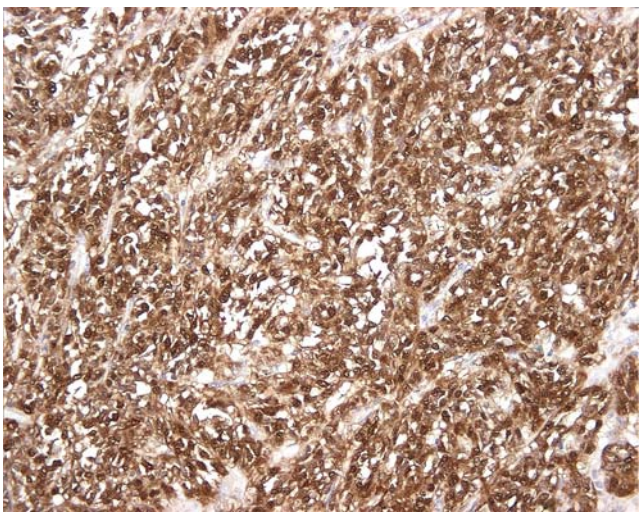

Fig. 3 Strong and diffuse nuclear and cytoplasmic S-100 protein immunoreactivity

Microscopically, a common feature of CCS of the gastrointestinal tract is its infiltrative growth pattern: the tumors are mainly centered in the submucosa and muscularis propria and extend into the mucosa and into the subserosal fat. The histologic features closely resemble those of its soft tissue counterpart. The tumor discloses a rather uniform pattern composed of nests or fascicles of rounded, polygonal, or fusiform cells with abundant clear or eosinophilic cytoplasm, delineated by thin to dense fibrous septa. The nuclei are typically vescicular with prominent nucleoli. Within genetically confirmed CCSs, the presence of CD68-positive osteoclast-like multinucleated giant cells has been reported in four [2, 9, 13, 29] of 16 cases, whereas multinucleated HMB-45-positive tumor cells, which are commonly seen in soft tissue CCS, were described only in one case [4]. At this point, it is worthwhile to mention the description of a major number of gastrointestinal neoplasms containing CD68-positive osteoclast-like multinucleated giant cells with morphological features resembling those of CCS lacking molecular confirmation. Six cases of osteoclast-rich tumors of the gastrointestinal tract resembling CCS were reported by Zambrano et al. [29] (only one case was studied by cytogenetics) and a further case was described by Huang et al. [17]. All reported cases, including those with cytogenetic/molecular studies, were found to be vimentin- and S-100 protein-positive and negative for other melanocytic markers, thus giving rise to the question whether these osteoclast-rich CCS-like tumors of the gastrointestinal tract should be considered a entity separate from classic S-100 protein-positive, HMB45-positive CCSs of soft tissues [23]. If we analyze the immunoprofile of all gastrointestinal tumors with features resembling CCS with or without osteclast-like giant cells (see below), and with or without molecular confirmation, it seems evident that the expression of melanocytic markers is only rarely described. Thus, the lack or low expression of melanocytic differentiation could be related mainly to the gastrointestinal location and not to a new osteoclast-rich entity. Moreover, intracellular melanin pigment has been

Table 1 Clear cell sarcoma of the gastrointestinal tract (review of genetically confirmed cases)

References	Age/sex	Site	Size (cm)	Gross findings	Regional and metastatic spread at diagnosis	Positive immunohistochemical results (selected data)	Molecular confirmation	Outcome
[5]	37/M	Ileum	6.5	Ulcerated mass	No	S-100 protein+, vimentin+	Karyotype	Liver metastasis at 24 months (resected) and at 46 months, AWD (46 months)
[10]	74/M	Transverse colon	2	Ulcerated mass	No	S-100 protein+, vimentin+, HMB-45+	RT-PCR	Liver metastasis at 9 months (resected), NED at 15 months
[22]	30/M	Stomach	4	Ulcerated mass	Yes (regional LNs, peritoneum)	S-100 protein+, vimentin+, NSE+, CD99+	Karyotype and FISH	Liver, pancreatic and Douglas cul-de-sac metastasis at 4 months, AWD at 18 months
[29]	15/F	Jejunum	5	Polypoid lesion	Yes (mesenteric LNs)	S-100 protein+, vimentin+	Karyotype	DOD at 16 months
[1]	57/M	Jejunum	6.5	Ulcerated mass	No	S-100 protein+, HMB-45+, Melan-A+, tyrosinase+	FISH	NED at 11 months
[27]	35/M	Ileum	1.8	Ulcerated mass	No	S-100 protein+, tyrosinase+ (focal), Melan-A+ (focal)	RT-PCR	Liver metastasis at 2 months, DOD at 15 months
[28]	21/F	Ileum	7	Ulcerated mass	Yes (mesenteric LNs)	S-100 protein+, vimentin+	FISH	NR
[4]	85/F	Ileum	NR	NR	Yes (mesentery)	S-100 protein+, Melan-A+, HMB-45+	RT-PCR and FISH	DOD at 1 month
[9]	41/M	Jejunum	8.7	Polypoid lesion	No	S-100 protein+, vimentin+, bcl-2+ (weak)	FISH	Liver metastatic lesions at 6 months
[13]	16/M	Ileum	5	Ulcerated mass	No	S-100 protein+, EMA+ (focal)	Karyotype and RT-PCR	Multiple mesenteric and omentum metastasis and lung nodule at 2 weeks, pulmonary ilar and esophageal LNs at autopsy (DOD at 11 months)
[2]	81/F	Ascending colon	NR	NR	Yes (regional LNs)	S-100 protein+, vimentin+ (focal), NSE+, CD56+, synaptophysin+	FISH and RT-PCR	Peritoneal implants and liver metastasis at 5 years (AWD)
[2]	42/F	Ileum	NR	NR	No	S-100 protein+, vimentin+ (focal), NSE+, CD56+, synaptophysin+	FISH and RT-PCR	No significant follow-up available (recent case)
[2]	42/F	Ileum	3.7	NR	Yes (mesentery)	S-100 protein+, vimentin+ (focal), NSE+, CD56+, synaptophysin+	RT-PCR)	No significant follow-up available (recent case)
[2]	51/F	Jejunum	NR	NR	NR	S-100 protein+, vimentin+, NSE+	FISH	Peritoneal and liver metastasis, AWD
[2]	18/F	Jejunum	NR	NR	NR	S-100 protein+, vimentin+, NSE+	RT-PCR	Local recurrence
Present case	31/F	Ileum	2.8	Ulcerated mass	Yes (regional LNs)	S-100 protein+, vimentin+	FISH	No significant follow-up available (recent case)

LNs Lymph nodes, *NED* no evidence of disease, *AWD* alive with disease, *DOD* died of disease, *NR* not reported

observed in less than half of the reported cases, thus supporting the recent observation [2] that most of these tumors have somehow lost the potential to differentiate along the melanocytic lineage, as more clearly evidenced by immunohistochemical results (see below).

Concerning the immunohistochemical profile, whereas most soft tissue CCSs express melanocytic markers, a high percentage of these tumors in the gastrointestinal location do not. Among genetically confirmed CCSs (Table 1), melanocytic markers were found to be positive only in four cases. Conversely, positive immunostaining for S-100 protein has been reported in all published cases; the expression of other neuroectodermal markers, such as NSE, CD56, and synaptophysin has been encountered in only few cases.

The distinctive cytogenetic character of CCS is the recurrent t(12;22)(q13;q12) translocation, resulting in the EWS-ATF1 fusion, first described in soft tissue cases [30]. This translocation has never been observed in any other neoplasm until very recently [14, 15]. In fact, fusion of the EWS and ATF1 genes has been described in three cases of angiomatoid fibrous histiocytoma; however, this latter tumor type lacks the melanocyte-specific splice form of the MITF gene [14, 15]. We now know that although CCS carries a recurrent distinctive translocation and lacks mutations of the BRAF gene, which are so common in melanoma, immunohistochemical, and ultrastructural studies suggest that it is, like melanoma, a neuroectodermal tumor with melanocytic differentiation. The melanocytic differentiation has been demonstrated by a microarray-based gene expression profiling study by Segal et al. [26], which showed that CCSs do not cluster with other high grade soft tissue sarcomas but with melanoma, thus representing a distinct genomic subtype of melanoma. Moreover, the up-regulation in most CCSs of genes, such as MITF and SOX10, involved in melanocytic differentiation, was also confirmed by the same authors [26].

In the recent study by Antonescu et al. [2], a novel recurrent variant fusion involving the EWS gene, EWS-CREB1, has been demonstrated in a series of three CCSs of the gastrointestinal tract. CREB1 is a gene that encodes another CREB family member closely related to ATF1. The three cases with EWS-CREB1 fusion showed only low or absent expression of melanocytic differentiation genes thus suggesting that evidence of melanocytic differentiation in CCS with EWS-CREB1 fusion is either absent or much less than in classic CCS with EWS-ATF1 fusion, suggesting the notion that melanocytic features are not a necessary finding in this type of sarcoma.

The above-reported findings on EWS-CREB1-positive CCS, together with the inconsistent immunohistochemical expression of melanocytic markers observed in both EWS-CREB1-positive and EWS-ATF1-positive gastrointestinal CCS cases, may once more suggest that the gastrointestinal location, rather than the fusion transcript type, might determine the lack of melanocytic differentiation [2]. The lack or low expression of melanocytic differentiation in either EWS-CREB1-positive and EWS-ATF1-positive CCSs of the gastrointestinal tract support the concept of a common histogenesis of these tumors from a gastrointestinal neuroectodermal precursor cell that might have lost the capacity to differentiate along the melanocytic lineage, in contrast to the neuroectodermal precursor cell of CCS of soft tissues [2].

Because of its unusual site of presentation, together with the inconsistent expression of melanocytic markers, the diagnosis of CCS of the gastrointestinal tract may be difficult. In view of its location and mesenchymal appearance, an obvious consideration is that of gastrointestinal stromal tumors (GISTs), which account for most of mesenchymal tumors at this site. These tumors usually arise within the gastric wall and the small intestine and are less frequently seen within the colon and rectum. Most GISTs are CD117-positive and CD34-positive and although CD117-negative cases have been reported, an overwhelmingly strong S-100 protein-positivity makes this hypothesis unlikely. Based on morphology, and particularly on immunohistochemical stains (strong positivity for S-100 protein), the most intriguing possibility to be ruled out is that of a metastatic melanoma. Metastatic spread of melanoma of the skin and eyes to the gastrointestinal tract has been reported. The differential diagnosis between CCS and melanoma is of particular importance, in spite of different therapeutic modalities. An extensive systemic examination, together with the lack of metastasis in other sites, could exclude a melanoma, although cases of metastatic melanoma of unknown origin have been reported. The immunohistochemical profiles of CCS and melanoma overlap, providing no aid to the differential diagnosis between these neoplasms. It has been recently stressed that CD117-positivity may prove useful in differentiating deep soft tissue or visceral lesions with melanocytic differentiation, as positive staining results would exclude CCS, but are compatible with metastatic melanoma [11]. Nonetheless, in these instances, a firm diagnosis of CCS relies mainly on cytogenetics or molecular diagnostic investigations by the demonstration of the EWS-ATF1 or EWS-CREB1 fusions and the lack of BRAF gene mutations. Among other neoplasms that may enter the differential diagnosis, strong positivity for S-100 protein excludes the hypothesis of a leiomyosarcoma whereas more challenging is the differential diagnosis with the epithelioid variant of malignant peripheral nerve sheet tumor (MPNST) which shows strong S-100 protein immunoreactivity. This latter eventuality may be ruled out mainly by molecular diagnostic investigations, as the expression of neural markers (other than S-100 protein)

has been reported in few cases of CCS [2, 22], whereas, no translocation involving the EWS or ATF1 gene locus has been found to date in MPNST. Another differential diagnostic alternative that may be considered is the rare clear cell tumor known as PEComa, of which few cases have been described in the gastrointestinal tract [12, 20]. This last entity is immunohistochemically characterized by the expression of both melanocytic markers and muscle markers, in particular smooth muscle markers, whereas S-100 protein is typically negative. When melanocytic differentiation is not evident, various types of tumors, including neuroendocrine tumors, poorly differentiated carcinomas, paragangliomas, and the rare renal-type clear cell sarcoma, of which one case has been described in the terminal ileum [18], should be distinguished mainly by immunohistochemical studies.

In summary, CCS of the gastrointestinal tract is a rare entity, with most cases affecting the small bowel. The clinical behavior of this tumor seems to be aggressive with an overall high incidence of local (regional lymph nodes, peritoneum, and mesentery) and distant (more frequently liver) metastasis. The morphology of these tumors, with or without osteoclast-like giant cells, closely resembles that of CCS of soft tissue, and their common histogenesis is confirmed by the presence of the typical EWS-ATF1 transcript fusion. In view of the rarity and unusual site of this tumor, and since most cases at this location lack the immunohistochemical expression of melanocytic markers, we emphasise the pivotal role of cytogenetics and/or molecular techniques for the correct evaluation of these lesions.

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CASE REPORT

Mucinous carcinoma of thyroid gland. Report of a primary and a metastatic mucinous tumour from ovarian adenocarcinoma with immunohistochemical study and review of literature

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Abstract Mucinous carcinoma of the thyroid gland is an uncommon tumour that from the histological point of view, resembles mucinous carcinoma of others sites. Although a mucinous appearance has sometimes been reported in association with cases of typical thyroid carcinoma, true mucinous carcinoma is exceptionally rare. We describe two cases of thyroid tumours with mucinous differentiation studied with immunohistochemistry. Both cases disclosed a similar histological appearance, with small nests and sheets of malignant epithelial cells associated with extensive extracellular mucin that substituted and entrapped the follicular parenchyma of the thyroid. Thyroglobulin and focally thyroid transcription factor (TTF) 1 were positive in one case. From these findings, we classified this tumour as primary mucinous thyroid carcinoma. Thyroglobulin and TTF-1 were negative in tumour cells of second case; on the contrary, positivity to the carcinoembryonic antigen and CA-125 was strong and generalized. However, successfully, the patient presented ascites associated to right ovarian mass. In this case, thyroid tumour represents the first clinical sign of an ovarian mucinous adenocarcinoma, and it has not been previously described in literature. Both patients died after few months to diagnosis. In conclusion, primary and secondary mucinous carcinoma are rare and unusual tumours of the thyroid gland that can be a cause of pitfall in differential diagnosis. In these cases, for a correct diagnosis, a complete clinical history, restricted histological criteria and immunohistochemical panel are necessary.

Keywords Mucinous carcinoma · Thyroid gland · Immunohistochemistry · Differential diagnosis

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Introduction

Mucinous thyroid tumours are very rare and sometimes can occur in association with a typical thyroid carcinoma (follicular and papillary) but does not represent a true primary thyroid carcinoma [1, 2, 6, 8, 10]. This latter is exceptionally rare, with only six cases reported in literature, and histologically are indistinguishable from mucinous carcinoma of others sites [3, 5, 7, 9]. We describe two cases of a mucinous tumour of the thyroid where one is a primary carcinoma as demonstrated by immunohistochemistry and the second represents a first clinical manifestation of a mucinous carcinoma of the ovary.

Case reports

Case 1

A 62-year-old female patient, presented in January 1999 with fever, weight loss, odynophagia and a rapidly enlarging

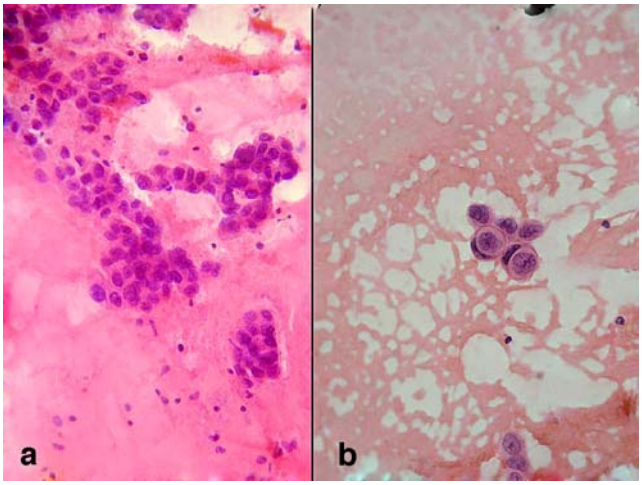

Fig. 1 FNA (case 1) shows scant amount of colloid material containing cohesive groups of malignant epithelial cells (**a**); an additional feature was the presence of malignant cells with features of signet ring cells (**b**) (hematoxylin–eosin, 40×)

mass of thyroid region. She was euthyroid, and ultrasonography confirmed a solid mass involving both lobes of the thyroid and bilateral lymph nodes. A fine-needle aspirate (FNA) of the mass and of the bigger lymph node was performed, which suggested poorly differentiated thyroid carcinoma. The patient underwent total thyroidectomy with resection of lymph nodes. Post-operative tomography revealed a metastatic lymph node at the right side of the neck and at the mediastinum. It was negative for lung metastasis. The adenopathy was irresectable because it was adherent to the vessels. Six months later, the patient died of disease, and the autopsy did not reveal others sites of disease.

Case 2

A 59-year-old woman had first sought medical attention in March 2004 when she was noted to have several enlarged thyroid glands. Ultrasound examination of the anterior region of the neck showed a diffuse enlargement of the thyroid gland associated with cervical lymph nodes. FNA was performed in our Department of Pathology using a standard technique followed by total thyroidectomy for malignancy. Over the next 3 months, the patient had developed subclavian, deep cervical lymph nodes and ascites.

The serum level of CA125 and carcinoembryonic antigen (CEA) were elevated. Computed tomography showed a right adnexal mass with peritoneal implants and retroperitoneal lymph nodes; no other mass is identified in the abdominal cavity. The patient died 4 months later. Autopsy was not allowed.

Material and methods

In both cases, FNA of the thyroid gland was performed using a 23-gauge needle under ultrasonographic control. The cytological smears were air dried and stained with May–Grunwald–Giemsa. Others slides were fixed in ethanol and stained with the Papanicolau method and hematoxylin–eosin.

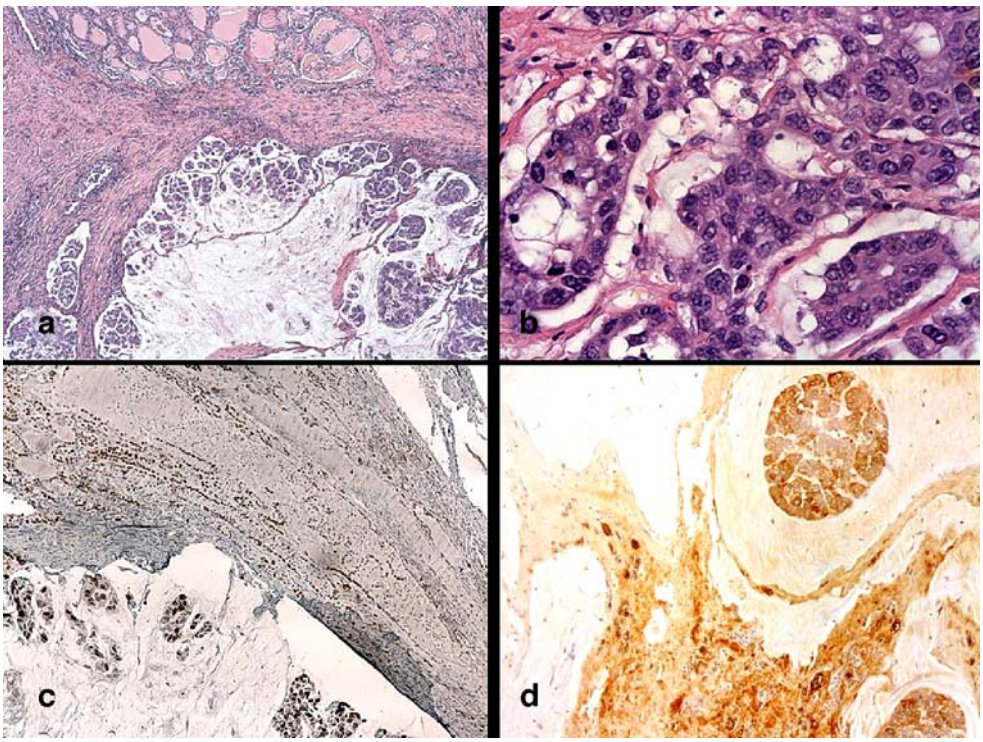

Fig. 2 **a** A large pool of extracellular mucin (hematoxylin–eosin, 10×). **b** Solid areas of poorly differentiated carcinoma with glandular differentiation (hematoxylin–eosin, 40×). **c** Tumour cells were immunoreactive with TTF-1 (immunoperoxydase, 20×) and **d** thyroglobulin (immunoperoxidase, 40×)

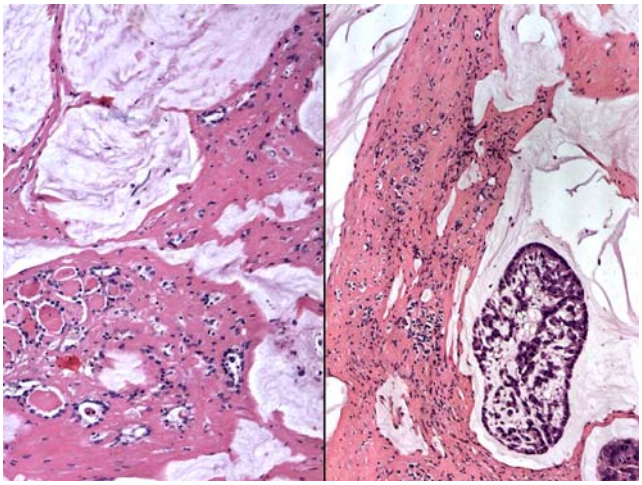

Fig. 3 In case 2, the tumour was predominantly composed of areas of large amount of paucicellular mucus (*left*), sometimes extracellular mucin entrapped residual atrophic thyroid follicles (*right*) (hematoxylin–eosin 20×)

In case 2, cytological effusion from ascites was used for cytological smears like previously reported, and after clearing of the supernatant from the cell pellet, the cell deposit was fixed in formol sublimate for processing into cell blocks.

Both surgical specimens were fixed in 10% buffered formalin and embedded in paraffin; 4-μm-thick, formalin-fixed, paraffin-embedded tissue sections of both cases were stained with hematoxylin–eosin, mucicarmine, Alcian blue and periodic acid-Schiff (PAS) before and after diastase treatment for light microscopic examination.

For immunohistochemical studies, consecutive sections were stained with antibodies to thyroglobulin, CK7, CK20, CK MNF116, Thyroid transcription factor 1(TTF-1), CEA, calcitonin and Ki67/MIB 1 (Dako, Carpinteria, CA) in case 1 and CA-125 (Dako) in case 2.

Results

Thyroid aspirates of case 1 were moderately cellular, with scant amount of colloid material containing cohesive groups of variable size of malignant epithelial cells (Fig. 1a); an additional feature was the presence of small groups of mucin-producing cells also with features of signet ring cells (Fig. 1b).

In case 2, FNA showed large streaks of mucin with small aggregates or sheets of malignant epithelial cells.

Some groups of neoplastic cells showed a papillary-like finding, but there was no evidence of a central vascular core.

Grossly, in both cases, the tumours were described grossly as firm to soft, solid mass and adherent to surrounding structures. The cut surface was smooth or slightly bulging also with a ‘fish-flesh’ or a focal gelatinous appearance.

Histologically, both cases disclosed a very similar appearance, with areas of abundant extracellular mucin around strands, small solid nests and trabeculae of epithelial malignant cells, an image similar to that of mucinous carcinoma of the breast. The mucus was positively stained for mucicarmine, Alcian blue and PAS before and after diastase treatment.

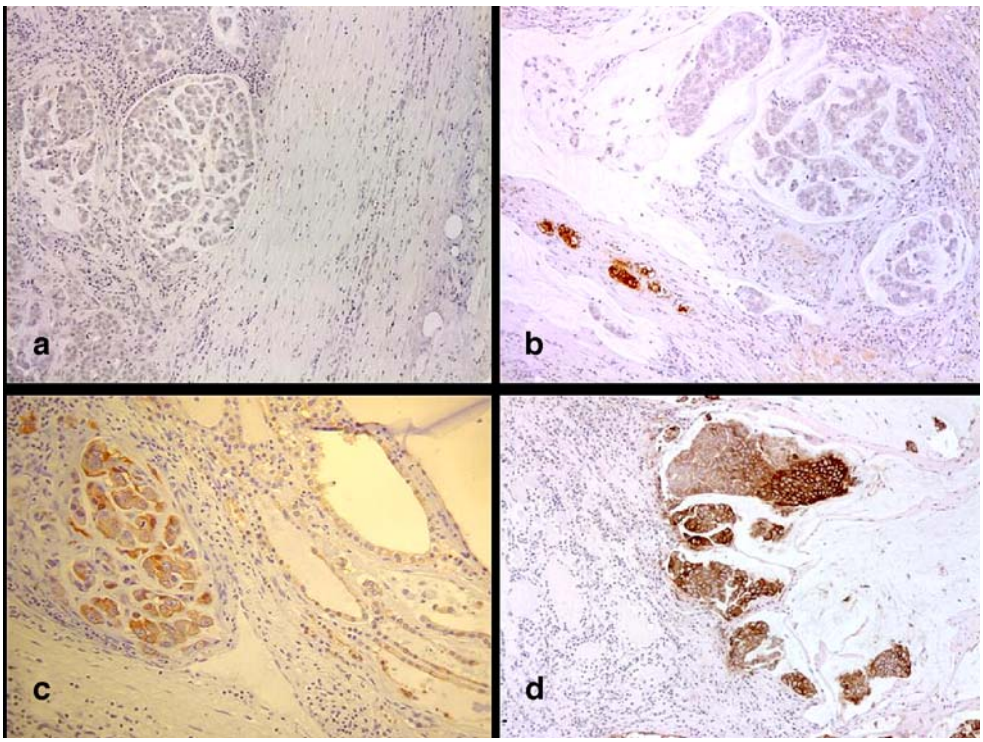

Fig. 4 Immunohistochemically, neoplastic cells are negative for **a** CK20 and **b** thyroglobulin but show strong reactivity for **c** CEA and **d** CA125 (immunoperoxidase, ×20)

Table 1 Summary of previous cases

Authors	Age	Sex	Immunochemistry	Follow-up
Diaz-Perez et al.	44	M	ND	AWD 8 Yr
Sobrinho-Simoes et al.	–	–	Thy+	–
Mizukami Y et al.	58	M	Thy+	Cervical and axillary metastases
Kondo T et al.	82	F	Thy+, TTF1+	DOD 4 Yr
Present case	62	F	Thy+, TTF1−/+	DOD 4 Yr

M male, *F* female, *Thy* thyroglobulin, *ND* no data, *AWD* alive without disease, *Yr* year, *DOD* died of disease

In case 1, large pool of extracellular mucin (Fig. 2a) progressively merged with loose nests of carcinoma with mucinous stroma and solid areas of poorly differentiated carcinoma also with glandular differentiation (Fig. 2b). The tumours grew in an aggressive manner and invaded the neighbouring cervical structures and skeletal muscle of the neck. There were no areas of typical thyroid carcinoma noted.

In this case, there was immunoreactivity to epithelial markers (CK MNF116, CK7), focally to TTF1 (Fig. 2c) and thyroglobulin (Fig. 2d) but not to calcitonin and CK20.

A diagnosis of primary thyroid mucinous carcinoma was made.

In case 2, the tumour was predominantly composed of areas of a large amount of paucicellular mucus and focally of mucin-containing gland-like structures, sometimes extracellular mucin entrapped residual atrophic thyroid follicles (Fig. 3). Vascular invasion was also evident. Immunohistochemically, neoplastic cells showed a positive reaction for cytokeratin (CK MNF116), CA-125, CEA and CK7 but did not show reactivity for CK20, epithelial membrane antigen, calcitonin, thyroglobulin and TTF-1 (Fig. 4).

Furthermore, malignant epithelial cells in cytological smears from cell blocks of ascites showed a consistent positivity for CA-125 and CK7 but was negative for CK20.

A diagnosis of thyroid metastasis from a mucinous adenocarcinoma of the ovary was made.

Discussion

Mucin production in thyroid gland is an unusual event but can occur in a wide variety of thyroid lesions (follicular neoplasm, papillary carcinoma, anaplastic and medullary carcinoma) [1, 2, 6, 8, 10]. Generally, in these cases, the tumour appears characterized by a pool of mucin, often extracellular and admixed with areas of typical thyroid carcinoma. These tumours do not represent a true primary thyroid mucinous carcinoma. In fact, this latter is extremely rare (only four cases reported), and histologically, it is indistinguishable from a mucinous carcinoma at other sites [3, 5, 7, 9]. In these case, areas of papillary or follicular carcinoma are not always evident. Among five cases studied, all are thyroglobulin and/or TTF-1 positive (Table 1).

Furthermore, in our case (case 1), the tumour was composed of nests of epithelial cells also with glandular differentiation that progressively merged with neoplastic areas of mucinous stroma and a pool of extracellular mucin, in the absence of findings of typical thyroid carcinoma. Tumour cells were positive for thyroglobulin and focally for TTF-1. Finally, follow-up examination has not demonstrated another neoplasm in others sites. From these features, we classified this tumour as primary mucinous thyroid carcinoma.

In the presence of neoplastic epithelial clusters or glands accompanied by a large mucus pool involving thyroid parenchyma, a metastasis from a mucinous adenocarcinoma arising in others sites should be considered in differential diagnosis. Generally, in this case, a correct diagnosis is easy because the metastatic cells are often associated with obvious widespread metastatic disease elsewhere.

Uncommonly, metastatic carcinoma in the thyroid gland can represent the first manifestation of the systemic disease [4].

In our case 2, we describe a previously unreported case of metastatic mucinous adenocarcinoma of the thyroid gland that represents the first evidence of an ovarian carcinoma. Furhtermore, in this case, the tumour was composed of strands and small nests of malignant epithelial cells with

Table 2 Criteria for differential diagnosis in primary and secondary mucinous thyroid carcinoma

	Histology	Immunohistochemistry	Clinical history
Primary MTC	Mucus pool with transition to solid and glandular areas	Thy+ and/or TTF-1+	Local recurrences
Secondary MTC	Large mucus pool with small clusters of epithelial cells	Thy−, TTF-1−	Tumour in others sites, lymph nodes and visceral metastasis
Other type	Mucus pool and typical thyroid carcinoma	Thy+, TTF-1+	Not relevant

extensive extracellular mucin. The presence of mucinous features in a thyroid mass in the absence of a previous history of a primary tumour in the other side may represent an even greater cytological and histological challenge to the pathologist. In fact, this tumour was first recognized as a primary mucinous carcinoma of the thyroid. A key feature that can help in the differential diagnosis is the absence of solid or glandular areas that progressively merged with areas of mucinous stroma and a pool of extracellular mucin, such as case 1. If a primary thyroid tumour is suspected, an immunohistochemical study on the histological specimens or on the aspirate material is necessary to provide a final diagnosis.

In fact, in our case, a negative immunostaining for thyroglobulin and TTF1 but positive for CA125 and CEA favour metastatic ovarian carcinoma over the primary thyroid adenocarcinomas. Then, the result of the diagnosis was confirmed by successive appearances of ascitis with an ovarian mass. In conclusion, mucinous carcinoma are rare and unusual tumours of the thyroid gland that can be a cause of pitfall in differential diagnosis. For a diagnosis of primary carcinoma-restricted histological criteria, an immunohistochemical panel and a complete clinical history are necessary. Particularly, from the histological point of view, the presence of solid and glandular clusters of neoplastic cells that progressively merge with areas of mucin deposition in the stroma and with a large pool of mucus, in the absence of areas of typical thyroid carcinoma, should favour a diagnosis of primary mucinous carcinoma (Table 2). Nevertheless, also in the absence of a history of metastatic disease or distant primary carcinoma, the possibility of a secondary thyroid involvement in mucinous adenocarcinoma could be considered.

For this motive, in the presence of uncommon and/or rare thyroid tumours, for it to be defined, diagnosis could only be established after the careful immunohistochemical study.

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CASE REPORT

Primary retroperitoneal mucinous cystadenoma with sarcoma-like mural nodule

A case report and review of the literature

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Abstract Primary retroperitoneal cystadenomas are extremely rare. This is the first report in literature to describe a primary retroperitoneal cystadenoma with a sarcoma-like mural nodule. A 45-year-old woman complained of a left-sided abdominal mass. A computed tomography scan revealed a cystic mass with a mural nodule, which seemed to originate from the tail of the pancreas. At laparotomy the cyst was not adhered to the pancreas but localized retroperitoneally. Histologic examination showed a mucinous cystadenoma with only foci of borderline malignancy with a mural "sarcoma-like" nodule. In view of the surgical and histopathological findings, the mucinous cystadenoma was regarded as primary retroperitoneal. This case demonstrates that in the era of radiological preoperative refinement, pathological diagnosis remains of utmost importance, especially for rare cases.

Keywords Retroperitoneum · Mucinous cystadenoma · Mural nodule

Introduction

Mucinous cystadenomas of the ovary are clinically and histopathologically well-established and common tumors. Primary retroperitoneal mucinous cystadenomas are extremely rare. Such tumors are histologically similar to ovarian mucinous cystadenomas. Their histogenesis is still unclear. We report a case of primary retroperitoneal mucinous cystadenoma with foci of borderline malignancy containing a mural "sarcoma-like" nodule.

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Case report

Clinical history

A 45-year-old, para 2, woman presented at the emergency room with a 3-week history of left-sided abdominal pain. She had felt a mass in the left lower quadrant 2 days before. Her clinical history included endometriosis and a car accident. The mass was progressive but not painful. Apart from the palpable mass of 15 cm in the left lower abdomen, physical examination was unremarkable. Ultrasonography demonstrated a 15-cm cystic mass with a 3.8-cm nodule in its wall. The uterus was normal in size, and internal ultrasonography showed small ovaries. Carcinoembryonic antigen, cancer antigen (CA) 125, and CA 19-9 levels were within normal limits. The next day, a contrast-enhanced computed tomography scan of the abdomen revealed a 15-cm left-sided cystic mass, which seemed to originate from the tail of the pancreas (Fig. 1a). The cystic mass showed a 4-cm nodule in its wall (Fig. 1b) and was suspected for a cystic papillary adenocarcinoma. At laparotomy, the cyst was not adhered to the pancreas and could be easily separated from its location near the tail without opening the pancreatic capsule. Vascularization appeared to arise from the mesentery of the left colon. It was localized in the retroperitoneal space extending caudally from the spleen to the lower abdomen with medial displacement of the left colon. Total resection of the cyst was performed, and the specimen was sent for histopathological examination.

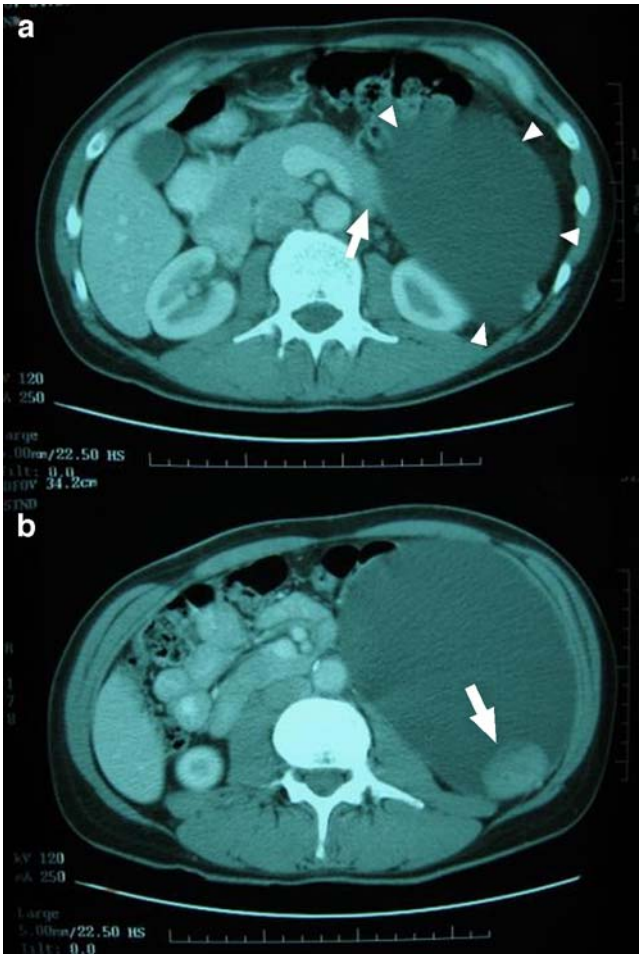

Fig. 1 Contrast enhanced CT scan shows images of a pancreatic cyst (*arrowheads*) originating from the tail of the pancreas (**a**) and a mural nodule within the wall of the cystic mass (**b**)

Further inspection showed two normal ovaries. Her postoperative recovery was uneventful. One year after surgery, the patient was without signs of recurrence or metastasis.

Materials and methods

The specimen was fixed in 4% buffered formalin. Representative samples were routinely processed and embedded in paraffin blocks. Four-micrometer-thick sections were stained with hematoxylin and eosin and with parallel routine immunohistochemical procedures. The antigens tested by immunohistochemistry were: pan-keratin, keratin Cam 5.2, cytokeratin 7, cytokeratin 10, cytokeratin 18, cytokeratin 20, epithelial membrane antigen, vimentin, desmin, actin, myosin, CD34, CD68, CD99, CD117, S-100 protein, and bcl-2.

Pathological findings

The specimen consisted of a unilocular cyst measuring 20×11 cm with a smooth surface. The content was a watery mucinous material. The wall was thin with a smooth gray-white inner surface and contained a circumscribed bean-shaped solid mural nodule of 3.5×3.5×2.5 cm, which showed a brown-yellow and focally hemorrhagic cut surface. Microscopically, most of the cyst was lined by single-layered tall columnar cells, abundant clear cytoplasm, and small basally located nuclei (Fig. 2a).

Occasionally (over less than 1% of the surface), the epithelium showed slightly atypical proliferation with

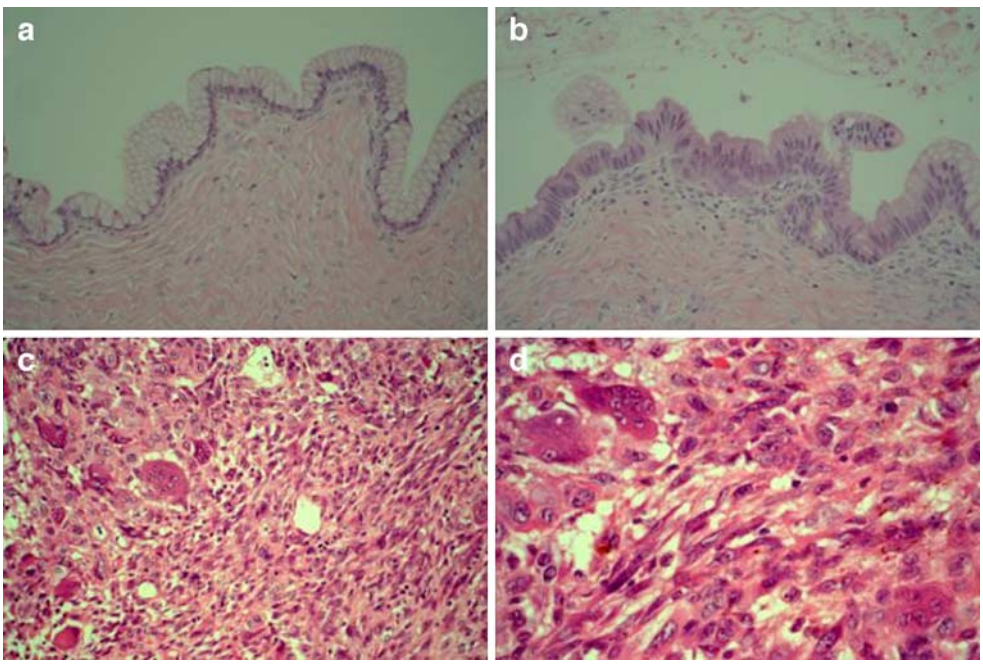

Fig. 2 Cyst wall with typical tall columnar mucous-secreting epithelium and fibrous wall (**a**). Low cellular proliferation with some stratification of the cells and slight nuclear atypia (**b**). Heterogenous proliferation of pleomorphic cells, spindle cells, osteoclast-like giant cells, and some mononuclear cells with some pigment (**c**). Detail of sarcoma-like nodule (**d**)

glandular budding, tufting of the epithelium, decreased cytoplasmic mucin, some stratification of slightly irregular nuclei, and an occasional mitoses (Fig. 2b). There was no infiltrative growth but foci of borderline malignancy. The nodule was well circumscribed without vascular invasion and consisted of a heterogenous population of spindle-shaped cells, pleomorphic cells with bizarre nuclei, mixed mononuclear inflammatory cells, benign osteoclast-like giant cells, and foci of hemorrhage. There were mitotic figures, including some atypical forms (Fig. 2c and d). The sarcoma-like cells proved to be keratin negative (Table 1).

Discussion

Mucinous cystadenomas can be located in the ovaries, pancreas, and in the retroperitoneum. The mucinous cystadenoma presented was localized retroperitoneally near the pancreas but was clearly not adhered to it. Because normal-appearing ovaries were found, the cystadenoma was thought to be primary retroperitoneal.

According to the literature, symptoms are nonspecific, and most patients complained of an abdominal distension or mass with or without pain [9]. Mucinous cystadenomas were relatively large, varying from 10 to greater than 20 cm in diameter, which is large enough to cause symptoms like abdominal fullness [9]. Preoperative diagnosis is very difficult, not because the tumors are often overlooked in the differential diagnosis but also because no sensitive methods or reliable markers are available [2]. As retroperitoneal mucinous cystadenomas are histologically similar to mucinous cystadenomas of the ovary, the ultrasonographic image pattern is in general of no help in distinguishing between ovarian and retroperitoneal origin. In our case, the diagnosis of the retroperitoneal mucinous cystadenoma could not be established preoperatively by ultrasonography or computed tomography. Although ultrasonography, computed tomography, or magnetic resonance imaging can detect retroperitoneal cysts, the diagnosis of mucinous cystadenoma is seldomly made preoperatively. The usual preoperative differential diagnosis consists of ovarian cyst, cystic mesothelioma, cystic lymphangioma, nonpancreatic pseudocyst, and renal cyst [4, 9, 18]. Although aspiration is a good method for delineating the nature of the cyst, cytologic analysis of the aspirated fluid frequently fails to reveal the type of epithelial cells lining the cyst. Therefore, exploratory laparotomy with complete excision of the cyst is usually indicated both for diagnosis and treatment [2].

Table 1 Immunohistochemical results of the sarcoma-like cells

Antigen	Result
Pan-keratin	–
Keratin Cam 5.2	–
Cytokeratin 7	–
Cytokeratin 10	–
Cytokeratin 18	–
Cytokeratin 20	–
Epithelial membrane antigen	–
Vimentin	+
Desmin	–
Actin	+/− (some)
Myosin	–
CD34	–
CD68	+
CD99	–
CD117	–
S-100 protein	–
bcl-2	+/− (some)

Retroperitoneal mucinous cystadenomas are histologically similar to mucinous cystadenomas of the ovary. The histogenesis of these tumors is still unclear. Four main hypotheses have been proposed [2, 14]. According to the first three hypotheses, the tumor arises either from ectopic ovarian tissue, although ovarian tissue was only rarely found [14], from a teratoma in which the mucinous epithelium has overgrown all other components or from urogenital remnants. The most widely accepted theory suggests coelomic metaplasia as the causal agent, whereby tumors arise from invagination of the peritoneal mesothelial layer that undergoes mucinous metaplasia with cyst formation [3, 9]. Such origin rather than from ectopic ovarian tissue is supported by the occurrence of such a tumor in a male patients [5, 8, 10, 17].

Primary mucinous tumors of the retroperitoneum are very uncommon. These tumors can be classified into three clinicopathologic types: mucinous cystadenoma, mucinous cystic tumor of borderline malignancy, and mucinous cystadenocarcinoma. Our case was diagnosed as a primary retroperitoneal mucinous cystadenoma with only foci of borderline malignancy and a mural “sarcoma-like” nodule.

Mural nodules have been described in ovarian and pancreatic mucinous cystic tumors [7, 16]. Mural nodules may be malignant representing anaplastic carcinoma, containing a predominant population of cytokeratin-positive cells with high-grade malignant nuclei, or a genuine soft tissue-type sarcoma [7, 15, 16]. Benign pseudosarcomatous mural nodules are composed of a heterogeneous cell population of epulis-type giant cells, atypical spindle cells with bizarre nuclei and mitotic figures, mixed inflammatory cells, and signs of hemorrhage and necrosis. In these cases, immunohistochemical staining shows a weakly or focally cytokeratin positivity in the pseudosarcomatous cells.

We performed a literature review using Embase and Medline starting in 1966 and identified approximately 45 cases of retroperitoneal mucinous cystadenoma and 25 cases of mucinous cystadenocarcinoma. Only eight cases of mucinous

Table 2 Cases of primary retroperitoneal mucinous cystadenomas of borderline malignancy

Study (year)	Age (years)	Sex	Symptom	Size, image	Tumor marker	Preoperative diagnosis	History	Pathology	Extracystic extension	Therapy	Outcome
Nagata et al. [11] (1987)	41	F	Abdominal swelling, pain	12×10×9 cm	UD	UD	UD	MCAbor	No	TR	UD
Banerjee and Gough [1] (1988)	47	F	Abdominal mass	10 cm (US) next to spl.	ND	Lt adrenal tumor	App Hyst	MCAbor	No	TR, resection spl.+lt adrenal	NED
Motoyama et al. [10] (1994)	63	M	Abdominal pain	6 cm (US), under rt kidney	High CEA in cystic fluid	Rt renal cyst	NR	MCAbor	No	ND	NED
Pearl et al. [13] (1996)	33	F	Abdominal swelling, pain	Large, unilocular lt (CT)	ND	ND	NR	MCAbor	No	LR	NED, 10 months
Papadogiannakis et al. [12] (1997)	33	F	Abdominal mass	13×9 cm (US+CT)	ND	Mesenteric cyst	NR	MCAbor	No	TR	NED, 12 months
Chen et al. [2] (1998)	48	F	Abdominal fullness	15×13×9 cm (CT)	ND	Mesenteric cyst	NR	MCAbor	No	LR	NED, 8 months
Gutsu et al. [6] (2003)	41	F	Flank pain, abdominal distension	21×16 cm rt (CT)	ND	Retroperitoneal cyst	NR	MCAbor	No	TR	NED, 18 months
Matsubara et al. [9] (2005)	36	F	Abdominal distension	12×8 cm rt (CT)	CA 125:51 CA 19-9: 55	Ovarian cyst	NR	MCAbor	No	TR, App, Myo	NED, 6 months
Present case (2007)	45	F	Abdominal pain	15 cm (US+CT)	CEA, CA125, CA19-9: normal	Cystic papillary adenocarcinoma or mucinous cystic neoplasm	Endom	MCAbor	No	TR	NED, 12 months

F Female; *M* male, *UD* unknown data, *NR* not remarkable, *MCAbor* mucinous cystadenoma borderline malignancy, *TR* tumor resection, *LR* laparoscopic resection, *NED* no evidence of disease, *US* ultrasonography, *spl.* spleen, *App* appendectomy, *Hyst* hysterectomy, *Myo* myomectomy, *Endom* endometriosis, *rt* right, *lt* left

cystadenoma with borderline malignancy have been reported (Table 2). Thus, our case is the ninth case of a retroperitoneal mucinous cystadenoma of borderline malignancy. By our knowledge, however, combination with a mural "sarcoma-like" nodule has not been described earlier in the literature. The patient should be followed. However, in this case, long-term follow-up seems not warranted regarding the only focal aspect (<1% of the surface) of borderline malignancy of the cyst and the benign reactive nature of the mural nodule.

Appendix

Article Précis

A 45-year-old woman complained of a left-sided abdominal mass, which turned out to be a primary retroperitoneal cystadenoma with mural nodule.

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LETTER TO THE EDITOR

The neonatal Fc receptor (FcRn) expression in the human skin

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The neonatal Fc receptor, FcRn, has a wide expression within the human body, including adult cells; however, it is not ubiquitously expressed (reviewed in [2]). The receptor is involved both in IgG transcytosis as well as recycling, being able to bind both free and antigen complexed IgG molecules (reviewed in [4]).

Cauza et al. [1] have shown that the neonatal receptor is present in the human epidermal keratinocytes, using both biopsies and cultivated keratinocytes. Furthermore, they were able to demonstrate that most of the FcRn molecules are expressed intracellularly, in acidified vesicles [1], and that the receptor is functional within these cells. However, the FcRn role in keratinocytes in terms of recycling, transcytosis, or perhaps both remains speculative.

In this paper, we show by immunohistochemistry that FcRn expression in human skin is not restricted only to keratinocytes but has a wider expression.

Tissues were obtained from five human cadavers (harvested in an interval of 24 h from the time of death), and a surgical biopsy was performed for diagnostic purposes (nevus). Immunohistochemistry was done on consecutive skin sections as previously described [2]. The epithelial cells were evidenced with an antihuman cytokeratins antibody (clone MNF116, Dako). As expected, the antibody stained the epithelial cells of the hair follicles and sebaceous glands and the keratinocytes (data not shown). The FcRn-staining pattern proved to be more complex. The epithelial structures, evidenced by the cytokeratins targeting, proved to be positive as well for the Fc neonatal receptor (Fig. 1). Furthermore, we were able to show that the cytokeratin-negative, S-100-positive melanocytes (data not shown) [3] express also this receptor (Fig. 2). Histiocytes and dendritic cells were also shown to be FcRn positive by others [1, 5]. The characterization of the exact nature of the cells scattered throughout the investigated tissues was beyond the goals of this study. However, we can speculate that the isolated FcRn-positive cells in our skin sections are histiocytes and dendritic cells.

The presence of FcRn in endothelial cells was investigated by labeling sequential sections with anti-FcRn and respectively anti-CD34 II (clone QBEnd, Dako) antibodies, as well as double labeling with HRP/DAB (brown) for FcRn, and Alkaline Phosphatase/FastRed (Dako, Denmark) (red) for CD34 II. Consistent with the results obtained on human mammary gland sections [2], blood vessels are either negative for the neonatal Fc receptor or express this molecule at a level that is undetectable by the immunohistochemical method (Fig. 3).

IgG is the main antibody isotype in the extravascular body spaces. Hence, the wide expression of the neonatal Fc receptor throughout the organism, including in various cell types of the skin, does not come as a surprise. Although the receptor was proven to be functional in keratinocytes [1], the FcRn function (recycling, transcytosis, or both) in the skin structures remains to be elucidated. On the other hand,

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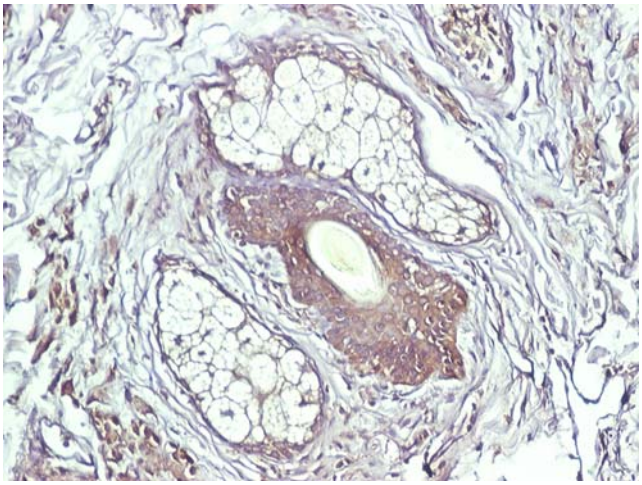

Fig. 1 Normal hair follicle and sebaceous gland. Positive staining for FcRn (200×)

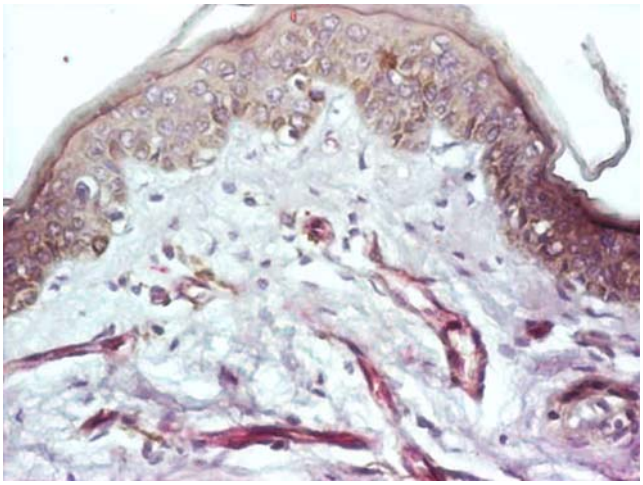

Fig. 3 Normal skin. Double labeling for FcRn (*brown precipitate*) and CD34 II (*red*; 200×)

given the fact that we were unable to identify the presence of the neonatal Fc receptor in the endothelial cells, we raise the question of potential alternative mechanisms capable of transporting IgG antibodies across the blood vessel walls.

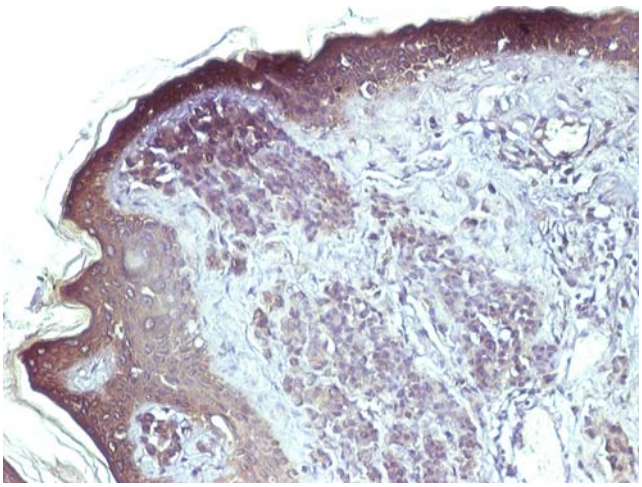

Fig. 2 Skin with nevus. Positive staining for FcRn both in keratinocytes and melanocytes (100×)

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ERRATUM

The spectrum of persistence of testicular blastema and ectopic testicular parenchyma: a possible result of focal delay in gonadal development

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Unfortunately the addresses were published with errors.
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REVIEW AND PERSPECTIVE

Pancreatic intraductal papillary-mucinous neoplasms: a new and evolving entity

Snezana Andrejevic-Blant · Markus Kosmahl · Bence Sipos · Günter Klöppel

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Abstract For a long time, intraductal tumors of the pancreas were neglected because they were misdiagnosed as mucinous cystadenocarcinoma, ordinary ductal adenocarcinoma, or chronic pancreatitis. Only in recent years have they been recognized as clinical and pathological entities. Most common are the intraductal papillary-mucinous neoplasms. Although they show an adenoma-carcinoma sequence, they have proved to have a more favorable prognosis than ductal adenocarcinoma, when resected in a preinvasive state. Recently, it has become clear that they constitute a heterogeneous group with at least four subtypes. Their stratification reveals that the various intraductal papillary-mucinous neoplasm subtypes have different biological properties with different prognostic implications.

Keywords Intraductal papillary mucinous neoplasm · Pancreas · Outcome

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Historical notes and a rising incidence

The pancreatic tumors that are characterized by an intraductal origin and growth pattern include intraductal papillary-mucinous neoplasms [3, 24, 41], intraductal tubular carcinomas [22, 45], intraductal tubular adenomas of the pyloric type [6], and intraductal acinar cell carcinomas [15]. Most common are the intraductal papillary-mucinous neoplasms (IPMNs). They are mucin-producing epithelial tumors that usually show a papillary architecture and are associated with dilatation of the ducts. Before 1990, these tumors were thought to be rare. They first started to be recognized approximately 20 years ago [31, 36, 39], when the many different names given to the tumor in the 1980s were replaced by the term IPMN [41]. This name was also introduced in the classification of exocrine pancreatic tumors propagated by the World Health Organization (WHO) [24] and the fascicles of the Armed Forces Institute of Pathology [44]. Since then, IPMNs have been reported with increasing frequency (Table 1) and currently account for about 7% of clinically diagnosed pancreatic neoplasms and up to 16% of surgically resected pancreatic neoplasms and for almost 50% of pancreatic cysts found incidentally [8, 17]. When only the cystic tumors of the pancreas are considered, IPMNs take first place, with a frequency of 24% [26].

The new incidence data on IPMNs raise the question whether their increase in number is real or not. Of course, it is difficult to accept that IPMNs might have been overlooked in the past, not only clinically but also morphologically. There are good reasons, however, to believe that IPMNs did always exist and did not really increase in frequency. One reason is related to the rapid improvements in modern imaging techniques, which enable more precise recognition of cystic lesions, even if they are small and

Table 1 Incidence data on intraductal papillary-mucinous neoplasms

Author	Period 1	Number of incidence	Period 2	Number of incidence
Sohn et al. [43]	1987–2001	58	2001–2003	78
Wada et al. [47]	1988–2000	63	2001–2003	37
Our series	1981–2000	55	2001–2007	50

asymptomatic. Another is connected with the decreasing risk of pancreatic surgery. The most important fact, however, may be that until 1999, the distinction between IPMNs and mucinous cystic neoplasms (MCNs) was unclear, so that many IPMNs were classified as MCNs [50] or regarded as ductal adenocarcinomas or chronic pancreatitis.

Adenoma-carcinoma sequence

In IPMNs, the normal ductal epithelium is replaced by mucin-producing columnar cells showing papillary proliferations and variable degrees of cellular atypia, even within an individual neoplasm. They are graded according to the most atypical area as IPMN with low grade dysplasia (adenoma), IPMN with moderate dysplasia (borderline), and IPMN with high grade dysplasia (carcinoma in situ). An invasive component may be found in 38–50% of the cases [7, 26, 40, 43]. Progression from adenoma to carcinoma is estimated to occur at about 5 years [43]. IPMNs therefore provide a model of neoplastic progression from a benign intraductal neoplasm through increasing grades of dysplasia to invasive carcinoma.

Prognosis after resection

Between 80 and 90% of IPMNs are surgically resectable. For these IPMNs, a 5-year survival rate of 77–100% was reported, provided the tumors did not have an invasive component (Table 2). By contrast, IPMNs with an invasive component had a 3- to 5-year survival rate of only 36–46% [12, 16, 43] (Table 2). Interestingly, the survival rate did not appear to be dependent on the grade of dysplasia in the IPMN if there was no invasive component [12, 16, 43, 47]. These data imply that the overall outcome of IPMNs therefore largely depends on the presence of an invasive component. If the tumor is already invasive, criteria for a poor outcome are lymph node involvement, vascular invasion, and bilirubin elevation [13]. A comparison of the prognosis of all patients with invasive IPMNs with that of patients with ductal adenocarcinoma reveals that patients with IPMNs survive longer than those with ductal adenocarcinomas [29, 40, 43].

Several studies have reported recurrences after resection of noninvasive IPMNs, some of which revealed only moderate dysplasia [12, 43, 47, 48]. The recurrences were either local or metastatic (Table 2). To explain the recurrences, particularly the local ones, it has to be assumed that either tumor tissue was overlooked at the pancreatic resection margin, or an invasive component remained undetected in the resected specimen, or there was multifocal disease. The last possibility has to be considered if the surgical margins were negative and the recurrence occurred in the pancreatic remnant. This has been observed only in a few cases [40]. When metastatic recurrences occur, it is most likely that they resulted from inadequate sampling that failed to detect an invasive component. Regarding the impact of a positive resection margin on IPMN recurrence, it is interesting to note that it has been reported that even IPMNs with positive margins did not recur during a median follow-up period varying from 19–40 months [13, 48]. The reason for this phenomenon might be that the growth of the remaining intraductal tumor tissue is so slow that clinical symptoms

Table 2 Five year survival rate and recurrence in 349[a] intraductal papillary-mucinous neoplasms

	Noninvasive 77–100%	Invasive 36–46%
No recurrence	93–98.7%	52–70%
Recurrence	1.3–7%	30–48%
Local	up to 6%	8–48%
Distant (metastases)	1%	12%
Both	0%	10–48%

[a] [12, 43, 47]

Table 3 Histopathological data on 105 intraductal papillary-mucinous neoplasms collected during a period of 26 years

IPMN	Gastric (*n*=27) (26%)	Intestinal (*n*=57) (54%)	Pancreatobiliary (*n*=7) (7%)	Oncocytic (*n*=14) (13%)
Noninvasive (65%)	*n*=20 (74%)	*n*=35 (62%)	*n*=3 (43%)	*n*=10 (72%)
Adenoma	9	12	1	5
Borderline	7	18	2	1
Carcinoma in situ	4	5	0	4
Invasive (35%)	*n*=7 (26%)	*n*=22 (38%)	*n*=4 (57%)	*n*=4 (28%)

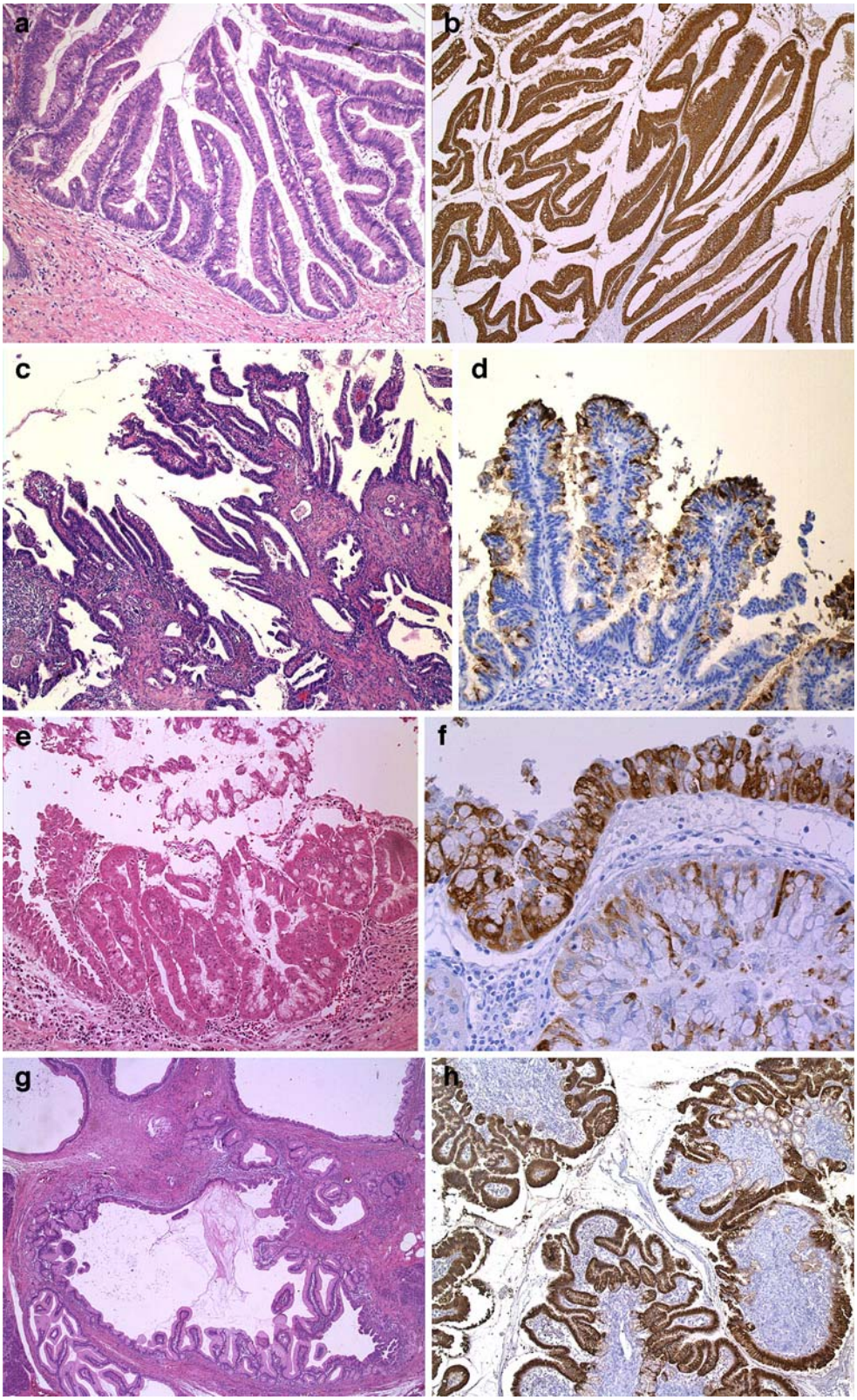

Fig. 1 Histological subtypes of intraductal papillary-mucinous neoplasms of the pancreas and their usual mucin pattern. **a**, **b** IPMN of the intestinal type positive for MUC2; **c**, **d** IPMN of the pancreatobiliary type positive for MUC1; **e**, **f** IPMN of the oncocytic type showing scattered positivity for MUC2; **g**, **h** IPMN of the gastric type, positive for MUC5

only appear after a follow-up period of 2 to 3 years. However, even if intraductal recurrences may take a long time to become clinically apparent, a positive margin in any IPMN case should lead to further tissue resection.

Histological type and prognosis

In 1991, it was reported that the invasive component of IPMNs corresponded either to an ordinary ductal adeno-

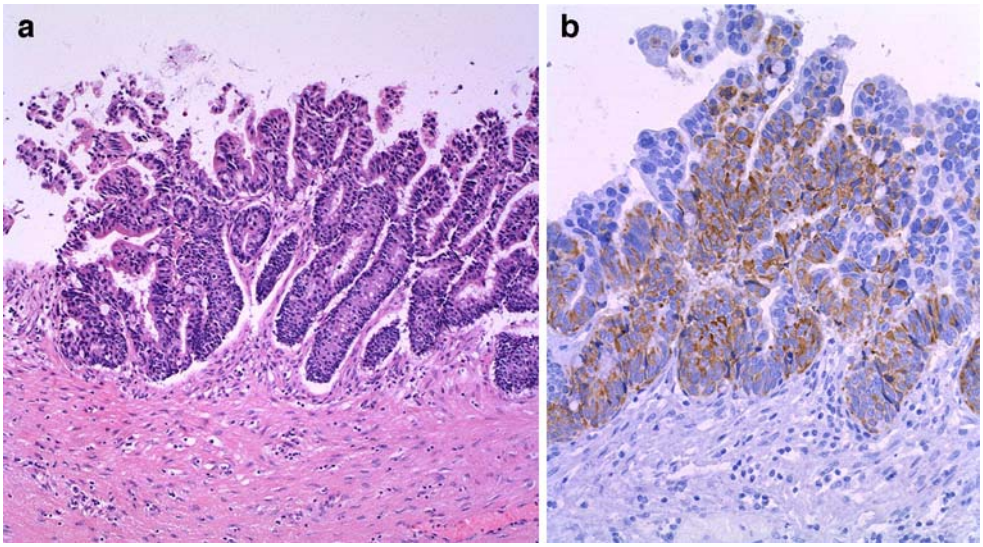

Fig. 2 IPMN of the gastric type showing severe cellular atypia and MUC5 positivity

carcinoma or, more frequently, to that of a mucinous (colloid) carcinoma [49]. This observation suggested that IPMNs form a group of heterogeneous neoplasms. A further argument for the heterogeneity of IPMNs was the detection of IPMNs in branch ducts rather than in the main duct, where most of the IPMNs are found. Finally, it was recognized that IPMNs differ in their histological and cytological features and in their mucin profile [3, 4, 18, 28, 32, 33]. Currently, four subtypes of IPMN can be distinguished: an intestinal type, a pancreatobiliary type, an oncocytic type, and a gastric type [18].

The most common type of IPMN is the intestinal type (Table 3). It usually occurs in the main duct of the pancreatic head [9] and shows a villous growth pattern similar to that of villous adenoma in the colon. It also expresses MUC2 and CDX2 but not MUC1 (Fig. 1a,b). When this IPMN type becomes invasive, the invasive component resembles mucinous (colloid) carcinoma [5, 28], a tumor of which at least 80% is composed of pools of extracellular mucin containing single cells or strands of neoplastic glandular epithelium or even a small component of signet ring cells. Patients with colloid carcinoma have a 55% 5-year survival rate after resection [5]. These tumors therefore seem to be much less aggressive than ordinary ductal adenocarcinomas.

The pancreatobiliary type of IPMN is much rarer than the intestinal type IPMN (Table 3). It shows complex arborizing papillae and expresses MUC1 only (Fig. 1c,d). Its invasive component usually corresponds to a conventional ductal adenocarcinoma. The prognosis of this type of IPMN, if invasive, seems to be similar to that of ductal adenocarcinoma and therefore poorer than that of the intestinal type of IPMN [4].

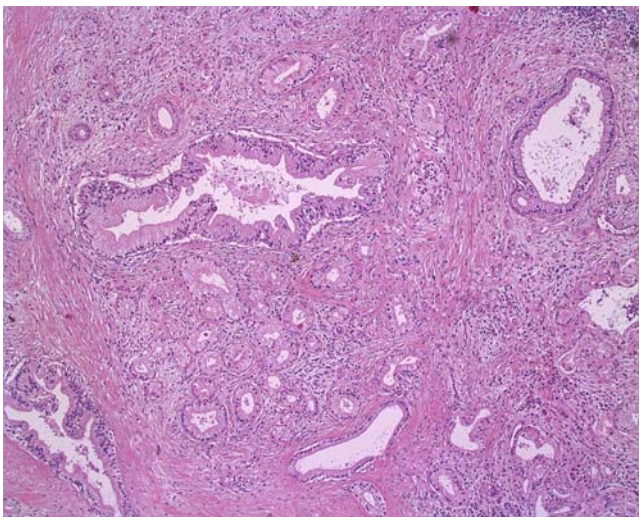

Fig. 3 PanIN-1 lesion associated with lobular fibrosis

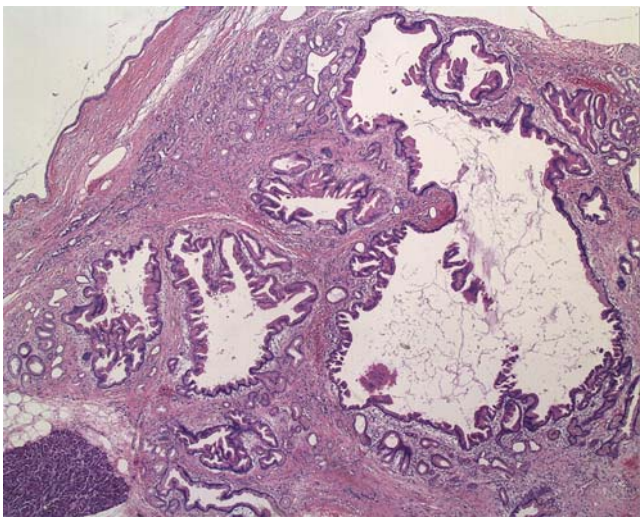

Fig. 4 Small multicystic duct-associated lesion in the periphery of pancreatic tissue suggestive of an IPMN of the gastric type but difficult to distinguish from a large PanIN-1 lesion

The oncocytic type of IPMN (also called intraductal oncocytic papillary neoplasm [2]) shows the same complex papillae as the pancreatobiliary type, but the lining cells reveal strongly eosinophilic cytoplasm. In addition, there are often numerous goblet cells. The tumor cells express MUC1 and MUC2 inconsistently (Fig. 1e,f). With fewer than 20 cases reported in the literature to date [2, 20, 34, 35, 37, 38, 42], the clinical and pathological behavior of this type is still unclear. Most of the cases (94%) were diagnosed as carcinoma, some of them with an invasive component or even distant metastases [37]. As the follow-up in this patient group is very short, no relevant data are available yet on survival and outcome.

The gastric type of IPMN exhibits papillary projections lined by epithelial cells resembling gastric foveolar cells and shows pyloric gland-like structures at the base of the papillae. These cells express MUC5 (Fig. 1g,h), while MUC1, MUC2, or CDX2 positivity is only occasionally observed. The gastric type of IPMN corresponds to the branch duct type, which occurs in the periphery of the pancreatic parenchyma, most often in the uncinate process, where it usually presents as a multicystic lesion with cysts no larger than 3 cm [9]. The gastric type seems to be less aggressive, i.e., less invasive, than the other IPMN subtypes [10, 25, 33, 46] but may show severe cellular atypia in a few cases (up to 25%) [46] (Table 3, Fig. 2) The size of the lesion was unrelated to the grade of cellular atypia [46].

Interestingly, pancreatic intraepithelial neoplasia (PanIN)-like complexes are frequently observed next to gastric type IPMNs. This raises the question whether IPMNs of the gastric type are a focal accentuation of a diffuse disease rather than a localized lesion. They might therefore also be related to the small peripheral cystic changes described by Kimura et al. [21] in non-neoplastic pancreata or the patchy lobular fibrosis associated with PanIN-1B lesions described by Detlefsen et al. [14] (Fig. 3). If this were the case, IPMNs of the gastric type would actually be large PanIN-1 lesions. An argument for this assumption is that both IPMNs of the gastric type and PanIN-1 lesions stain for MUC5 in the absence of MUC1 and MUC2 positivity. This assumption would also explain why it is difficult to distinguish PanIN lesions from some IPMNs [27] (Fig. 4), despite a consensus definition of both lesions [19].

Although the malignant potential of IPMNs of the gastric type seems to be rather low, it has to be pointed out that the fibrocystic changes that have been described in pancreata removed from patients with a strong family history of pancreatic cancer [11, 30] are similar, if not identical, to IPMNs of the gastric type and their associated PanIN lesions. This implies that IPMNs of the gastric type/PanIN-1 lesions are not innocuous lesions but have a malignant potential.

Summary and perspectives

The significance of IPMNs among the pancreatic tumors has increased greatly in recent years because of their improved recognition, both clinically and histopathologically and their much better prognosis than ordinary ductal adenocarcinomas. Moreover, they appear to fall into four subtypes that have special biological properties with prognostic implications. Of particular interest in relation to the development of ductal adenocarcinomas is the fact that the so-called gastric type IPMNs seems to occur in pancreata from patients with a strong family history of pancreatic cancer. Furthermore, it is of interest that the pancreatic IPMNs have their counterparts in IPMNs of the biliary duct system, where the same subtypes may occur [1, 23, 51, 52]. The treatment of choice is resection, but future trials may reveal that the extent of resection could depend on the IPMN subtype.

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REVIEW AND PERSPECTIVE

Lymphatic vessels are present in phosphaturic mesenchymal tumours

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Abstract Oncogenic osteomalacia (OO) is an acquired form of hypophosphataemic osteomalacia, which is associated most commonly with the development of a benign phosphaturic mesenchymal tumour mixed connective tissue type (PMTMCT). PMTMCTs are generally well vascularised tumours, and many have in the past been classified as haemangiomas and haemangiopericytomas. Although these tumours show some morphological variation, it has been proposed that they represent a distinct histopathological entity. Our aim in this study was to determine by immunohistochemistry the vascular profile of PMTMCT. Using monoclonal antibodies directed against several vascular markers, including the lymphatic endothelial cell antigens LYVE 1 and podoplanin, we found that PMTMCTs, in contrast to haemangiomas and haemangiopericytomas, contain lymphatic vessels. Taken with previous observations that PMTMCTs over express FGF23 and other gene products, this finding provides further evidence that most osteomalacia associated mesenchymal tumours represent a discrete pathological entity.

Keywords Phosphaturic mesenchymal tumour · Lymphatics · Vascular tumour · Osteomalacia

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Introduction

Oncogenic osteomalacia (OO) is a rare syndrome characterised by hypophosphataemia that occurs in association with a number of tumours, particularly mesenchymal tumours of soft tissue and bone [5, 21, 22]. In this syndrome, there is increased phosphate excretion, decreased plasma 1,25 dihydroxy vitamin D_3 levels and osteomalacia, which does not respond to vitamin D therapy. These biochemical and clinical abnormalities resolve when the tumour, which may be very small, is resected [5, 7].

OO may occur at any age and has been reported to occur in association with a variety of different tumour types. Most cases are associated with mesenchymal neoplasms of soft tissue or bone, but a few cases have been reported in association with other tumours, such as carcinomas and lymphomas, as well as in association with neurofibromatosis, polyostotic fibrous dysplasia and epidermal naevus syndrome [1, 7, 10, 13, 14]. The various types of mesenchymal tumour associated with OO have been categorised into four distinct morphological patterns [22]: (a) a primitive appearing mixed connective tissue tumour, (b) an osteoblastoma-like tumour, (c) a non ossifying fibroma-like tumour and (d) an ossifying fibroma-like tumour. The most common of these tumour types is the mixed connective tissue variant, which is generally designated as phosphaturic mesenchymal tumour, mixed connective tissue type

(PMTMCT). OO-associated mesenchymal tumours have common histopathological features and have been shown to over-express fibroblast growth factor 23 (FGF23), a protein that inhibits renal tubular epithelial phosphate transport [8, 9]; on this basis, it has been proposed that these tumours constitute a single pathological entity.

Although malignant tumours associated with OO have been reported, most PMTMCTs are benign tumours [7]. PMTMCTs exhibit some morphological variation but generally contain a proliferation of spindle shaped mesenchymal cells that lie in a well vascularised connective tissue matrix; this matrix may contain areas of calcification, cartilage and bone formation as well as scattered osteoclast like giant cells [21, 22]. The vascular component of PMTMCTs is often very prominent, and many of these lesions have in the past been classified as a type of vascular tumour, usually a haemangiopericytoma or some benign form of haemangioma [7].

To examine whether the vascular profile of PMTMCT is unique, we analysed the antigenic phenotype of endothelial cells in eight cases of PMTMCT and compared it with that of haemangiomas and haemangiopericytomas. As it has previously been shown that lymphatic vessels are not typically found in haemangiopericytomas and (capillary, cavernous or epithelioid) haemangiomas [23], we were particularly interested in determining whether PMTMCTs contain lymphatic vessels, which we identified in this study by expression of two highly specific lymphatic endothelial cell markers, LYVE 1, a novel homologue of the CD44 glycoprotein, which is a receptor for hyaluronan [3] and podoplanin, a heavily O glycosylated glycoprotein that plays a role in cell and matrix adhesion [4].

Materials and methods

Eight cases of PMTMCT, five cases of capillary haemangioma, five cases of cavernous haemangioma and five cases of haemangiopericytoma were obtained from the archives of the Histopathology Departments of the Nuffield Orthopaedic Centre, Oxford, the Royal National Orthopaedic Hospital, London, and Emory University, Atlanta. Clinical details of the PMTMCT cases are shown in Table 1; four of these cases have previously been reported and were found to be FGF23 positive by immunohistochemistry [8]; the remaining four cases had elevated serum FGF23 levels. Paraffin embedded sections (5 μm) were cut onto silane-coated or charged slides and incubated at 37°C for 24 hours before staining.

All immunohistochemical staining was performed using an indirect immunoperoxidase technique (ChemMate Envision, Dako, UK). Tissue sections were dewaxed and re hydrated by successive immersion in xylene, graded ethanol and water. Antigen retrieval was performed by microwave treatment (700 W, 2×4 min) in Target Retrieval Solution (Dako). Endogenous peroxidase was blocked by 0.2% (*v*/*v*) hydrogen peroxide in 80% ethanol and protein block serum before 30-min incubation with a mouse anti-human LYVE 1 monoclonal antibody, which was generated against a soluble LYVE 1 Fc fusion protein [3]. Sections were also stained with a monoclonal antibody to the lymphatic endothelial cell marker, podoplanin [4]. Tumour sections where also stained immunohistochemically against the blood vessel endothelial cell markers, Factor VIII related antigen/von Willebrand factor (vWf), CD31 and CD34 using respectively a polyclonal antibody to vWf and the monoclonal antibodies JC70 and QBend10 (Dako). Antigens were detected by incubation with labelled polymer and diaminobenzidine (Dako). The sections were then counterstained with haematoxylin, dehydrated, cleared and mounted.

Normal sections of skin and lymph node were used as positive control tissue; these contained both lymphatic and blood vessels. Negative controls consisted of sections stained with primary antibody diluent alone or substitution of an appropriate irrelevant antibody for the tumour under investigation [e.g. MNF116 anti-cytokeratin antibody (Dako) for primary neoplasms and non-neoplastic lesions of bone].

Table 1 TMCT cases analysed in this study

Case no.	Age (years)/ sex	Site	LYVE 1/podoplanin staining
1	53 Female	Foot	+/+
2	60 Female	Femur	+/+
3	54 Male	Groin	+/+
4	48 Male	Thigh	+/+
5	58 Male	Popliteal fossa	+/+
6	52 Male	Bone	+/+
7	30 Female	Ilium	−/−
8	37 Female	Wrist	+/+

Results

All eight cases of PMTMCT showed a number of common histological features. The tumours were composed of a proliferation of spindle shaped tumour cells, which showed minimal to moderate nuclear pleomorphism and little or no mitotic activity (Fig. 1). All the tumours were highly vascular; there were numerous prominent thick or thin-walled vessels, some of which were dilated and had a haemangiopericytoma like appearance. Tumour cells lay in a fibrous matrix, which focally showed a variable degree of

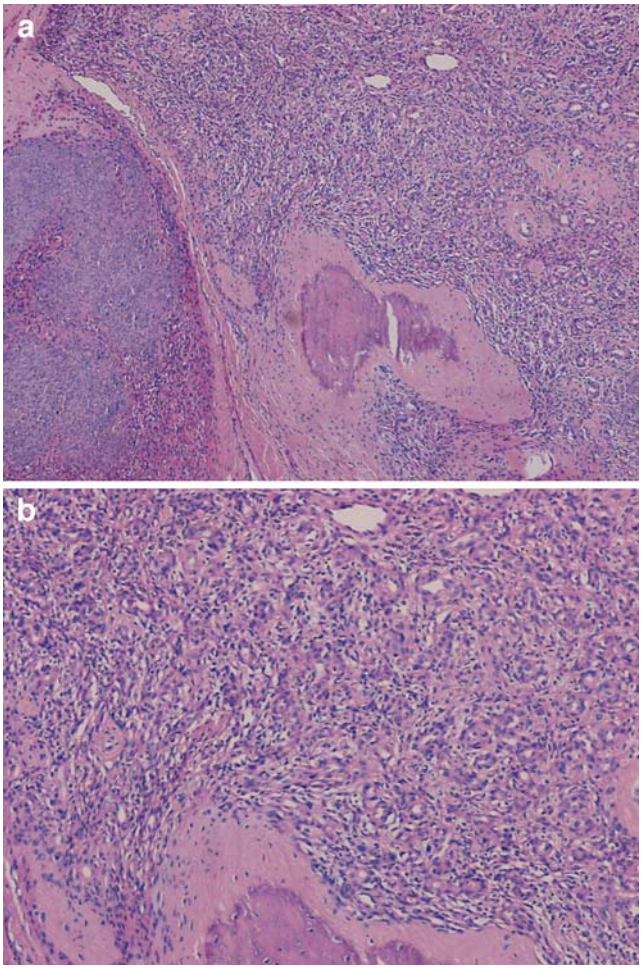

Fig. 1 **a** Low and **b** high-power photomicrographs of a representative PMTMCT of soft tissues showing a well-vascularised spindle cell tumour in which there is focal ossification. The bone trabeculae have a prominent osteoid seam

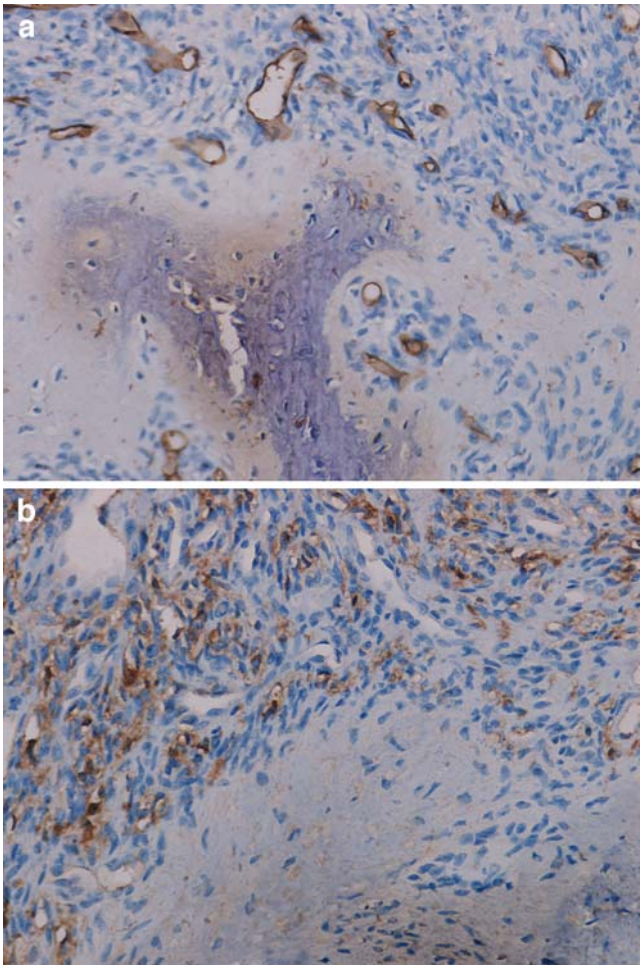

Fig. 2 **a** LYVE-1 staining of endothelial cells lining lymphatic vessels and **b** CD34 staining of endothelial cells lining vascular channels in a PMTMCT of soft tissues in which there is focal ossification

mucinous or myxoid degeneration. Some tumour contained focal areas of ossification with formation of bone trabeculae, some of which were covered by wide osteoid seams (Fig. 1).

The five cases of soft tissue PMTMCT contained numerous vessels, which were lined by endothelial cells that stained for CD34, CD31, and vWf. These tumours also contained scattered LYVE 1^+/podoplanin$^+$ lymphatic vessels (Fig. 2); these vessels also reacted variably for CD34, CD31 and vWf. Spindle shaped mesenchymal tumour cells, giant cells and other cells not lining vascular spaces did not express LYVE 1 or podoplanin.

The number of LYVE 1^+/podoplanin$^+$ lymphatic vessels in the PMTMCTs examined varied depending on the degree of vascularity seen in these tumours. In general, the more vascular the PMTMCT, the more lymphatics were observed. LYVE 1^+/podoplanin$^+$ lymphatic vessels were most numerous in two haemangioma-like lesions, which contained many capillary sized blood vessels as well as scattered dilated vascular spaces lined by endothelial cells which were LYVE 1^-/podoplanin$^-$ but vWf$^+$, CD31$^+$ and CD34$^+$. Examination of serial sections revealed that some LYVE 1^+/podoplanin$^+$ endothelial cells also expressed vWf, CD31 and CD34 to a variable extent. The remaining three soft tissue PMTMCTs contained mainly a proliferation of spindle shaped mesenchymal tumour cells amongst which were scattered small capillary sized vessels, including some lined by LYVE 1^+/podoplanin$^+$ endothelial cells; these vascular channels did not contain red cells and stained for CD34 as well as variably for CD31 and vWf.

Three cases of PMTMCT arose in bone. Two of the tumours contained capillary sized and larger thin walled vessels, which did not contain red cells, and were lined by LYVE 1^+/podoplanin$^+$ endothelial cells (Fig. 3). These tumours contained focal areas of bone formation and dystrophic calcification associated with the presence of reactive osteoclast like giant cells. Endothelial cells lining the vascular channels containing red cells were positive for CD34 and showed variable staining for CD31 and vWf. The remaining bone PMTMCT was a spindle cell tumour in

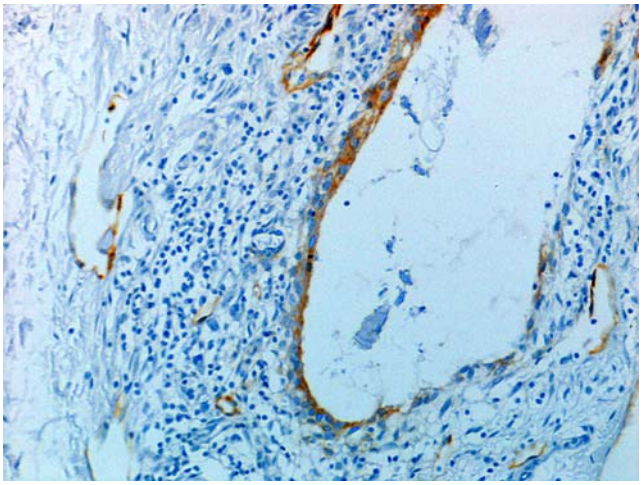

Fig. 3 Podoplanin staining of lymphatic endothelial cells lining a dilated lymphatic vessel in a PMTMCT of bone

which there were scattered small vessels that were $CD34^+$, $CD31^+$ and vWf^+ but LYVE 1^-/$podoplanin^-$.

In positive control lymph node and skin tissues, endothelial cells lining lymphatic vessels were positive for LYVE 1 and podoplanin; these vessels showed variable staining for CD31, CD34 and vWf. Endothelial cells lining blood vessels also expressed CD31, CD34 and vWf. These antigens were also expressed by endothelial cells in capillary and cavernous haemangiomas, as well as haemangiopericytomas; there was no staining of vessels for LVYE 1 or podoplanin in these lesions.

Discussion

Identification of lymphatic endothelial cell markers has led to a re-assessment of the histogenesis of several tumours, such as Kaposi's sarcoma and angiosarcoma [4, 23]. In this study, we have shown that, in contrast to capillary/cavernous haemangiomas and haemangiopericytomas [23], most PMTMCTs contain lymphatic vessels as demonstrated by expression of the specific lymphatic endothelial cell markers, LYVE 1 and podoplanin.

PMTMCT is the most common type of osteomalacia associated tumour, which occurs in soft tissues [7, 22]. This tumour behaves in a benign fashion (although malignant forms have been reported) and is often small, slow growing and difficult to localise. It is characterised by the presence of variable numbers of primitive appearing mesenchymal stromal cells that lie in a well vascularised connective tissue stroma that may contain scattered osteoclast like giant cells. It is thought that mesenchymal cells in these tumours are the source of the hormonal factor that causes the osteomalacia. Immunohistochemical studies have not found evidence of epithelial, neural or neuroendocrine differentiation in the mesenchymal cells of this tumour, and in the present study, we found that PMTMCT mesenchymal cells did not express lymphatic endothelial cell markers. The vascularity of OO-associated PMTMCTs is often very prominent, and on this basis, many of these lesions have in the past been categorised as a type of vascular tumour [7, 21, 22]. Most of the vascular elements in the eight PMTMCTs studied in our series were lined by blood vessel endothelial cells that expressed CD31, CD34 and vWf; these antigens are expressed by lining endothelial cells in benign and malignant vascular tumours (including haemangiomas and haemangiopericytomas) [23]. CD31, CD34 and vWf are also known to be expressed by lymphatic endothelial cells [11, 16, 20], and we found that LYVE 1^+/$podoplanin^+$ lymphatic vessels in PMTMCTs also expressed these antigens. Our finding of lymphatic vessels expressing these antigens in most PMTMCTs shows that these tumours have a distinct vascular profile, which distinguishes them from morphologically similar vasoproliferative tumours; this observation is in keeping with the hypothesis that PMTMCTs represent a discrete pathological entity.

FGF23, a gene known to be mutated in autosomal dominant hypophosphataemic rickets, is abundantly expressed in PMTMCT as shown by protein and mRNA expression. Increased levels of FGF23 have been found in patients with OO and polyostotic fibrous dysplasia [15, 19] and were noted in four of the PMTMCT cases we studied. Although the precise relationship between FGF23 expression in PMTMCTs and OO is not certain, many (but not all) of these tumours are clearly distinguished by over expression of this gene product. FGF23 is expressed in mesenchymal tumour cells and not in endothelial cells in PMTMCTs [8]. FGF23 is degraded by PHEX, a membrane - bound endopeptidase mutated in X linked hypophosphataemia. It has been suggested that OO-associated mesenchymal tumours may produce sufficient FGF23 to overwhelm native PHEX levels. The precise role of FGF23 in the pathogenesis of OO is uncertain, but most FGFs are potent stimulators of angiogenesis in vitro and in vivo [2]. It is not known if the presence of lymphatic vessels in PMTMCT is associated with over expression of FGF23, but several FGFs or their receptors are known to influence lymphangiogenesis. Fibroblast growth factor receptor 3 (FGFR3) expression is upregulated in lymphatic endothelial cells by PROX1, a transcription factor which is essential for the development of the lymphatic system [18]. FGF1 and FGF2 have also been shown to promote the proliferation, migration and survival of lymphatic endothelial cells in vitro [6, 12].

In addition to FGF23, tumours that cause OO are distinguished from other mesenchymal tumours by expression of a number of gene products, which are related to bone matrix formation (DMP 1 and fibronectin), mineralisation (MET) and mineral ion transport (Pit 1/Glvr 1 and Ank A)

[6]. Some OO-associated tumours also show increased expression of several genes involved in tumourigenesis and/or inflammation, including factors associated with matrix adhesion such as CD44 and osteopontin [6, 17]. Expression of these factors is of interest as both LYVE 1 and podoplanin play a role in cell/matrix adhesion. LYVE 1 is a well characterised homologue of the CD44 glycoprotein, which acts as a receptor for cell/matrix hyaluronan [3]. It is possible that in PMTMCTs mesenchymal tumour cells over express a number of gene products, which promote the generation of matrix components that induce the proliferation of lymphatic vessels, a feature which distinguishes these tumours from typical haemangiomas and haemangiopericytomas.

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ORIGINAL ARTICLE

KPNA2 protein expression in invasive breast carcinoma and matched peritumoral ductal carcinoma in situ

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Abstract The aim of this study was to evaluate protein expression of Karyopherin alpha 2 (KPNA2) in invasive breast cancer and matched ductal carcinoma in situ (DCIS) and to correlate it with clinicopathological data, including patient survival. KPNA2 protein expression was assessed by immunohistochemistry in breast tissue samples, containing invasive carcinomas, DCIS, and adjacent histologically benign breast tissues. A polyclonal goat KPNA2 antibody was used for immunostaining of 83 clinicopathologically characterized cases. For statistical analysis, staining of at least 10% of nuclei was considered KPNA2 positive. Immunohistochemical detection of KPNA2 in invasive carcinoma showed a significant correlation with higher tumor stage, positive lymph node status, higher tumor grade, and negative ER status. Concordantly, KPNA2-positive tumors (31.3%) showed significantly shorter disease-free survival times (69 months vs 118 months; $p=0.007$). KPNA2 protein expression was also detected in DCIS (21.3%) adjacent to invasive tumor and correlated with nuclear grade ($p=0.013$). Expression of KPNA2 in invasive breast cancer correlates with conventional prognostic parameters and shorter disease-free survival. Additionally, KPNA2 is overexpressed in DCIS, particularly high grade lesions, which emphasizes its potential role in carcinogenesis of invasive breast carcinomas.

Keywords Breast cancer · DCIS · KPNA2 · Prognostic marker · Immunohistochemistry

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Introduction

Breast cancer is the most common malignant neoplasm of women in the Western world, with an estimated number of 178,480 new cases of breast cancer among US women in 2007 [4]. Recent diagnostic and therapeutic advances are encouraging, but at the same time, increasingly detailed prognostic information is required, which should exceed conventional prognostic parameters, i.e., age, nodal status, tumor size, tumor grade, tumor type, and receptor status. This condition seems to be met by several promising molecular markers like Kallikrein 5 [20], urokinase plasminogen activator and its inhibitor [9], tissue inhibitor of metalloproteinase 1 [10], Ep-CAM [17], osteopontin [12],

CD24 [8], SFRP1 [7], survivin [13, 16], and Karyopherin alpha 2 (KPNA2) [2, 15, 19].

Several gene expression profiling studies demonstrated overexpression of KPNA2, a member of the karyopherin alpha protein family, in breast cancer tissue [2, 15, 19]. Proteins of the karyopherin alpha family play a central role in nucleocytoplasmic transport. They act as an adaptor in the nuclear import of macromolecules by binding cargoproteins, which contain a classical nuclear localization signal, thereby linking them to the transport protein karyopherin beta. A strong nuclear KPNA2 immunoreactivity was observed in breast cancer cells compared to a weak or absent staining in normal breast tissue by Dahl et al. [2]. They also demonstrated an independent negative correlation between nuclear KPNA2 protein expression in the primary tumor and overall survival of breast cancer patients.

So far, no data on KPNA2 protein expression in preinvasive lesions of the breast are available. We, therefore, aimed to investigate the expression of KPNA2 immunohistochemically in a cohort of clinically characterized breast cancer samples and matched peritumoral ductal carcinoma in situ (DCIS) to verify the prognostic properties in invasive cancer and to focus on KPNA2 expression in adjacent DCIS.

Materials and methods

Patients

The study included 83 patients with breast cancer, diagnosed at the Institute of Pathology, University Hospital Charité, Berlin, between 1991 and 1997. Patient age at the time of diagnosis ranged from 30 to 80 with a mean of 58 years. Clinical follow-up data, including overall survival and disease-free survival, were available for all cases. The median observation time for overall survival was 78 months for patients still alive at the time of analysis (range 3–162 months). Fourteen patients (16.9%) died during follow-up, and 31 patients (37.3%) experienced disease progression, defined by either metastatic disease or local recurrence.

The selection of cases for this study was based on availability of tissues. Patients with systemic disease (pM1) at the time of diagnosis were excluded. Histological typing of tumors was carried out according to the criteria of the World Health Organization. Tumor stage was determined according to the guidelines of the UICC [14]. Tumors were graded according to Bloom and Richardson in the modification of Elston and Ellis [3]. Data regarding the estrogen receptor (ER) status, the expression of Her-2/neu (c-erbB2), and the proliferative fraction (Mib-1) were gathered from the archival pathology reports. The clinicopathological data of the cases are described in Table 1.

Table 1 Clinicopathological characteristics of primary breast carcinomas

Variable	No. (%)
Patient age	
<60 years	47 (56.6)
≥60 years	36 (43.4)
pT status	
pT1	51 (61.4)
pT2	24 (28.9)
pT3	6 (7.2)
pT4	2 (2.4)
pN status	
pN0	34 (41.0)
pN1	21 (25.3)
pN2	14 (16.9)
pN3	13 (15.7)
Histological grade	
G1	19 (22.9)
G2	40 (48.2)
G3	24 (28.9)
Histology	
Ductal	78 (94)
Lobular	5 (6)
Estrogen receptor	
Negative	19 (22.9)
Positive	55 (66.3)
Unknown	9 (10.8)
HER2 expression	
0/1+	45 (54.2)
2+/3+	19 (22.9)
Unknown	19 (22.9)

Immunohistochemistry

Immunohistochemistry (IHC) staining was performed using formalin-fixed, paraffin-embedded archival tissues. After sectioning, tissue sections were applied to slides, deparaffinized with xylene, and gradually rehydrated. Antigen retrieval was performed using a microwave oven (10 min at 250 W). The primary antibody anti-KPNA2 (goat polyclonal SC6917, Santa Cruz Biotechnology; dilution 1:200) was incubated at room temperature for 1 h. Detection was carried out using an avidin–biotin peroxidase method with 3,3′-diaminobenzidine as chromogen (ChemMate detection kit, DAKO, Glostrup, Denmark). Brief nuclear counterstaining with hematoxylin completed the procedure.

While normal testicular tissue served as a positive control for KPNA2 IHC, unspecific binding of the secondary antibody was excluded by omitting the primary antibody.

Evaluation

Evaluation of the immunohistochemical stainings was independently carried out by two pathologists who were

unaware of the outcome of the disease. Areas of invasive carcinoma, DCIS, and adjacent histologically benign breast tissue were considered separately. KNPA2 positivity was defined as strong nuclear staining in at least 10% of cells.

Statistical analysis

Data were analyzed using the software package SPSS, version 13.0. Spearman's rank correlation was calculated, and Fisher's exact test and χ^2 test were applied to evaluate the statistical significance of association between expression of KPNA2 and clinicopathological variables.

For univariate survival analysis, cumulative survival curves were calculated according to the Kaplan–Meier method. Differences in survival were assessed with the log-rank test. The impact of KPNA2 positivity, patient age, histologic tumor type, pT status, pN status, tumor grade, ER status, and cerbB2 status on overall survival time and disease-free survival time was analyzed.

Results

KPNA2 immunostaining showed a predominantly nuclear staining pattern, restricted to epithelial cells of normal breast glands, DCIS, and invasive breast cancer (Fig. 1). Additionally, a weak-to-moderate cytoplasmic staining was seen in some cases, but was not subjected to further analysis. Connective tissue cells, blood vessels, and inflammatory cells did not display KPNA2 immunoreactivity.

KPNA2 immunostaining in normal breast tissue

Normal breast tissue adjacent to tumor was present and evaluable in 78 (94%) patients. Nuclear staining was only seen in ten (12.0%) samples. As the stained nuclei never exceeded 1%, all samples were considered KPNA2 negative.

KPNA2 immunostaining in intraductal carcinoma

Seventy-five (90.4%) invasive breast cancer specimens contained peritumoral DCIS, of which 40 cases (53.3%) were of non-high grade and 35 (46.7%) were of high grade. Staining of KPNA2 in at least 10% of nuclei was detected in 16 samples (21.3%), whereas 59 samples (78.7%) were KPNA2 negative. High-grade DCIS displayed KPNA2 staining in at least 10% of nuclei significantly more frequently (n=12, 34.3%) than non-high-grade DCIS (n=4, 10.0%; p=0.013). According to this, 23 samples of high-grade DCIS (65.7%) and 36 samples of non-high-grade DCIS (90.0%) were KPNA2 negative. KPNA2 immunoreactivity in DCIS correlated with KPNA2 expression in adjacent invasive carcinomas (cc=0.725; p<0.001).

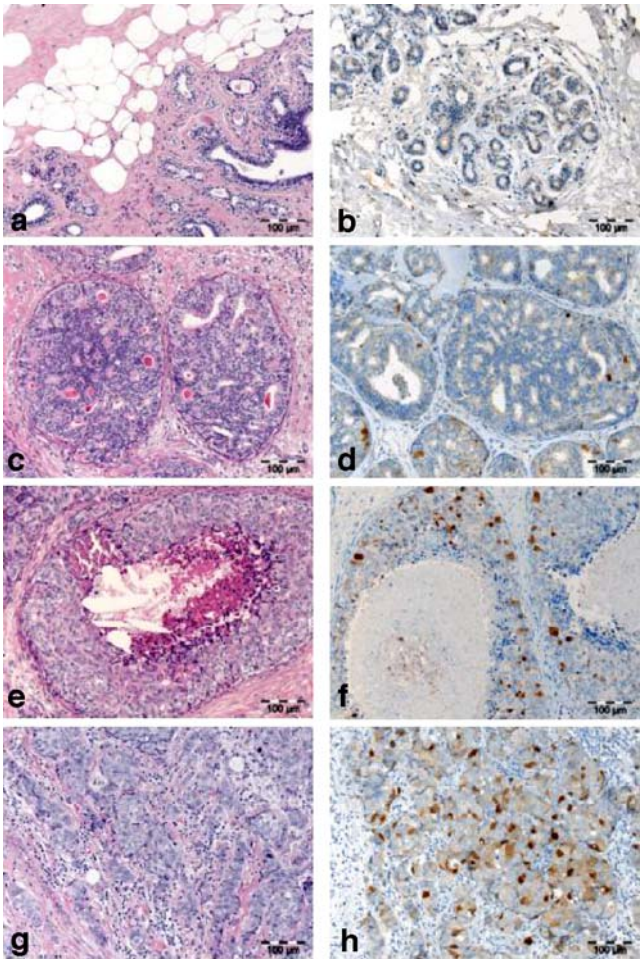

Fig. 1 Hematoxylin and eosin staining and KPNA2 immunohistochemistry. **a** Normal glandular breast tissue (HE). **b** Normal glandular breast tissue displaying luminal epithelium with only single KPNA2-positive nucleus and weak-to-moderate cytoplasmic staining. **c** Non-high-grade DCIS (HE). **d** Few KPNA2-positive nuclei and weak cytoplasmic staining in non-high-grade DCIS. **e** High-grade DCIS (HE). **f** High-grade DCIS with markedly increased nuclear KPNA2 staining. **g** Invasive ductal carcinoma (HE). **h** Strong nuclear KPNA2 staining and moderate cytoplasmic staining in invasive ductal carcinoma. **a**–**h** Original magnification ×200

KPNA2 immunostaining in invasive breast carcinomas

KPNA2-positive immunostaining was seen in 26 (31.3%) invasive carcinomas, the median percentage of positive nuclei being 15%, with a maximum of 40%. KPNA2 positivity of invasive carcinoma revealed a statistically significant correlation with higher pT status, nodal status, histological grade, and a negative ER status (Table 2).

KPNA2 immunoreactivity in invasive carcinomas and univariate survival analysis

Patients with KPNA2-positive breast carcinomas (≥10%) had an estimated mean disease-free survival time of 69 months (95% confidence interval, 47–92 months)

Table 2 Correlation of KPNA2 immunoreactivity in invasive breast cancer with conventional clinical or tumor parameters

KPNA2	DCIS	pT status	pN status	Grading	ER status	HER2
Correlation coefficient	0.725	0.321	0.390	0.470	−0.316	0.148
Significance (two-sided)	0.000	0.003	0.000	0.000	0.006	0.244
N	75	83	82	83	74	64

compared to 118 months (95% confidence interval, 100–135 months) in patients with negative KPNA2 staining (<10%; $p=0.007$; Fig. 2). For overall survival, no significant differences were noted (data not shown).

Discussion

Nucleocytoplasmic transport mechanisms have been the target of numerous studies for they are involved in many cellular processes, such as gene expression, cell-cycle progression, and signal transduction [1]. Regulating the subcellular distribution of macromolecules, nuclear transport mechanisms may also control their functions. There is increasing evidence that these mechanisms may contribute to malignant cell transformation, which makes their elements interesting targets for further evaluation [11]. One of these elements is KPNA2, an adaptor protein, which mediates the nuclear import of macromolecules with a classical nuclear localization signal. Linking a cargoprotein and Karyopherin beta, KPNA2 initiates the transport through the nuclear core complex. Thakur et al. [18] demonstrated that one of the proteins whose subcellular distribution may be influenced by KPNA2 is BRCA1, thereby connecting KPNA2 expression with breast carcinogenesis.

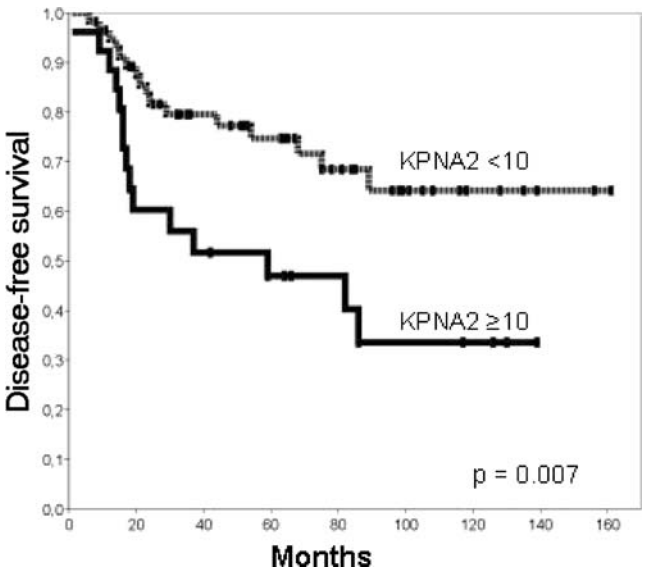

Fig. 2 Survival analysis. Kaplan–Meyer curves regarding disease-free survival of patients with KPNA2 expression in less than 10% of nuclei (*dashed line*) vs patients with KPNA2 expression in at least 10% of nuclei in invasive carcinomas (*bold line*)

Another cargoprotein of KPNA is TP53. Kim et al. [5] identified a truncated form of KPNA in a breast cancer cell line and showed that overexpression of this truncated form results in cytoplasmic accumulation of TP53, whereas overexpression of intact KPNA leads to a transactivation of TP53-responsive genes in the nucleus.

Several gene expression profiling studies reported overexpression of KPNA2 in breast cancer [2, 15, 19]. Sotiriou et al. [15] found an association of KPNA2 overexpression with higher histologic tumor grade, which matches our findings on protein level. Dahl et al. were the first to evaluate KPNA2 protein expression in breast cancer and observed a strong nuclear immunohistochemical staining in breast cancer cells compared to a weak or absent staining in normal breast tissues. They also demonstrated an independent negative association between KPNA2 expression in the primary tumor and overall survival in breast cancer patients which was particularly valid in the group of node-positive cases. Importantly, KPNA2 expression appears to be characteristic of the basal-like subtype of breast cancers, which has an immediate impact on adjuvant therapy planning [2].

The present study confirms our former results in an independent and well-characterized breast cancer cohort, using the cutoff value applied by Dahl et al. [2]. KPNA2 expression in at least 10% of nuclei was seen in 31% of invasive carcinomas, whereas none of the matched normal tissues was KPNA2 positive. Nuclear KPNA2 staining was significantly correlated with higher tumor stage, lymph node status, higher tumor grade, and negative ER status. Consistent with the results reported by Dahl et al., survival analysis revealed that patients with KPNA2-positive breast carcinomas had significantly shorter disease-free survival times, which clearly validates the prognostic value of KPNA2 in breast cancer.

Further investigation of KPNA2 expression in preinvasive lesions of the breast appears mandatory to establish the time point of KPNA2 upregulation in the multistep process of mammary carcinogenesis. To our knowledge, the present study includes the first description of KPNA2 protein expression in matched DCIS and invasive lesions of the breast. Staining of KPNA2 in at least 10% of nuclei was detected in 21% of patients with DCIS. KPNA2 immunoreactivity in DCIS and invasive carcinomas correlated significantly. The portion of KPNA2-positive cases increased successively, comparing adjacent benign tissues (0%), DCIS (21.3%), and invasive carcinomas (31%). This indicates, that upregulation of KPNA2 may be an early

event in carcinogenesis, which is also assumed by Klein et al. [6], who found KPNA2 upregulated early in a mouse model of breast cancer. This hypothesis is corroborated by the fact that samples showing positive KPNA2 staining in at least 10% of nuclei were significantly less frequent in the group of low-grade DCIS (10%) compared to those cases displaying adjacent high-grade DCIS (34.3%). The portion of KPNA2-positive cases in the latter group was almost equivalent to the portion of KPNA2-positive invasive carcinomas, which suggests that KPNA2 overexpression is preceding invasion. Clearly, further studies are needed to clarify the functional role of KPNA2 in breast carcinogenesis.

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ORIGINAL ARTICLE

Flat DIN 1 (flat epithelial atypia) on core needle biopsy: 63 cases identified retrospectively among 1,751 core biopsies performed over an 8-year period (1992–1999)

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Abstract Uniform management of flat DIN 1 (flat epithelial atypia) on core needle biopsy (CNB) concerning surgical excision or clinical follow-up are lacking. In a retrospective review of 1,751 CNB over an 8-year period, we found 63 cases with flat DIN 1 as the most advanced lesion; follow-up was available in 55 cases. Of the 63 patients, 24 had a subsequent biopsy for 15 days to 10 years after the initial CNB, an infiltrating carcinoma was found in nine (14.3%) patients, seven (11.1%) in the ipsilateral, and two (3.2%) in the contralateral breast. Five underwent an excisional biopsy of the ipsilateral breast within less than 3 months of the initial CNB; none had either an invasive or intraepithelial carcinoma. Based on our findings, we consider flat DIN 1 a marker of slightly increased risk for subsequent development of invasive breast carcinoma. When flat DIN 1 is found on CNB as the most advanced lesion after mammographic correlation, an excisional biopsy is not mandatory; however, close follow-up is advised with repeat mammograms for early detection of any clinically occult carcinoma in the vicinity of flat DIN 1 that may have been missed by the CNB.

Keywords Flat epithelial atypia · Flat DIN 1 · Core needle biopsy · Breast carcinoma · Ductal intraepithelial neoplasia

Introduction

Flat epithelial atypia is an intraductal alteration characterized by replacement of the native epithelial cells by a single or three to five layers of mildly atypical cells. The ducts involved are variably distended and often contain intraluminal microcalcifications or secretory material [33]. Azzopardi, using the term "Clinging carcinoma, monomorphous type," provided a thorough description of this lesion in 1979 [1]; however, the lesion remained widely unrecognized and/or ignored, until recent years when its neoplastic nature was confirmed at the molecular level [18]. Because of the often subtle cytologic atypia and absence of architectural alterations, it can be easily mistaken for normal breast tissue and has been misinterpreted as a component of fibrocystic changes, as blunt duct adenosis and normal breast for many years. Over the past 5–6 years, there has been a surge of interest in flat epithelial atypia with the increasing frequency of its detection in breast biopsies performed for mammographically detected microcalcifications. A number of reports have referred to this lesion by a wide variety of terms including columnar alteration with prominent apical snouts and secretion [10], atypical cystic lobules [14, 23], columnar cell change with atypia, and columnar cell hyperplasia with atypia [29]. Genetic evidence supports the idea that flat epithelial atypia represents one of the earliest recognizable neoplastic alterations in the breast [18, 30]. These genetic findings coupled with the frequent finding of this alteration as the milieau in which micropapillary and cribriform ductal carcinoma in situ (DCIS) develop further supports its inclusion in the ductal intraepithelial neoplasia (DIN) classification. The rationale and advantages in the use of the DIN classification have been previously stated [32, 36]. As we have used the DIN classification proposed by one of the authors, modifications to the DIN 1 group of lesions as described in the World Health Organization (WHO) 2003 classification [33] have been incorporated into the DIN classification (Table 1). The modified classification that we currently use applies the designation of flat DIN 1 for flat

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Table 1 DIN (ductal intraepithelial neoplasia) and traditional terminology in the classification of intraductal proliferative lesions

DIN classification (currently used by the authors)	DIN classification (2003 WHO) [33]	Traditional terminology [33]
Low risk DIN	Usual ductal hyperplasia	Usual ductal hyperplasia
Flat DIN 1	DIN 1a	Flat epithelial atypia
DIN 1 (lesion ≤2 mm)	DIN1b	Atypical ductal hyperplasia (ADH)
DIN 1 (lesion >2 mm)	DIN 1c	DCIS grade 1
DIN 2	DIN 2	DCIS grade 2
DIN 3	DIN 3	DCIS grade 3

epithelial atypia. Despite the increasing recognition of this lesion, the clinical significance and management of flat DIN 1, particularly when found on core needle biopsy (CNB), are unknown, and uniform management guidelines for surgical excision or clinical follow-up are lacking. The current study presents the follow-up and frequency of subsequent invasive breast carcinoma among patients with pure flat DIN 1 discovered in a retrospective review of 1,751 CNB over an 8-year period.

Materials and methods

The files of the Yale University Department of Pathology were searched for breast CNB evaluated between January 1992 and December 1999. CNBs showing other than invasive carcinoma, DCIS, lobular intraepithelial neoplasia (LIN), and/or fibroadenomas were retrieved from the files, and all initial diagnostic hematoxylin-and-eosin-stained slides were reviewed. The 2003 WHO definition of flat epithelial atypia as a change "characterized by replacement of the native epithelial cells by a single layer of mildly atypical cells often with apical snouts, or proliferation of a monotonous atypical cell population in the form of stratification of uniform, cuboidal to columnar cells generally up to 3–5 cell layers with occasional mounding" was used [33]. Flat epithelial atypia, with even a single arcade or micropapillary formation, was considered as atypical ductal hyperplasia (ADH) and excluded.

Cases with pure flat DIN 1 as the most advanced lesion on CNB were included. Medical records were reviewed to verify the absence of any history of ipsilateral breast carcinoma or clinically evident malignancy at the time of biopsy. Follow-up was obtained from the medical records, and the pathology files were searched for subsequent surgical procedures, and hematoxylin-and-eosin-stained slides from subsequent breast biopsies were also retrieved and reviewed.

The number of cores/core fragments present in the initial CNB and the extent of flat DIN 1 was recorded. The cases were grouped according to the extent of flat DIN 1 present in the fragments examined, in those having less then five ductules (or only one terminal duct lobular unit [TDLUs]) involved, five to ten ductules (two TDLUs) involved, and greater than ten ductules (greater than two TDLUs) involved.

Results

A total of 1,751 CNB were performed and evaluated in the period selected. After excluding biopsies with the diagnosis of invasive carcinoma, DCIS, LIN, and/or fibroadenomas, a total of 736 CNB were retrieved and reviewed.

Among 736 CNB reviewed, 63 (8.5%) qualified as flat DIN 1 and met the inclusion criteria. The patients, all women, ranged in age from 39 to 75 with a median age of 52 years. The mammographic abnormalities leading to CNB included calcifications in 49 cases (76%) and density/mass in 14 cases (24%). Follow-up was available in 55 patients. Mean follow-up was 6.2 years (range 1–11 years; Figs. 1 and 2).

The average number of cores/fragments received for pathologic examination was 13 (range 4–26). In 14 (22%) cases, less than five ductules (only one TDLU) were involved, in 20 (32%) cases, five to ten, and in 29 (46%) cases, greater than ten ductules were involved.

Of the 63 patients, 24 had a subsequent breast biopsy (core or excision), 16 in the ipsilateral and four in the contralateral breast, and four in both the ipsilateral and contralateral breasts (Table 2). The interval between the initial CNB and subsequent biopsy was 15 days to 10 years. An infiltrating carcinoma was found in nine (14.3%) patients in a subsequent biopsy, seven (11.1%) in the ipsilateral breast, and two (3.2%) in the contralateral breast (Fig. 3). The mean interval between the initial CNB and the invasive carcinoma was 3.7 years (range 2–9 years) for the ipsilateral tumors. Both cases with contralateral tumor were found 7 years after the initial CNB. In one patient, DIN 2 (DCIS grade 2) was found in the contralateral breast 2 years after the initial CNB.

The mean age of the patients who developed subsequent invasive carcinoma in the ipsilateral breast was 53 years (range 39–63 years) at the time of diagnosis of flat DIN 1, and both women who developed invasive carcinoma in the contralateral breast were 51 years of age.

Among the seven patients who developed subsequent ipsilateral invasive carcinoma, five (71%) had greater than ten ductules (greater than two TDLUs) involved by flat DIN 1 in the initial CNB; the number of fragments in these cases was five in two cases and 7, 12, and 16 in the remaining three. In the other two patients (29%) who had

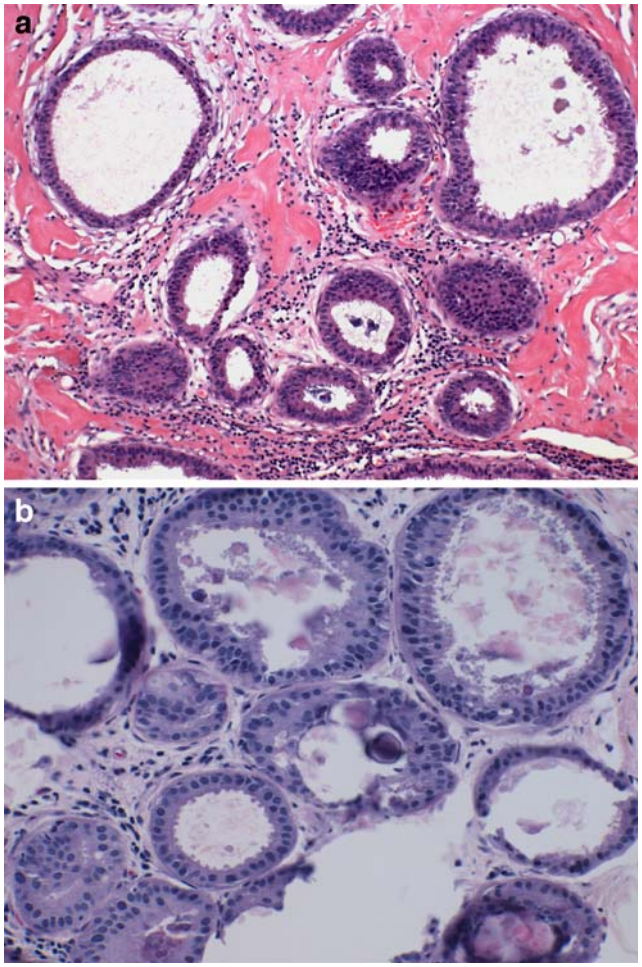

Fig. 1 **a** Case 32, flat DIN 1 on CNB, variably distended acini lined by one to three layers of monotonous cells. No evidence of breast carcinoma in 8 years of available follow-up. **b** Case 42, flat DIN 1 with microcalcifications, no evidence of breast carcinoma in 3 years of available follow-up

four and six core fragments for evaluation, less than five ductules (less than one TDLU) were involved.

All the subsequent ipsi and contralateral infiltrating carcinomas were of ductal type. Five were moderately differentiated and two well differentiated in the ipsilateral breast; in six of the seven cases, flat DIN 1 was present associated with the invasive carcinoma or in the surrounding tissue. Both contralateral invasive carcinomas were moderately differentiated; flat DIN 1 was present in the adjacent breast tissue in both cases. Flat DIN 1 was also present in the subsequent excision showing DIN 2 in the contralateral breast of a third patient.

Two of the seven patients who developed a subsequent ipsilateral invasive carcinoma (cases 15 and 20) had an "interval" biopsy showing DIN 1 (ADH) in between the initial CNB and subsequent invasive carcinoma. Case 15 had two subsequent excisional biopsies, 1 month and 3 years after the initial CNB with the last biopsy 5 years before the invasive carcinoma. Case 20 had a subsequent CNB followed by an excisional biopsy 2 years after the initial CNB and 3 years before the invasive carcinoma.

Five of the 24 women underwent an excisional biopsy of the ipsilateral breast within less than 3 months of the initial CNB; one 15 days later, three 1 month later, and one 2 months later (cases 9, 13, 14, 15, and 63); none of these patients had either an invasive or intraepithelial carcinoma in their excision. The reason for the excisional biopsy after the CNB in these cases was the finding of a radial scar on CNB in one case and the presence of a mass or density on mammography unexplained by the CNB findings. The findings in the excisional biopsy ranged from concurrent flat DIN 1 and LIN in two cases (cases 9 and 63), DIN 1 less than 2 mm (ADH) and a radial scar in one case (case 14), DIN 1, less than 2 mm (ADH) in one case (case 15), and familial colorectal cancer with calcifications in the remaining case (case 13).

Five (7.8%) of 63 women with flat DIN 1 on core biopsy developed subsequent ipsilateral invasive carcinoma without any intervening biopsies that contained a more advanced process (ADH or DCIS).

Discussion

This study is based on a retrospective assessment and identification of 63 examples of flat DIN 1 as the most advanced lesion in core biopsies performed before the widespread recognition of this lesion. Although limited by its retrospective nature and relatively small number of cases, it offers insight into the natural history of these lesions. Because flat epithelial atypia was not recognized as a pathologic

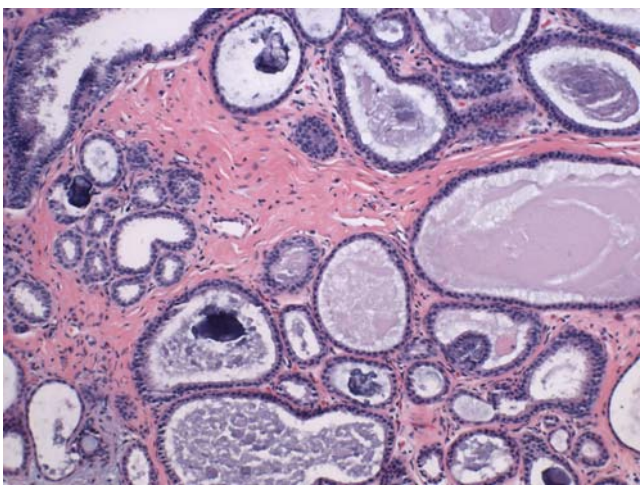

Fig. 2 Flat DIN 1 (case 41), terminal duct lobular unit with distended acini lined predominantly by a single layer of monotonous cells. Luminal calcifications are a frequent finding in flat epithelial atypia leading to mammographic detection. Lifting of the epithelium by stromal calcifications can occur as seen here in the *lower right* (*arrow*) and should not be considered as epithelial tufts or arcades

Table 2 Summary of clinicopathologic data of patients who underwent subsequent breast biopsy after initial CNB

Case #	Age	Subsequent biopsy/ specimen type	Time interval after initial CNB	Laterality I/CL	Findings leading to subsequent biopsy	Pathologic findings in subsequent biopsy
1	43	Excision	5 years	I	Microcalcifications	DIN 1 (FEA and ADH)
4	57	Excision	2 years	I	Mass	IDC, DIN 2 (DCIS 2)
5	45	Excision	6 years	I	Mass	DIN 1 (ADH)
		CNB	7 years	I	Mass on US	Low risk DIN (IDH)
6	54	CNB/excision	2 years	I	Microcalcifications	IDC, DIN2 (DCIS 2), Flat DIN 1, LIN
7	63	CNB/excision	9 years	I	Mass	IDC, Flat DIN 1 (FEA)
8	61	Excision	1 year	I	Mass	Flat DIN 1 (FEA)
9	54	Excision	1 month	I	Mass	Flat DIN 1(FEA), LIN
		Excision	9 years	CL	Mass Microcalcifications	Sclerosing adenosis, low risk DIN (IDH)
10	54	Excision	6 years	I	Palpable mass	IDC, Flat DIN 1 (FEA)
13	50	Excision	1 month	I	Mass	FCC
		CNB/Excision	10 years	CL	Microcalcifications	DIN 1 (FEA and ADH)
14	62	Excision	15 days	I	Radial scar on CNB	DIN 1 (ADH), radial scar
15	49	Excision	1 month	I	Mass	DIN 1 (ADH)
		Excision	3 years	I	Mass	DIN 1 (ADH)
		Excision	8 years	I	Mass	IDC, Flat DIN 1 (FEA), LIN
17	51	CNB/excision	7 years	I	Mass	DIN 1 (FEA and ADH)
		CNB/excision	7 years	CL	Mass	IDC, DIN 2 (DCIS 2), Flat DIN 1
20	39	CNB/excision	2 years	I	Microcalcifications	DIN 1 (FEA and ADH)
		CNB	5 years	I	Microcalcifications	IDC, DIN 2 (DCIS 2), DIN1 (FEA + ADH)
27	62	Excision	1 year	I	Microcalcifications	FCC
35	55	Excision	4 years	I	Mass	IDC, Flat DIN 1 (FEA)
36	44	Excision	4 years	I	Microcalcifications	Flat DIN1(FEA), FCC, papilloma
37	75	CNB	6 years	CL	Microcalcifications	Flat DIN 1(FEA), LIN
39	51	CNB	7 years	CL	Mass	IDC, Flat DIN 1 (FEA)
47	47	CNB	6 years	I	Microcalcifications	Fibroadenoma
51	62	CNB	3 years	I	Microcalcifications	Low risk DIN (IDH)
		Excision	2 years	CL	Mass	DIN 2 (DCIS 2)
54	49	Excision	3 years	CL	Mass/ microcalcifications	DIN 1 (FEA and ADH)
55	49	Excision	6 years	CL	Mass on US	FCC
58	51	CNB/excision	5 years	I	Microcalcifications	DIN 1 (FEA and ADH)
63	53	Excision	2 months	I	Microcalcifications	Flat DIN 1 (FEA), LIN

CNB Core needle biopsy, *I* ipsilateral breast, *CL* contralateral breast, *DIN* ductal intraepithelial neoplasia, *FEA* flat epithelial atypia, *IDC* infiltrating duct carcinoma, *DCIS* ductal carcinoma in situ, *LIN* lobular intraepithelial neoplasia, *FCC* fibrocystic changes, *ADH* atypical ductal hyperplasia, *IDH* intraductal hyperplasia

alteration at the time of initial review, no immediate (within 3 months) excisional biopsy was performed in 58 of the 63. Follow-up was available for 55 of the women.

Flat DIN 1 as the most advanced lesion was identified in 3.6% of CNB (63 of a total of 1,751), but this percentage is likely to be higher in current practice as a result of the widespread use of mammography as a screening tool and more abundant sampling (increased number of cores removed per case).

In 73% of the cases, flat DIN 1 was identified in biopsies performed for the evaluation of microcalcifications found on screening mammogram. The frequent association of flat epithelial atypia with microcalcifications is well documented in the literature [10, 25].

There is limited knowledge concerning the bilaterality and multifocality of flat epithelial atypia. Flat DIN 1 was identified in the contralateral breast in six of the eight cases (75%) in which a subsequent biopsy of the contralateral breast was performed, including two associated with invasive carcinoma and three with more advanced intraepithelial lesions (ADH and DCIS; Fig. 4). This finding seems to indicate that flat DIN 1 is often bilateral. In 29 (46%) of the CNB greater than ten ductules showed flat DIN 1, and in the excision specimens, flat DIN 1 was seen in a multifocal

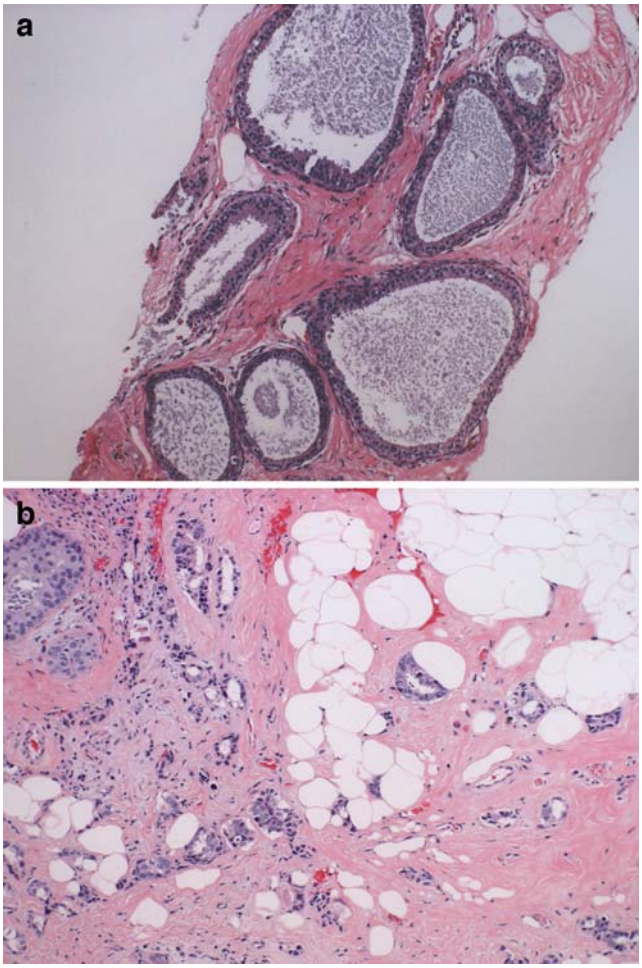

Fig. 3 **a** Flat DIN 1 on CNB, distended acini lined by one to two cell layers of monotonous mildly atypical cells, containing secretory material (case 20). **b** Ipsilateral infiltrating duct carcinoma 5 years after initial the CNB

distribution. The high frequency of bilaterality and multifocality noted for flat DIN1 is quite similar to that observed with lobular intraepithelial neoplasia.

Flat DIN 1 was found adjacent to invasive carcinoma in eight of the nine subsequent invasive carcinomas and in the case of contralateral DIN 2. Flat DIN 1 is often noted in the background with gradual transition to micropapillary and cribriform DIN 1 (DCIS grade 1) illustrating an increase in cell volume with progression but no change in the cytologic features [1, 10, 11, 24, 28]. This observation has been proposed as morphologic evidence for flat DIN 1 representing a precursor lesion of invasive and in situ carcinoma [21, 29]. Moinfar et al. [18] identified the same genetic alterations (loss of heterozygosity) in flat DIN 1 and adjacent in situ and infiltrating carcinoma; this has been confirmed in subsequent studies [30]. The morphologic findings confirmed at the molecular level indicate that flat DIN1 reflects a neoplastic alteration with the potential—often unrealized—to progress slowly to a more advanced process and ultimately a low-grade invasive carcinoma.

Studies with follow-up information on biopsies with only flat DIN 1 as the most advanced atypia, whether in excisional or core biopsies, are limited. Eusebi et al. [9] identified 21 cases of "clinging carcinoma" in a retrospective review of more than 4,000 breast biopsies initially diagnosed as benign between 1965 and 1971. However, the study makes no distinction between clinging carcinoma of the monomorphous type (flat DIN 1) and more proliferative micropapillary DIN 1 or a pleomorphic variant (unequivocal and higher-grade DCIS). Of these 21 patients, two died of breast cancer: One presented several years after the initial biopsy with advanced ipsilateral breast cancer and died 12 years after the biopsy, and the second patient presented with invasive duct carcinoma 6 years after the initial biopsy and died 4.9 years later. Another two were found to have persistent clinging carcinoma, one in the ipsilateral breast 9 years later and the other in the contralateral breast 11 months later. Bijker et al. [2], analyzing the data of the European Organization for

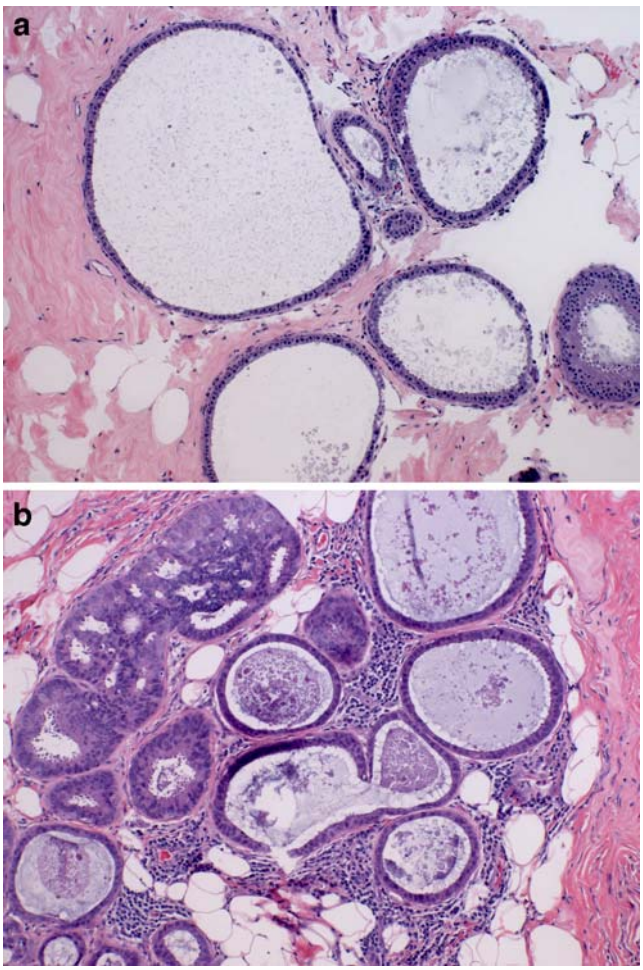

Fig. 4 **a** Flat DIN 1 on CNB (case 54). **b** Excisional biopsy of the contralateral breast 3 years after initial CNB showing DIN 1 (ADH) in a background of flat DIN 1. One duct has a cribriform proliferation of monotonous mildly atypical cells similar to the cells lining the ducts in the areas of flat epithelial atypia

Research and Treatment of Cancer trial 10,853 (excision with or without radiotherapy), reported no recurrences among 59 women with well-differentiated DCIS with clinging architecture after a median follow-up of 5.4 years.

Of the 63 patients identified as having flat DIN 1 on CNB retrospectively in this study, seven developed an invasive carcinoma in the ipsilateral breast indicating an absolute risk of 11.1%. The absolute risk was higher in the ipsilateral breast compared to 3.2% in the contralateral breast, with a mean follow up of 6.2 years available for 55 of the 63 patients. The results from the study by Eusebi et al. [9] indicate an absolute risk of 9.5% with a mean follow-up of 16.7 years, but this study was inclusive of lesions beyond flat DIN 1. Although available data do not permit assessment of the precise level of risk of developing subsequent carcinoma, they appear to indicate an increased risk of developing carcinoma compared to the absolute risk in women with intraductal hyperplasia (IDH), reported as 2.6% in the study by Tavassoli and Norris [34] and 4% by Dupont and Page [8], and perhaps closer to that of ADH. In the study by Tavassoli and Norris [34], 9.8% of women with ADH developed invasive carcinoma with a mean follow-up of 12.4 years; however, the development of subsequent invasive carcinoma after a diagnosis of ADH varies in the literature from 3.7 to 22% [3, 7, 15]. In patients with ADH, 25 [33] to 44% [26] of subsequent invasive carcinomas occur in the contralateral breast; in comparison, the percentage of contralateral invasive carcinoma in patients with flat DIN 1 is lower, 3.2% in our study. It should be pointed out that all previous studies cited here for flat DIN 1, IDH, and ADH were based on the assessment of excisional biopsy samples; therefore, disease extent as well as absence of more advanced pathology was more definitively established compared to core biopsies.

There is limited available data regarding the significance of flat DIN 1 detected on CNB and its management. Most of the data regarding flat epithelial atypia in core biopsies are limited to abstracts that looked at the findings in subsequent excisions [4, 5, 12, 16, 20]. Based on this limited data, a subsequent excision showed a more advanced lesion, (DCIS or invasive carcinoma) in approximately one third of cases. It should be noted, however, that given the variation in terminology used to designate this lesion and the inclusion in many of these studies of more advanced lesions with arcades, epithelial tufts, and cribriform architecture in addition to flat epithelial atypia (in essence inclusion of ADH among flat lesions), it is difficult to determine how many of the reported cases are truly pure flat epithelial atypia and how many represent atypical IDH. In five of the cases in our study, the initial core biopsy was followed by an excisional biopsy within less than 3 months, and none of these patients had either an invasive or intraepithelial carcinoma in the excision, but ADH was present in two cases; in these cases, lack of correlation between findings in the core and imaging may have prompted the excisional biopsy.

Because most published follow-up studies on flat epithelial atypia include ADH among the studied cases, the results are of limited value. Routine performance of excisional biopsy after the identification of flat epithelial atypia on CNB has been suggested by some authors [13, 21, 29] based on studies that have included ADH among the cases of flat epithelial atypia. Given the rather high frequency of flat epithelial atypia and lack of general familiarity with the lowest threshold for this lesion, attachment of undue clinical significance to this lesion could lead to an explosion of excisional biopsies for a lesion of still unknown consequence.

The current recommendation is that a surgical excision should be performed in cases showing ADH on a core biopsy, based on the reported finding of carcinoma in 33–87% of the subsequent excisional biopsies [6, 13, 27]. However, the frequency of subsequent carcinoma varies according to the method of biopsy used being lower with the use of the Mammotome compared to the automated biopsy gun and gauge of the needle. The current use of the Mammotome and the larger 8-ga needle provide a larger and/or more adequate sampling of the mammographically detected abnormalities. In our practice, when we encounter pure flat DIN 1 on CNB and is the only explanation for the mammographically detected abnormality, we perform three additional levels, beyond the usual three initial levels, to exclude a more advanced lesion deeper in the block. If arcades or micropapillae become apparent, the lesion should be diagnosed as ADH, and an excisional biopsy should be performed. This is a logical approach that many pathologists utilize intuitively when encountering early or borderline lesions (i.e., to evaluate areas suspicious for microinvasion). This is a minor additional step in the evaluation of a lesion of unknown biologic significance that has the potential of identifying a more advanced lesion, in which case an excision would be recommended, rather than recommending an excision in all cases. In our opinion, if additional levels fail to reveal a more advanced lesion, an excisional biopsy is not mandatory, but close follow-up is advised with repeat mammogram every 6 months for 2–3 years for early detection of any clinically occult invasive carcinoma or more advanced DIN (DCIS) in the vicinity of flat DIN 1 that might have been missed.

To get a true assessment of the clinical significance of flat DIN 1, only cases of pure flat lesions uncontaminated by more advanced proliferations in the form of focal arcades, micropapillae, and cribriforming should be studied, and standardization of the morphologic criteria and terminology are crucial to understand its biological significance and establish therapeutic guidelines.

Incorporation of the "columnar cell" in the designation of this lesion is misleading, and the value of grading or subcategorizing "columnar cell lesions" further is doubtful. The mature mammary epithelium varies from cuboidal to low columnar and columnar in shape with or without apical snouts in its many normal physiologic variations. Furthermore, flat epithelial atypia also varies from cuboidal/low columnar to columnar and from a single to multiple cell layers. Therefore, it is not clear what is accomplished by inclusion of "columnar" in the terminology or grading of these lesions. It is actually misleading as it may imply that only lesions with columnar cells should be placed in this category, although flat epithelial atypia also consists of cuboidal cells. Furthermore, the presence of cells with columnar morphology lining the ducts with unequivocal ADH can result (and has resulted) in incorrect inclusion of ADH in the category of flat DIN 1. The term "flat" refers to the overall architecture of the proliferation, which lacks any intraluminal protrusions in the form of arcades, bridges, or micropapillae and is essential in distinguishing this lesion from ADH.

Within the DIN classification system for intraductal proliferative lesions, flat epithelial atypia is designated as flat DIN 1. The common findings of flat epithelial atypia in association with ADH, low-grade DCIS, and well-differentiated infiltrating carcinoma, particularly tubular carcinoma, and the similarities in their cytologic features and genetic alterations have led to the concept that flat epithelial atypia represents one of the earliest morphologically recognizable intraepithelial neoplastic alteration of the breast [18] supporting its inclusion into the DIN classification and within the DIN 1 group; the cytologic features of the cells in the spectrum of DIN 1 (flat epithelial atypia, ADH, and low-grade DCIS) whether flat, forming partial, or complete duct involvement by micropapillae, arcades, or cribriform growth patterns in one or 30 ducts are identical.

The diagnosis and identification of flat epithelial atypia remains a diagnostic challenge in current practice with inter and intraobserver variability [22, 31]. Those lesions characterized by the presence of three to five cell layers are more readily recognizable (Fig. 5); those consisting only of a single layer are more difficult. Immunohistochemistry for high-molecular-weight cytokeratins CK-34betaE12 (CK903) and CK5/6 as used in the evaluation of problematic intraductal proliferations of the breast [17, 19] and estrogen receptors (ER) could be useful when considering the diagnosis of flat epithelial atypia. Negative immunoreaction for CK-34betaE12 (CK903) or CK5/6 along with intense and diffuse positivity for ER, as is often seen in flat epithelial atypia [35], would be in favor of the diagnosis; however, the final interpretation should be made on the overall morphologic features and not on the immunohistochemical findings alone. When in doubt, it is better to underdiagnose. It is also important to differentiate flat epithelial atypia from DIN 2 and 3, flat type (high-grade DCIS). Lesions with flat architecture but showing high-grade cytologic atypia with high nuclear pleomorphism (nuclear grades 2 and 3) with or without frequent mitotic figures should be designated as DIN 2 or 3 (DCIS grade 2 or 3) and excluded from the category of flat DIN 1 (flat epithelial atypia).

On the other hand, blunt duct adenosis, a form of adenosis characterized by mild distention of the ducts and generalized hypertrophy of all components of the TDLU (epithelial cells, myoepithelial cells, and often stroma) should be distinguished from flat epithelial atypia. In blunt duct adenosis, the myoepithelial cell layer is commonly prominent resulting in the impression of a proliferative lesion; however, there is only a single epithelial cell layer without any atypia. This distinction may be difficult, and in the past, not uncommonly, flat epithelial atypia was misinterpreted as blunt duct adenosis.

The use of standardized criteria for the diagnosis and common terminology is essential in establishing the significance and determining guidelines for management of this lesion, particularly on CNB. As we continue to study the biology of this lesion and more data emerge in the literature, we propose performing three additional levels, beyond the usual three initial levels, when flat DIN 1 is found as the most advanced lesion on CNB. If additional levels fail to reveal a more advanced lesion, an excisional

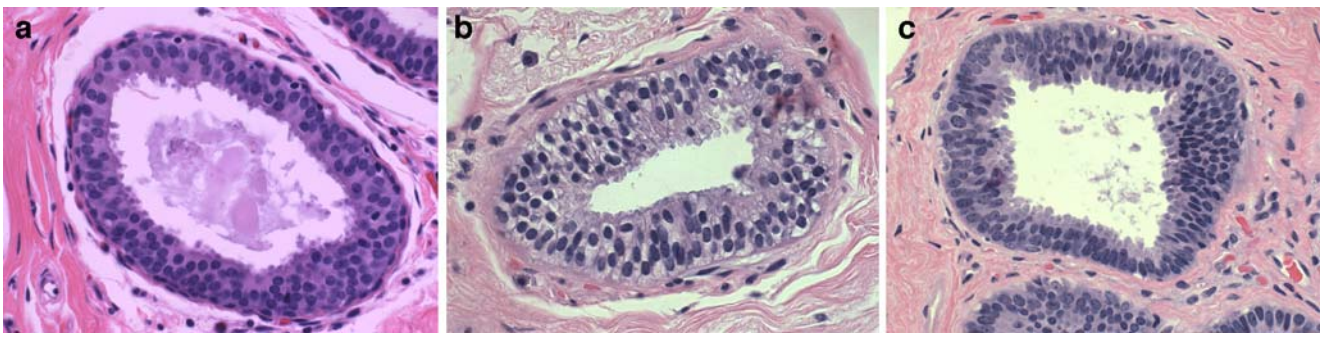

Fig. 5 **a**, **b**, **c** Three examples of multilayered Flat DIN 1, characterized by replacement of the native epithelial cell layer by two to four cell layers of monotonous mildly atypical cells

biopsy is not mandatory if there are no mammographic features of concern. Because clinically occult, more advanced DIN and low-grade carcinomas may be missed, close follow-up is advised with repeat mammogram every 6 months for 2–3 years for early detection of any such invasive carcinomas in the vicinity of flat DIN 1. Finally, after assessment of additional levels and the immunoprofile, if there remains doubts about the presence of flat DIN 1, it is better to underdiagnose this lesion or refrain from diagnosing.

Conclusion

Flat DIN 1 found on CNB is often in the setting of a biopsy performed for the evaluation of microcalcifications identified on screening mammogram. Cognizant of the retrospective nature of the study, based on our findings, flat DIN 1 is a marker of slightly increased risk for subsequent development of invasive breast carcinoma. However, follow-up studies of flat DIN 1 as the most advanced lesion are needed to better understand the significance of this lesion and establishing uniform guidelines in its management.

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ORIGINAL ARTICLE

Immunohistochemical expression of estrogen receptors alpha and beta in lobular neoplasia

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Abstract The designation lobular neoplasia (LN) of the breast includes atypical lobular hyperplasia and lobular carcinoma in situ. Estrogen receptors (ER) play a significant role in breast carcinogenesis. In the present study, ER-alpha and ER-beta status are evaluated in 30 breast tissue specimens from patients whose main lesion was LN. A standard immunohistochemical procedure, using monoclonal antibodies for ER-alpha and ER-beta, was applied to the lesion and the adjacent normal breast tissues, the latter serving as control. In all cases, both receptors were expressed in LN as well as in normal breast ducts and lobules. Concerning ER-alpha, the Allred score and the percentage of ER-alpha-positive cells were significantly higher in LN than in the adjacent normal breast tissue. On the contrary, regarding ER-beta, the Allred score and the percentage of ER-beta-positive cells were significantly lower in LN compared with normal adjacent breast tissue. Greater increase in the percentage of ER-alpha-positive cells was associated with a smaller reduction in the percentage of ER-beta-positive cells and vice versa (Spearman's rho=−0.5044, p=0.001). In conclusion, upregulation of ER-alpha and downregulation of ER-beta may represent two discrete molecular events in LN pathogenesis. Of notice, a mutually limiting interaction may exist between the two events.

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Keywords Lobular neoplasia · Estrogen receptor · Immunohistochemistry · Breast

Introduction

The term lobular neoplasia (LN), including the designations atypical lobular hyperplasia and lobular carcinoma in situ (LCIS), refers to the entire spectrum of atypical epithelial proliferation originating in the terminal duct-lobular unit, with or without pagetoid involvement of terminal ducts. Nowadays, it is widely known that LN represents a risk factor and a nonobligatory precursor for the subsequent development of invasive carcinoma in either breast, of either ductal or lobular type [10, 14, 17].

Estrogen receptor (ER) upregulation is a hallmark of cancer progression, at nearly all stages of breast carcinogenesis [25]. Two ERs exist: ER-alpha, located on chromosome 6q25.1, and ER-beta located on 14q22-24. A great number of isoforms/splice variants of ER-beta have been identified. Of notice, ER-beta seems to exhibit a pattern inverse to ER-alpha, declining during the progression from normal breast tissue to atypical ductal hyperplasia, ductal carcinoma in situ, and invasive ductal carcinoma [15, 18]. The ER-beta/ER-alpha ratio has emerged as an important risk factor; a low ER-beta/ER-alpha ratio seems to predict progression to carcinoma in nonatypical epithelial hyperplasia [19]. It is interesting to note that ER-beta gradual reduction has been reported to be more pronounced in invasive ductal than in invasive lobular carcinomas [20].

To date, there is scarcity of data with respect to the evaluation of ER-alpha and ER-beta status in patients whose main lesion is LN. To our knowledge, this is the first study focusing on the immunohistochemical expression of ERs alpha and beta in LN cases.

Materials and methods

Tissue specimens were taken from 30 patients with LN. The patients' age at operation ranged between 38 and 67 (median age=51 years). The diagnosis of LN was established by vacuum-assisted breast biopsy or by excisional breast biopsy. The cases of LN coexisting with atypical ductal hyperplasia, ductal carcinoma in situ, invasive lobular carcinoma, or invasive ductal carcinoma were excluded.

The ER-alpha and ER-beta receptors were immunohistochemically detected with the commercially available ER6f-11 and ER-beta (clone EMRO2; Novocastra supplied by Menarini) antibodies and visualized using an avidin–biotin detection system. Antigen retrieval was achieved in 0.001 M citrate buffer (pH=6.0) at 85°C for 18 h. Immunohistochemical assessment of ER-alpha and ER-beta was performed both in the lesion and the adjacent normal breast ducts and lobules, serving as control.

As far as ER-alpha and ER-beta evaluation is concerned, (1) Allred score was calculated [1], and (2) the percentage of positive cells (nuclei) was separately analyzed. In all cases, the area of maximum staining intensity was preselected on each slide and a minimum of 100 cells were evaluated in the designated area. The immunohistochemical evaluation was performed independently by two consultant histopathologists (AN and AL).

Statistical analysis was performed with STATA 8.0 statistical software. The statistic performed at each case is mentioned in parentheses in the text.

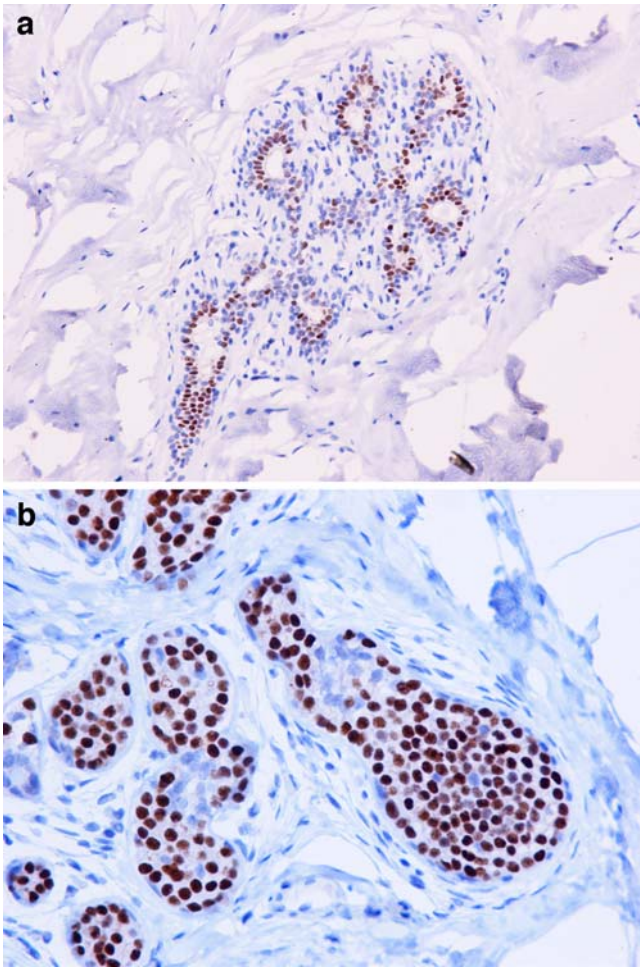

Fig. 1 Immunohistochemical nuclear expression of ER-alpha in normal breast (**a**; ×200) and in lobular neoplasia (**b**; ×400)

Results

Apart from the nuclear ER-alpha and ER-beta immunoreactivity in epithelial cells of normal ducts and lobules and in LN, some lymphocytes and myoepithelial cells were also stained (Fig. 1a,b, and 2a–c).

Concerning ER-alpha, Allred score was significantly higher in LN than in the adjacent normal ducts and lobules (mean ± SD=6.85±0.98 vs 6.24±1.04, respectively; p=0.003, Wilcoxon matched-pairs signed-ranks test). On the other hand, regarding ER-beta, Allred score was lower in LN than in the adjacent normal ducts and lobules (mean ± SD=6.95±0.67 vs 7.26±0.63; p=0.007, Wilcoxon matched-pairs signed-ranks test; Fig. 3).

The percentage of ER-alpha-positive cells was significantly higher in LN than in the adjacent normal ducts and lobules (mean ± SD=67.8±17.2 vs 59.7±18.6%, respectively; p=0.039, Wilcoxon matched-pairs signed-ranks test). On the contrary, the percentage of ER-beta-positive cells was lower in LN than in the adjacent normal ducts and lobules (mean ± SD=69.9±17.1 vs 80.7±12.0%; p<0.001, Wilcoxon matched-pairs signed-ranks test; Fig. 4).

Subsequently, the increase in ER-alpha percentage was calculated (percentage of positive cells in LN−percentage of positive cells in normal breast tissue), together with the decrease in ER-beta (percentage of positive cells in normal breast tissue−percentage of positive cells in LN). It interesting to note that the greater increase in ER-alpha expression was associated with a smaller reduction in ER-beta expression and vice versa (Spearman's rho=−0.5044, p=0.001). Accordingly, the increase in ER-alpha Allred score and the decrease in ER-beta Allred score were calculated, but the respective association did not reach statistical significance (Spearman's rho=−0.074, p=0.648).

Discussion

ER positivity is a well-established feature of LCIS [6, 13], but the profile of ER subtypes has not been evaluated in detail. This is the first study focusing exclusively on LN lesions and demonstrating a significant ER-alpha upregulation and ER-beta downregulation therein. The diptych ER-

alpha upregulation/ER-beta downregulation has become evident after the calculation of the Allred score and, more specifically, after the assessment of the percentage of ER-alpha- and ER-beta-positive cells.

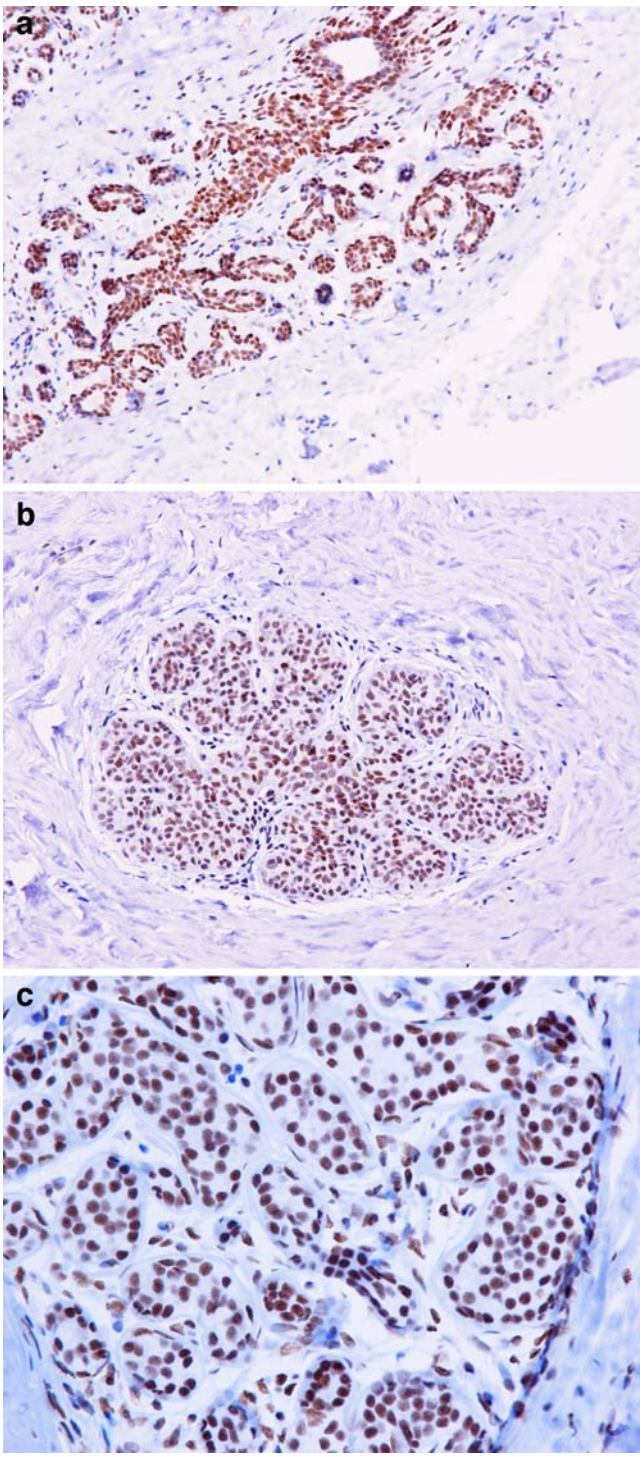

Fig. 2 Immunohistochemical nuclear expression of ER-beta **a** Strong intensity in normal breast (×200). **b** Decreased percentage of positive cells in lobular neoplasia, in comparison to the adjacent normal breast tissue (×200). **c** Strong intensity in lobular neoplasia (×400)

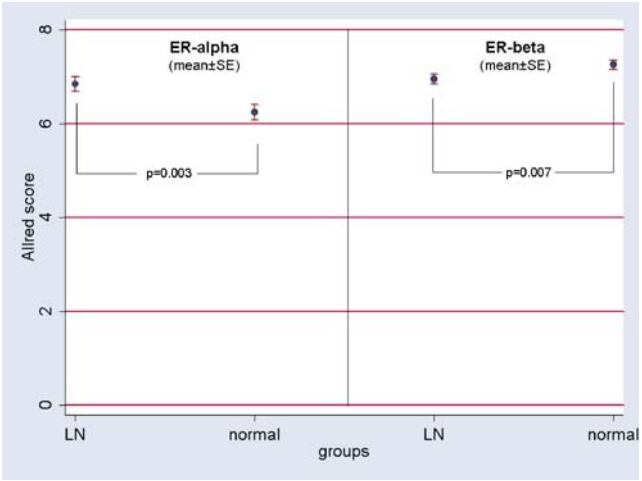

Fig. 3 Allred score in LN and normal adjacent breast tissue

The present finding has multivalent physiological implications. ER-alpha and ER-beta are two distinct receptor subtypes, differing in their transcriptional activity and putatively mediating differential estrogen effects [3]. As mentioned above, the importance of the ER-beta/ER-alpha ratio has been reported in the context of breast cancer [11]; ER-beta has been associated with favorable features in breast cancer [9] and may have a generally protective role [8]. Thus, the increase in ER-alpha expression together with ER-beta downregulation might represent an advantage of LN cells, pointing to a putative subsequent malignant transformation.

Concerning ER subtypes in LN, a hint dates back to 2001, when the decreased ER-beta/ER-alpha ratio was demonstrated in proliferative benign breast disease with atypia and in carcinomas in situ [15]. Of notice, however, that study did not make the distinction between ductal and lobular lesions, putatively because of the relatively small

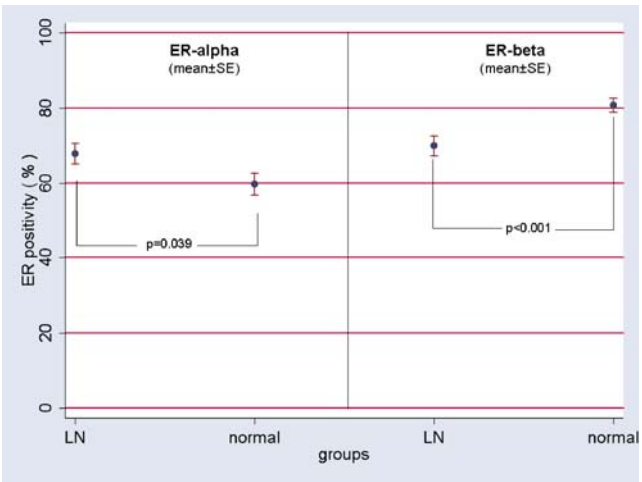

Fig. 4 Percentage of ER-alpha and ER-beta positivity in LN and normal adjacent breast tissue

sample size, and thus the ER subtype status in LN remained an undiscovered field.

Additionally, a rather striking finding emerged in our sample: A greater increase in ER-alpha positivity was associated with a smaller reduction in ER-beta positivity and vice versa. It seems thus that there is a mutually limiting link between the two molecular events, i.e., ER-alpha upregulation and ER-beta downregulation, at the phase of LN. This mutually limiting effect could prevent excessive, aberrant proliferation, which might have occurred in case of disproportionate ER-alpha upregulation or extreme ER-beta downregulation. Indeed, a sole event (concerning ER-alpha or ER-beta expression) may be needed at the phase of LN.

However, when the Allred score was calculated, the respective, mutually limiting, association did not reach statistical significance. This might be due to the fact that the intensity of the staining (a major constituent of the Allred score) is measured in a rather limited, confined 0–3 scale. Such a confined scale-range may render difficult to document statistically significant changes and may thus blur the association detected when the absolute percentage of positive cells was evaluated.

It would be of particular interest to evaluate whether the mutually limiting effect vanishes or persists in invasive lobular carcinoma. Despite controversial reports [12], marked ER positivity is an important feature of invasive lobular carcinoma, vis-à-vis invasive ductal carcinoma [2, 5, 7, 16], but the inter-relationships between ER-alpha and ER-beta therein have not been fully elucidated. For the future, it is also tempting to anticipate the evaluation of ER-beta isoforms in LN tissues because to date ER-beta isoforms have only been studied on breast cancer and breast cancer cell lines [23].

An interesting observation was that ER-beta was also expressed on some lymphocytes and myoepithelial cells. The expression of ER-beta on myoepithelial cells has been also reported in the past [4, 21]. Concerning lymphocytes, ERs have been found occasionally expressed in breast tissue lymphocytes [21]; ER-beta have been also identified in intraepithelial lymphocytes in human oviducts [24] and human cervix [22]. The significance of such findings remains to be fully elucidated in the future.

In conclusion, ER-alpha upregulation and ER-beta downregulation represent two discrete molecular events in LN pathogenesis. The mutually limiting interaction between the two events seems to characterize LN. Automated procedures involving quantification of ER-alpha and ER-beta expression, as well as the evaluation of ER-alpha and ER-beta messenger ribonucleic acid expression are needed to confirm the present findings.

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ORIGINAL ARTICLE

Mesometrial smooth muscle as an origin of female retroperitoneal (pelvic) leiomyomas

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Abstract Retroperitoneal (pelvic) leiomyomas have recently come to be recognized as distinctive lesions. Retroperitoneal leiomyomas occur almost exclusively in women, and past studies on these invariably emphasized a striking similarity between their histological features, with those of uterine leiomyoma, whereas their origin remains unknown. In this study, we took notice of mesometrial smooth muscle, which has been little known either clinically or pathologically, as a possible origin of tumor. Anatomically, the mesometrial smooth muscle was an accumulation of thin bundles (approximately 1 mm in thickness) that ran parallel to the oviduct. It was connected broadly with the lateral wall of the uterine body and ended in the pelvic floor. The mesometrial smooth muscle was present just beneath the serosal surface of the anterior aspect of the mesometrium and continuously transited from the smooth muscle bundles of the outer layer of the uterine myometrium. The muscle cells were immunopositive for smooth muscle cell markers and estrogen/progesterone receptors. In all of the six female retroperitoneal leiomyomas examined, hormone receptor-positive nontumorous smooth muscle layers were present in the periphery of the tumors, seemingly representing the mesometrial smooth muscles. In conclusion, we believe most retroperitoneal (pelvic) leiomyomas in females arise from the mesometrial smooth muscle.

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Keywords Mesometrium · Leiomyoma · Estrogen receptor · Progesterone receptor · Leiomyosarcoma

Introduction

Retroperitoneal (pelvic) leiomyoma is a rare but distinctive lesion. Although it was traditionally believed that most smooth muscle cell tumors of the retroperitoneum (which include a number of gastrointestinal stroma tumors) were malignant [13, 15], recent follow-up studies by Billings et al. [3] and Paal et al. [12] clarified the presence of benign smooth muscle tumor in the retroperitoneum and abdominal cavity. Characteristically, retroperitoneal leiomyomas occur almost exclusively in women in contrast to gastrointestinal, cutaneous, and somatic deep leiomyomas [8], which exhibit no difference between genders, and past studies on retroperitoneal leiomyomas invariably emphasized the striking similarity of their histological features with those of uterine leiomyoma [3, 12]. Some investigators called them "parasitic uterine leiomyoma," which means an implantation or detachment of uterine leiomyoma, but this idea dose not have any scientific basis [6, 18]. Thus, the cellular origin of retroperitoneal leiomyomas has not been elucidated.

The mesometrium is the largest part of a broad ligament that is attached to the uterus, ovarian ligament, and pelvic floor. The mesometrium includes a broad bundle of smooth muscle cells just beneath the thin peritoneal membrane, but this fact is not generally known. In the present study, we noted the mesometrial smooth muscle as an origin of retroperitoneal (pelvic) leiomyoma and studied the anatomical and immunohistochemical characteristics of mesometrial smooth muscle with an analyzation of retroperitoneal leiomyomas experienced in our laboratory. We believe that

most retroperitoneal leiomyomas in females seem to arise from the mesometrial smooth muscle, which belongs to an identical cell lineage of those of uterine myometrial cells.

Materials and methods

Gross and microscopic examination of the mesometrium was performed using autopsy cases from nine women aged 46 to 79. To examine the expression of estrogen and progesterone receptors in smooth muscle cells, various organs were sampled from a 72-year-old man who had died of pulmonary carcinoma and a 62-year-old woman who had died of polycystic kidney disease. The tissues sampled included the lung, stomach, urinary bladder, ureter, prostate, testis, seminal duct, uterus, ovary, oviduct, and vaginal wall. A piece of scrotum wall was obtained from a surgical specimen of a 58-year-old-man who underwent wide resection for primary squamous cell carcinoma of the scrotum.

Six retroperitoneal and two parauterine leiomyomas were retrieved from the surgical file of the Pathology Division of the Fukushima Medical University Hospital.

All tissues were processed routinely and embedded in paraffin. Sections that were 3 to 4 μm thick were cut and stained with hematoxylin–eosin. Immunohistochemical staining of formalin-fixed and paraffin-embedded sections was performed using the streptavidin–biotin method (LSAB kit, Dako Japan, Kyoto, Japan). The primary antibodies and their final dilutions were: α-smooth muscle actin (1A4, Dako Japan, 1:100), desmin (D33, Dako Japan, 1:50), *h*-caldesmon (h-CD, Dako Japan, 1:50), estrogen receptor-α (ER1D5, Immunotech Japan, Tokyo, Japan, 1:50), and progesterone receptor (1A6, Immunotech Japan, 1:20).

Results

Anatomy of the mesometrial smooth muscle

Grossly, the mesometrial smooth muscle was an accumulation of light tan-colored thin bundles covered by the surface serous membrane, when observed from the anterior aspect of the uterus. The bundles ran parallel with the oviduct and were connected broadly with the lateral wall of the uterine body (Fig. 1), and ended in the lateral to posterior pelvic wall involving the pararectal region.

Histologically, the mesometrial smooth muscle was present just beneath the serosal surface of the mesometrium (Fig. 2). It continuously transited from the smooth muscle bundles of the outer layer of the uterine myometrium and preserved an almost uniform thickness in the anterior aspect of the mesometrium (approximately 1 mm in thickness; Fig. 3a,b). Although uterine myometrium also transited to the posterior aspect of the mesometrium, smooth muscle cells soon became sparse in this region, unlike with the anterior mesometrial smooth muscle. The mesometrial smooth muscle was composed of an accumulation of rather loosely arranged small bundles of smooth muscle cells in the

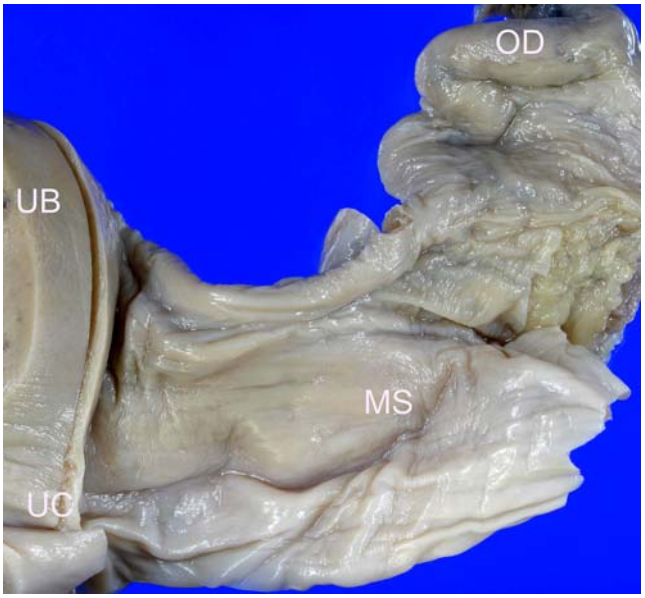

Fig. 1 Gross features of anterior aspect of the broad ligament including mesometrium (left side of 46-year-old female, autopsy case). Mesometrial smooth muscle (*MS*) was formed by an accumulation of light tan-colored thin bundles covered by the surface serous membrane, which ran parallel to the oviduct (*OD*). They were connected broadly with the lateral wall of the uterine body (*UB*). The right margin of the mesometrium was cut near the pelvic wall. *UC* Uterine cervix

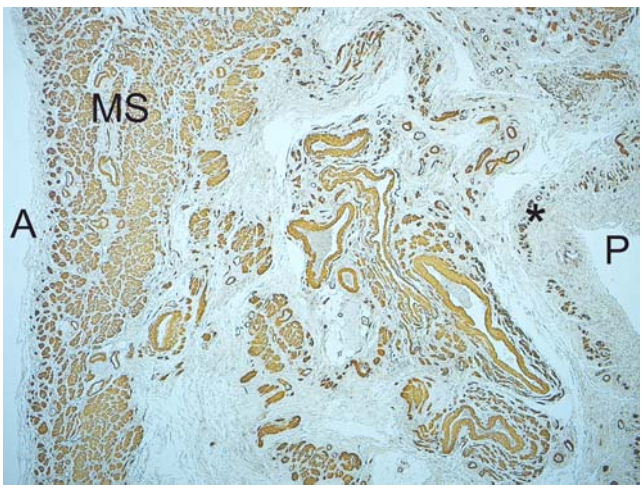

Fig. 2 Full thickness of the mesometrium in sagittal section. Immunostain for α-smooth muscle actin highlighted a dense smooth muscle layer (*MS*) in the anterior aspect of the mesometrium. Although scattered smooth muscle cells (*asterisk*) were present in the posterior aspect, they were almost restricted near the uterus. There were rich blood vessels and nerve fibers between these. *A* Anterior aspect, *P* posterior aspect, *MS* mesometrial smooth muscle. *Asterisk*, Scattered smooth muscle cells

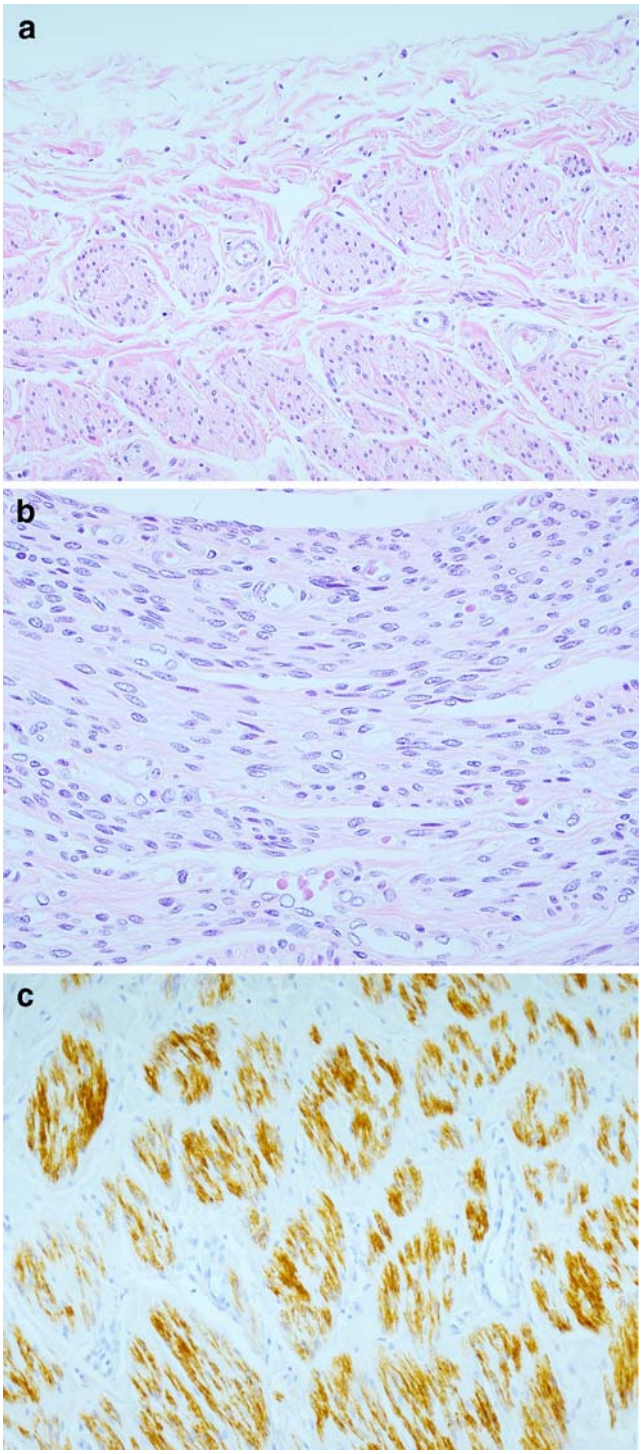

Fig. 3 High-power fields of the mesometrial smooth muscle cells. Cross-section (**a**) and longitudinal section (**b**). The cellular features were indistinguishable from those of myometrial smooth muscle cells. The cells were immunopositive for smooth muscle markers (**c** *h*-caldesmon)

sagittal section and showed a longitudinal arrangement almost parallel to the oviduct. Immunohistochemically, the smooth muscle cells were intensely positive for α-smooth muscle actin but were rather less intensely positive for *h*-caldesmon (Fig. 3c) and desmin. They consistently express estrogen/progesterone receptors to various degrees (Fig. 4).

Retroperitoneal and parauterine leiomyomas

Clinical information and histopathological features are summarized in Table 1. The age of patients ranges from 21 to 58. All but one case had a past history of uterine leiomyoma, and three patients had undergone a simple hysterectomy in the past. Four of the eight tumors were beyond 10 cm in size, and the minimum tumor size was 3.7 cm. The preoperative diagnosis was ovarian tumor in five cases, and the remaining three cases were diagnosed as retroperitoneal tumors.

Histological features of both retroperitoneal and parauterine leiomyomas were indistinguishable from those of uterine leiomyoma. The tumors often exhibited varying degrees of myxoid change, which was extensive in two tumors (cases 1 and 2). Extensive hyalinous change was observed in one case (case 7). Case 5 was a cellular type, and case 6 had rich lipomatous elements (lipoleiomyoma). In all of the retroperitoneal leiomyomas and a parauterine leiomyoma arising from the broad ligament, nontumorous smooth muscle layers were present in the periphery of the tumors (Fig. 5). Immunohistochemically, all retroperitoneal and parauterine leiomyomas and nontumorous muscle layers were positive for antibodies against estrogen/progesterone receptors.

Estrogen/progesterone receptors in the normal smooth muscles

Estrogen/progesterone receptor-positive smooth muscle cells were present in the uterine myometrium, stroma of the ovary, muscle layer of the oviduct, muscle bands of the

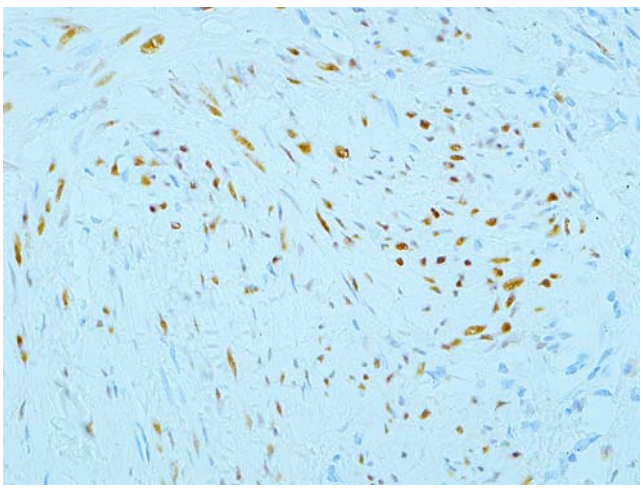

Fig. 4 The mesometrial smooth muscle cells consistently expressed hormone receptors (progesterone receptor staining)

Table 1 Case summary of retroperitoneal and parauterine leiomyomas

Cases	Age	Size (cm)	Location	Preoperative diagnosis	Past history of uterine myoma	Past history of surgery	Normal smooth muscle layer
Retroperitoneal leiomyoma							
1	21	Huge	Retroperitoneum	RT	−	−	+
2	35	9.0	Retroperitoneum	RT	+	Myomectomy	+
3	39	5.0	Pararectal	OVT	+	Myomectomy	+
4	47	7.0	Retroperitoneum	OVT	+	Hysterectomy	+
5	53	13.0	Retroperitoneum	OVT	+	Hysterectomy	+
6	58	11.0	Parasigmoid colon	RT	+	Hysterectomy	+
Parauterine leiomyoma							
7	52	15.0	Broad Ligament	OVT	+	−	+
8	55	3.7	Round ligament	OVT	+	Myomectomy	−

RT Retroperitoneal tumor, *OVT* ovarian tumor

broad (mesometrial smooth muscle) and round ligaments, and in the wall of the vagina. The vascular smooth muscle cells in these regions were consistently negative for hormone receptors. Smooth muscle cells included in the lung, stomach, urinary bladder, ureter, prostate, testicle, seminal duct, and scrotum were all negative for estrogen/progesterone receptors.

Discussion

Generally, leiomyoma preferentially arises from organs where rich smooth muscle cells are originally present. As is well known, the uterus is the most common site of leiomyoma, followed by the gastrointestinal tract and skin. In contrast, the origin of retroperitoneal leiomyoma remains unclear. Although there have only been a few large-series studies on retroperitoneal leiomyomas, these reports [3, 12] describe their distinctive clinicopathological features: (1) Retroperitoneal leiomyomas almost exclusively occur in women in contrast to gastrointestinal, cutaneous, and somatic deep leiomyomas, which exhibit no difference between genders; (2) They show a striking resemblance in their histology to uterine leiomyomas and immunohistochemically express estrogen/progesterone receptors. These characteristics of retroperitoneal leiomyoma strongly suggest an intimate relation to uterine leiomyomas. In contrast, it is noteworthy that retroperitoneal leiomyoma can occur in patients who have previously undergone a hysterectomy. Although retroperitoneal leiomyomas rarely arise in men, histological features for these are different from those of women, and the hormone receptor is negative [12]. Thus, retroperitoneal leiomyomas of men are thought to be different entities from those of women.

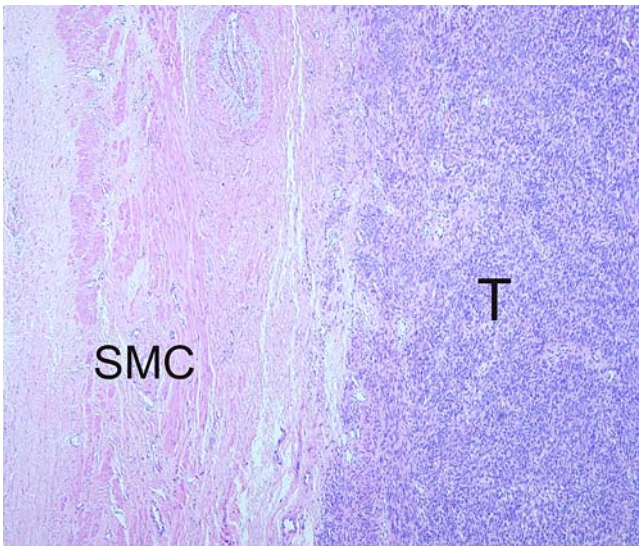

Fig. 5 In the periphery of cellular leiomyoma (*T*), a thin layer of smooth muscle cells (*SMC*) were seen, presumably mesometrial smooth muscle. Case 5

The purpose of the present study is to clarify the origin of retroperitoneal leiomyomas in women. The first clue to make us take notice of the mesometrial smooth muscle was the presence of a seemingly nontumorous, thin, smooth muscle layer adjacent to the tumor tissue in a case of retroperitoneal leiomyoma. Consequent analysis revealed that thin smooth muscle layers were identified adjacent to tumor tissue in all six retroperitoneal leiomyomas and in a parauterine leiomyoma. Moreover, observation of the female pelvis of autopsied cases clarified that the mesometrial and myometrial smooth muscles are a continuous structure with similar cellular characteristics including the expression of hormone receptors. The hypothesis that retroperitoneal leiomyomas originate from the mesometrial smooth muscle well explain the indistinguishable similarity of retroperitoneal and uterine leiomyomas. It can be accepted that the retroperitoneal leiomyomas exclusively

occur in female. Furthermore, retroperitoneal leiomyoma can occur in patients who have undergone hysterectomy, as the mesometrium is preserved through the various gynecologic surgeries. Although we could not explain the origin of intraabdominal leiomyomas located far from the pelvic cavity, we believe that most of pelvic leiomyomas, if not all, seem to arise from the mesometrial smooth muscle.

To date, descriptions of human mesometrial smooth muscle are rarely found and have been limited in literature either the anatomy or of reproductive medicine using animal models. Mossman [11] described in an anatomical study of the mammalian uterus that the development of mesometrial smooth muscle varies greatly between mammalian groups and that in some species, as in the porcupine, mesometrial smooth muscle forms a very conspicuous band. Liebgott [9] first studied the mesometrial smooth muscle systemically in the mouse and reported that the outer longitudinal myometrial layer extends onto the mesometrium as a bilaminar extension. This description corresponds to our observation in humans, while the mesometrial smooth muscle seems to be more developed in mice than humans [9]. As expected, uterine myometrium and mesometrial smooth muscle are intimately related, but they do not appear identical. Several studies using animal models reported that the effect of estrogen or prostaglandins administration was different between uterine myometrium and mesometrial smooth muscle [1, 10, 17].

Traditionally, parauterine leiomyomas including paracornual, parametrial, and parasalpingeal types has been distinguished from retroperitoneal leiomyoma [4, 5, 7]. Honore [7] defined parauterine leiomyoma as follows: (1) Their juxtauterine borders were easily separated from the uterus, (2) they were not covered externally by perimetrium directly continuous with that overlying the normal adjoining uterus, and (3) histologic study of the interface showed no direct continuity between the outer myometrium. Based on the observation of 22 parauterine leiomyomas, he reported that none were found in the distal perisalpinx or mesovarium [7]. Interestingly, Honore [7] mentioned that "It appears that smooth muscle in parauterine connective tissues, whether anatomically orthotopic or representing heterotopic myometrial rest, behaves oncologically like the myometrium, the closer it is to the uterus," while he seemed not to be aware of the mesometrial smooth muscle itself. It is likely that some parametrial leiomyomas arise from the mesometrial smooth muscle. Thus, retroperitoneal and parauterine leiomyomas are thought to be analogous tumors with close, if not identical, cell lineage.

In the present immunohistochemical study, it was confirmed that normal smooth cells with expression of hormone receptors were restricted in the reproductive and genital organs of women and that male smooth cells completely lacked hormone receptors in any organs examined immunohistochemically. This result correspond well to a description in a past report on the leiomyoma of deep soft tissues, in which no somatic leiomyomas, whether in men or women, expressed hormone receptors [3]. It seems certain that the smooth muscle cells and their neoplasm of female genital organs including the uterus and parauterine tissues, vagina, and vulva have different characteristics from those of other organs, e.g. gastrointestinal tract, skin, and soft tissue, and the former tumors often exhibit various secondary changes, high cellularity, and atypia as commonly seen in uterine leiomyomas. Therefore, leiomyomas of female genital organs should be distinguished from those arising from other organs. Although the origin of retroperitoneal and intra-abdominal leiomyomas in men has been unknown, these may originate from vascular smooth muscles.

Although most retroperitoneal leiomyomas are correctly diagnosed without difficulty, some cellular or atypical tumors may be problematic. Paal and Miettinen [12] said that well-differentiated retroperitoneal smooth muscle tumors that lack atypia and coagulation necrosis and have less than three mitoses/50 high power fields can be classified as at least probably benign. Criteria for these are principally based on an excellent study on uterine smooth muscle tumors carried out by Bell et al. [2]. Recently, more simplified criteria was proposed to differentiate problematic uterine atypical leiomyoma from leiomyosarcoma based on the differences in their cellular origin [16]. The expression of hormone receptors was thought to be the most easily recognizable characteristics of uterine leiomyomas and was exceedingly rare in the leiomyosarcomas of either the uterus or soft tissue [14, 19]. It seems that the criteria can be applicable to the retroperitoneal smooth muscle tumor in women. Paal and Miettinen [12] also argued that the immunostaining of hormone receptors is essential to the diagnosis because the 11 retroperitoneal leiomyosarcomas examined were all negative. In contrast, other criteria should be applied for tumors of men, because their cellular origin seems to differ from those in women.

In summary, mesometrial smooth muscle, which has been little known to date, was described with anatomical, histological, and immunohistochemical observations. It was clarified that mesometrial smooth muscle was continuous and that it had a structure intimately related to uterine myometrium. We believe most retroperitoneal leiomyoma in women originate from mesometrial smooth muscle.

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ORIGINAL ARTICLE

Tubulocystic carcinoma of the kidney: a new entity among renal tumors

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Abstract Tubulocystic carcinoma is a tumor entity, which is not yet included in the WHO-classification of renal tumors. We report a series of 11 cases of this tumor, 6 of which were examined in by immunohistochemistry using a panel of five antibodies (CK7, CK34βE12, CK19, CD10 and P504S). All patients were men. Each had renal tumor stage of pT1N0M0, with a diameter of 1.7 to 7 cm (mean, 3.3 cm). None of the patients presented with recurrence or metastases. Grossly, tumors were microcystic masses with a bubble-wrap appearance. Histological features included cysts and small tubules, separated by delicate septa and lined by flat to columnar or hobnail cells. The cyst and tubule epithelium showed immunohistochemical characteristics of both proximal and distal tubules. Tubulocystic carcinoma is a distinctive kidney tumor, with noteworthy macroscopic and microscopic characteristics, which can be distinguished from other cystic kidney tumors, including cystic nephroma, multilocular cystic renal cell carcinoma and some solid tumors with extensive cystic changes. More cases are needed to ascertain its prognosis. Tubulocystic carcinoma should be considered as a new subtype of renal cell carcinoma in the next revision of the WHO classification.

Keywords Tubulocystic carcinoma of the kidney · Cystic renal tumors · Low-grade collecting duct carcinoma · Immunohistochemistry

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Introduction

Tubulocystic carcinoma (TCC) of the kidney is a very recently described entity [10], which was not illustrated in the last WHO 2004 classification [6].

This rare tumor has been described over the last few decades as a variant of collecting duct carcinoma. In 1956, Masson [11] described a cystic tumor located in the central region of the kidney, with cysts lined by "hobnail cells"; he proposed the denomination "epithelioma bellinien". Thus, in the literature, such a tumor related to the Bellini ducts was translated as a collecting duct (or Bellini duct) carcinoma. In the third series of the AFIP fascicle, published in 1994, collecting duct carcinomas were illustrated either by multicystic tumors or by a number of high-grade solid tumors [13]. Since then, this tumor has been considered to be, and has been published as, a "low-grade collecting duct carcinoma" by McLennan et al. [9], Amin et al. [1], Srigley and Eble [16] and Farah et al. [7].

In 2004 at the USCAP meeting, Amin et al. [2] presented a series of 29 such tumors and renamed them "tubulocystic carcinomas of the kidney". They described a tumor with a very specific aspect. Macroscopically, tumors

Table 1 Clinical features

Case Number	Sex	Age (years)	Tumor size (cm)	Nuclear grade	Therapy	Stage pTNM	Recurrence or metastases
1	M	43	3	2	pN	T1N0M0	data not available
2	M	50	1.7	2	pN	T1N0M0	no
3	M	71	7	2	N	T1N0M0	no
4	M	66	2	2	pN	T1N0M0	data not available
5	M	70	3	2	N	T1N0M0	data not available
6	M	68	3	3	N	T1N0M0	data not available
7	M	54	7	2	N	T1N0M0	no
8	M	55	2.3	2	pN*	T1N0M0	no
9	M	51	2.2	2	N	T1N0M0	no
10	M	30	1.8	3	pN*	T1N0M0	no
11	M	80	3	2	N	T1N0M0	data not available

M man, *pN* partial nephrectomy, *pN** diagnosis on preoperative biopsy, *N* total nephrectomy

had a spongy-like cut surface; microscopically, they displayed a tubulocystic architecture. Four cases with ultrastructural and immunohistochemical studies were added to the previous series and presented at the 2005 USCAP meeting [14]. The authors showed that cells exhibited characteristics of proximal convoluted tubule cells (brush border, expression of CD10 and P504S) and of intercalated cells of the collecting duct (short microvilli, cytoplasmic interdigitation, expression of CK19 and parvalbumin), therefore indicating the absence of a relationship with collecting duct tumors.

Finally, at the end of 2005, McLennan and Bostwick [10] reconsidered their initial series of 13 cases published in 1997 and reported it in a large review entitled "recently described rare renal tumors". They divided it into two separate entities: five cases of mucinous tubular and spindle cell carcinoma and eight cases of TCC, which belonged partly to the series of Amin et al. [2].

Less than 40 cases of TCC have been reported in the literature. Our series comprises 11 cases of TCC of the kidney and includes, for 6 of them, an immunohistochemical study.

Materials and methods

Cases were personal cases or consultation cases (AV) and were reviewed by three pathologists (SA, MS and AV). The diagnostic criteria used were based on the description by Amin et al. [2] in 2004 and MacLennan and Bostwick [10] in 2005. A total of 11 cases of TCC were diagnosed over a period of 11 years (1995–2006). Clinical findings were obtained for all patients and follow-up was conducted for only six of them, with a mean time of 23 months (range 3–72 months). Tumors were staged and graded according to the TNM 2002 classification and Fuhrman's classification.

An immunohistochemical study was performed in six cases. The panel of markers included the following: CK7 (M7018, 1/50, Dako), CK34βE12 (M0630, 1/50, Dako),

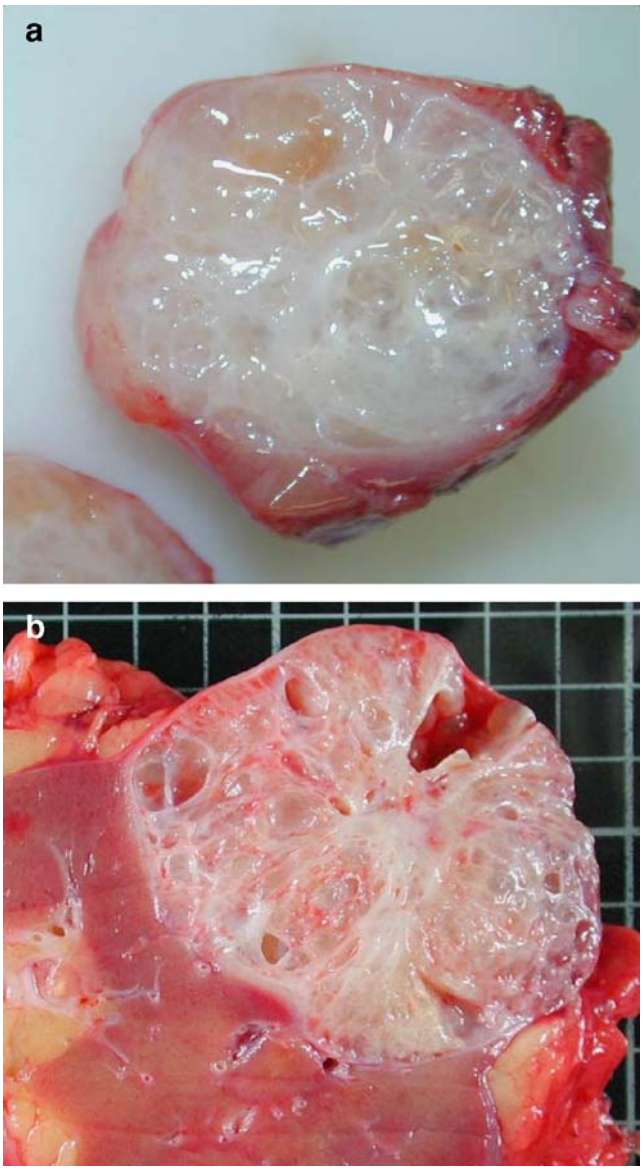

Fig. 1 **a** and **b**: Macroscopic aspect of two different cases of tubulocystic carcinoma of the kidney with both a similar white "bubble-wrap" appearance

CK19 (M0888, 1/25, Dako), CD10 (NCL-CD10-270, 1/10, Novocastra) and P504S (CP200C, 1/20, Biocare-Menarini). Two independent observers (SA and MS) graded the staining on all sections. Grading was as follows: − (no staining), ± (positive and focal staining) and + (positive and diffuse staining).

Results

Clinical findings

Clinical findings are listed in Table 1.

All patients were men, with ages ranging from 30 to 80 years (mean: 56.8 years). Each presented a unilateral renal tumor of 1.7 to 7 cm in diameter (mean: 3.3 cm). For two of them, the diagnosis was made on a pre-operative renal biopsy. All tumors were stage pT1N0M0. The treatment consisted of partial nephrectomy for six patients and total nephrectomy for five patients. No patient received adjuvant therapy. In our series with a mean follow-up of 23 months, none of the six patients presented with recurrence or metastases.

Pathologic features

All tumors showed remarkable uniformity in macroscopic aspects (Fig. 1). They were well delineated from normal parenchyma. The cut surface had a "bubble-wrap" appearance. All consisted of a white and spongy mass made of thin-walled translucent cysts that contained clear watery fluid. No solid components or hemorrhagic or necrotic areas were identified. The mass was confined to the kidney, with no renal capsule penetration or vascular invasion.

Microscopically, tumors were limited but not encapsulated (Fig. 2a). They were entirely composed of cyst structures of variable size and of small tubules (Fig. 2b). They were separated by delicate septa or by fibrotic stroma. The tubules and cysts were lined by flat to columnar eosinophilic epithelial cells (Fig. 2c), producing, in some areas, a "hobnail" appearance (Fig. 2d). Nuclei were regular, round or oval with some prominent nucleoli. Fuhrman nuclear grade was 2 in nine cases and 3 in two cases. No necrosis was seen, and mitotic figures were extremely rare. Neither clear cell areas, papillary projections or "ovarian-like" stroma were identified in any of the cases. All tumors were classified as pT1 (pTNM 2002).

Immunohistochemistry

Immunohistochemical staining results are summarized in Table 2.

Immunohistochemistry showed strong positivity for CK19, CD10 and P504S in all cases. The expression of CK7 was either weak or heterogeneous or both. Five cases were CK34βE12 negative, and in one case (case no. 8), a

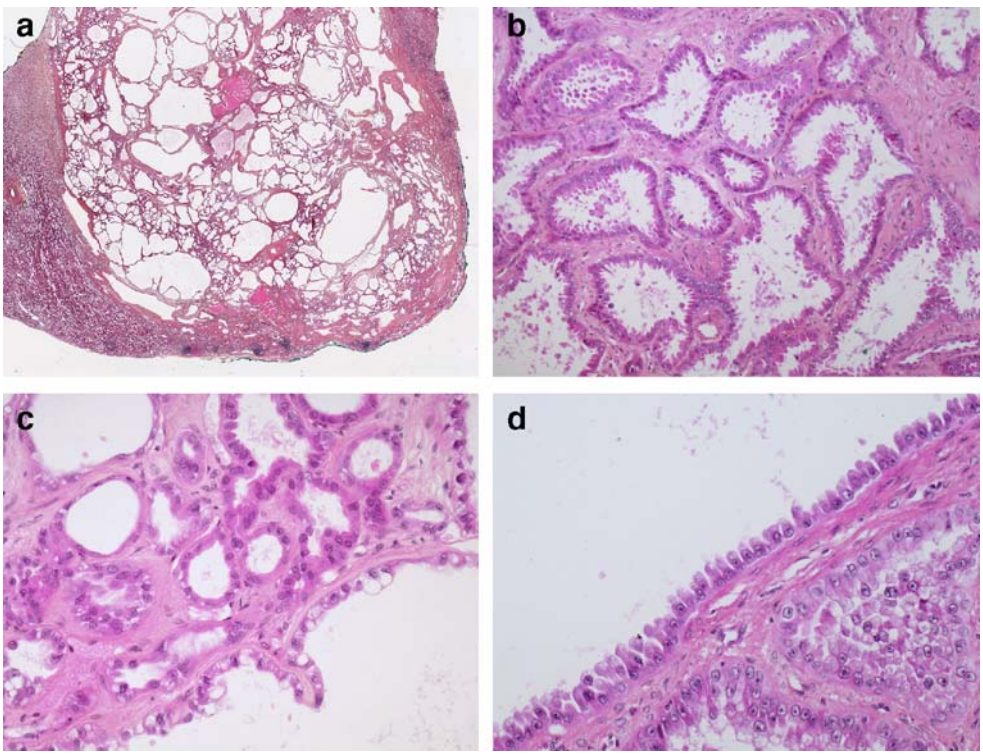

Fig. 2 Microscopic aspects of tubulocystic carcinoma of the kidney; tumor is quite well limited but not encapsulated (**a**; low power field) and is composed of both cyst structures and small tubules (**b**; low power field) which are lined by flat to columnar eosinophilic epithelial cells (**c**; high power field) or by "hobnail" cells (**d**; high power field)

Table 2 Immunohistochemical results

Case n°	CK7	CK34βE12	CK19	CD10	Racémace
1	–	–	+/–	+	+
7	Rare cells +	–	+/–	+	+
8	Rare cells +	+/–	+	+	+
9	Rare cells +	–	+	+	+
10	+/–	–	+	+	+
11	+/–	–	+/–	+	+

few cells were marked with this high molecular weight cytokeratin (Fig. 3).

Discussion

The present study illustrates a distinctive group of tumors renamed "tubulocystic carcinoma" in 2004 by Amin et al. [2].

It is a very rare tumor; to our knowledge, less than 40 cases have been described in the literature. Amin et al. [10], in their series of 29 patients, reported a strong male predominance (6/1). We found the same tendency: All our patients were men. These tumors seem to have a favourable prognosis. In the Amin series, follow-up was obtained for 22 of them, and the authors reported only two cases of metastases (bone and liver metastases). Interestingly, however, the two metastatic tumors displayed areas of clear cells. In our study, follow-up obtained for 6 out of 11 patients showed no recurrence or metastases, with a mean time of 23 months (range, 3–72 months).

In the past, TCC was considered to be a tumor of collecting duct origin and was sometimes confused with collecting duct (Bellini duct) carcinoma, an aggressive infiltrative solid tumor with poor prognosis. Therefore, an "evolving concept" of collecting duct carcinomas was proposed, and low-grade collecting duct carcinoma at the beginning of the spectrum corresponded to the current TCC [9]. However, immunohistochemical and ultrastructural studies demonstrated the poor relationship between TCC and collecting duct tumors [14]. These tumors show expression of proteins of proximal convoluted tubules (CD10 and P504S), distal tubules (CK19) and intercalated collecting duct cells (parvalbumin). Ultrastructurally, they display abundant microvilli with brush border organization as proximal convoluted tubule cells, but with short microvilli and cytoplasmic interdigitation, similar to intercalated cells of the collecting duct [14]. Those observations led to the conclusion that TCC epithelial cells show aberrant renal tubular differentiation. This coexistence of proximal and distal tubules characteristics has been reported in several renal tumors [3, 4, 8, 15] indicating that aberrant tubular differentiation may be a common occurrence in renal neoplasms regardless of their nephron origin. All reported cases present a homogeneous macroscopic and cytoarchitectural appearance: they have a spongy "bubble-wrap" appearance due to small to interme-

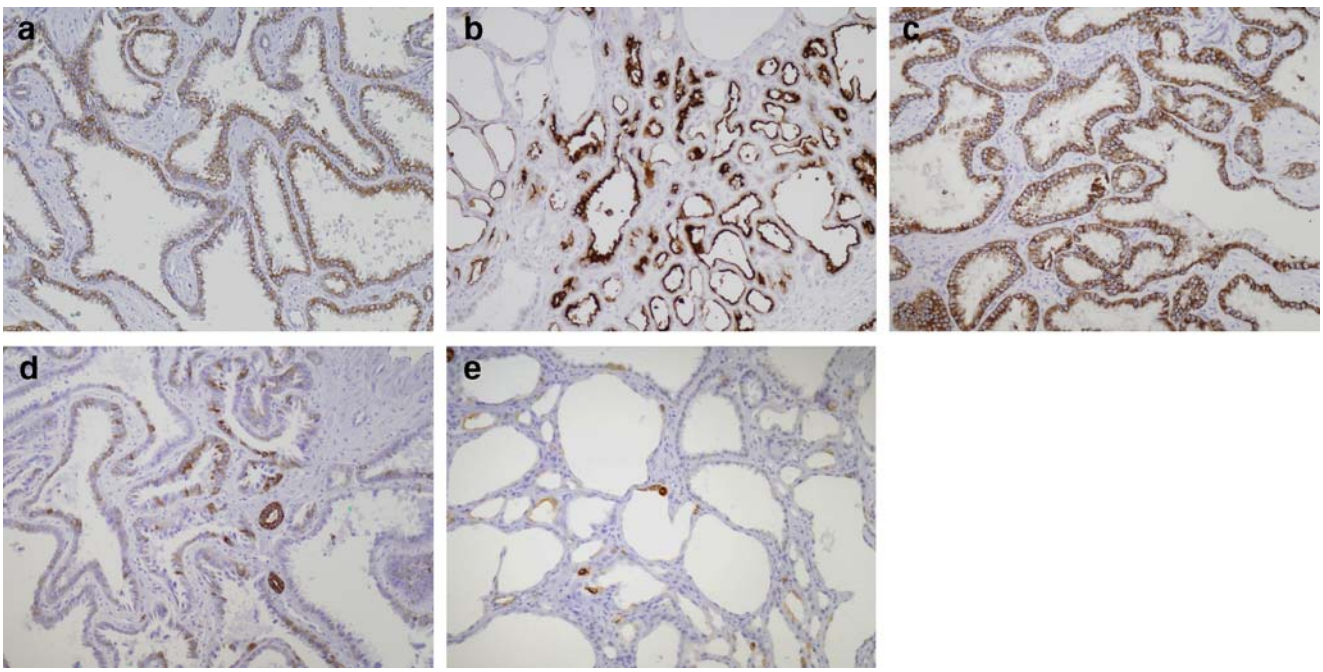

Fig. 3 Immunohistochemical features: strong positivity for CK19 (**a**), CD10 (**b**) and P504S (**c**), weak and heterogeneous positivity for CK7 (**d**) and heterogeneous positivity of few cells for CK34βE12 (**e**)

diate (and occasionally large) cystic structures dispersed within a thin fibrotic stroma.

Two main differential diagnoses among cystic renal neoplasms must be considered: cystic nephroma and multilocular cystic renal cell carcinoma. TCC and cystic nephroma are both composed of multiple cysts lined by a single layered cuboidal epithelium of eosinophilic cells with some areas of hobnail cells. In cystic nephroma, however, cysts are usually larger and contain neither tubules nor epithelial cells in the septa. Of interest, cystic nephroma shows a striking female predominance (8:1), whereas TCC occur more often in men [12].

Low-grade multilocular cystic renal cell carcinoma was described in the WHO 2004 classification as a new entity [6]. This tumor is a variant of clear cell carcinoma displaying large cysts lined by clear cells, separated by fibrous septa containing nests of clear cells [5]. Moreover, some chromophobe carcinomas and some oncocytoma may be entirely cystic with no solid areas, but cysts are lined, respectively, by chromophobe cells or, oncocytic cells. In the later, oncocytic cells are usually cuboidal with round central nuclear and are classically CD10 and CK7 negative by immunochemistry [17, 18].

In conclusion, TCC are a distinctive group of kidney tumors with a male predominance and noteworthy macroscopic (bubble-wrap appearance) and microscopic (cysts lined by hobnail cells and separated by a thin fibrotic stroma) characteristics. The examination of more cases is warranted so as to better understand the biology of this tumor, to ascertain its prognosis and to choose appropriate treatment. It is suggested that TCC will be described in the next revision of the WHO classification.

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ORIGINAL ARTICLE

Contraction of tubulointerstitial fibrosis tissue in diabetic nephropathy, as demonstrated in an in vitro fibrosis model

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Abstract Tubulointerstitial fibrosis in diabetic nephropathy (DN) was investigated using an in vitro tissue model of remodeling, to determine the pathogenic mechanism of fibrosis that leads to renal atrophy, i.e., renal failure. The remodeling model consisted of a renal fibroblast-populated collagen lattice (FPCL). The overexpression of transforming growth factor (TGF)-β1 in the diabetic kidney gave rise to FPCL contraction. FPCL relaxation was induced by the subsequent addition of cytochalasin D. The FPCL failed to contract when exposed to TGF-β1 plus Y27632, a Rho kinase inhibitor. TGF-β1 induced the phosphorylation of myosin light chains, and Y27632 blocked this activity. TGF-β1-induced FPCL contraction was suppressed by the addition of 2,3-butanedione monoxime, a myosin ATPase inhibitor. As shown in the video, the contraction rate of the projections of the cells in the FPCL was significantly greater in the TGF-β1 group than in the control group. Collectively, these results indicate that TGF-β1-induced FPCL contraction is attributable to actin–myosin interactions in the fibroblasts through the activation of Rho kinase, the phosphorylation of myosin light chains, and the subsequent activation of myosin ATPase. We propose that via these mechanisms, tubulointerstitial fibrosis generates tissue contraction that leads to renal atrophy and renal failure in DN.

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Keywords Diabetic nephropathy · Tubulointerstitial fibrosis · Fibroblast-populated collagen lattice · Contraction · Transforming growth factor β1

Introduction

Diabetic nephropathy (DN) is one of the most serious complications in diabetic patients. DN results in chronic renal failure. Morphologically, it is well known that glomerulosclerosis, which includes glomerular basement membrane thickening and mesangial expansion, and tubulointerstitial fibrosis occur at the early stages of DN. It has recently been demonstrated that the development of tubulointerstitial lesions is more closely correlated with a progressive decline in renal function compared to glomerular lesions [5, 40]. Tubulointerstitial fibrosis ultimately leads to renal failure through renal atrophy in DN. The mechanism by which fibrosis leads to renal atrophy has not yet been determined.

Transforming growth factor (TGF)-β1, which is a profibrogenic cytokine, is overexpressed in the DN kidney, especially in the interstitial cells and proximal tubular epithelial cells that are involved in tubulointerstitial fibrosis [29, 47, 52]. It is well known that TGF-β1 causes fibrosis in various disorders [6]. TGF-β1 stimulates the production of extracellular matrix (ECM), including type I collagen, by fibroblasts and myofibroblasts. In addition, TGF-β1 attenuates the degradation of ECM deposits, thereby promoting ECM accumulation. The inhibition of ECM degradation results from the reduced production of matrix metalloproteinases and the increased production of tissue inhibitor of metalloproteinase-1 and plasminogen activator inhibitor-1 by infiltrating cells and other types of cells [14,

32, 39]. TGF-β1-mediated intracellular signaling occurs mainly via the Smad pathway. TGF-β1 binds to the type II receptor, which as a consequence recruits and phosphorylates the type I receptor. The activated type I receptor then phosphorylates Smad2 and Smad3. Activated Smad2 and Smad3 form heteromers with the common partner Smad4 and then translocate into the nucleus to regulate target gene expression [11, 57].

On the other hand, it has been demonstrated that TGF-β1 evokes the contraction of granulation tissues [19, 44] or fibrotic tissues [28]. TGF-β1-induced tissue contraction has been revealed to occur via activation of the small G protein Rho, using an in vitro granulation or fibrosis tissue model that consists of a fibroblast-populated collagen lattice (FPCL) [51]. Although the pathway through which TGF-β1 activates Rho has not been clarified, it appears to be independent of the Smads [13, 33]. Activation of Rho induces the formation of stress fibers and focal adhesions [1, 49]. Rho also activates Rho kinase [30, 41, 42], which in turn gives rise to actomyosin contraction through phosphorylation of the myosin regulatory light chain (MLC) [2, 9]. Phosphorylation of MLC occurs in different ways depending on the cell type. In smooth muscle cells, the increased level of intracellular Ca^{2+} plays a dominant role in contraction. An increase in the level of intracellular Ca^{2+} activates Ca^{2+}-dependent MLC kinase, which is followed by the phosphorylation of MLC [25, 34, 53]. In nonmuscle cells, such as fibroblasts [8, 46, 50] and endothelial cells [20], cell contraction is predominantly generated in a Ca^{2+}-independent manner. MLC is directly phosphorylated by Rho kinase in these cells, independently of MLC kinase. For both MLC kinase-dependent and Rho kinase-dependent MLC phosphorylation, the phosphorylation site is Ser-19. In addition, Rho kinase phosphorylates the myosin-binding subunit of MLC phosphatase, resulting in the inhibition of MLC phosphatase activity [37]. As a consequence, contraction is induced through increased phosphorylation of MLC. In addition, several reports have shown that Rho kinase induces TGF-β signaling [10, 36, 48], leading to the positive feedback system. Ultimately, after contraction of the FPCL, the fibroblasts undergo apoptosis [12, 22, 54, 56].

To the best of our knowledge, the present study is the first to investigate the mechanism of fibrosis tissue contraction in DN using renal fibroblasts (NRK 49F cells) populated in a collagen lattice, in the form of an in vitro model of fibrosis tissue. Grinnell [21] has previously described that contraction of floating FPCL gives rise to a mechanically relaxed tissue whose cells have morphological and proliferative features resembling dermis or scar, whereas FPCL anchored to the bottom of a culture dish develop into a stressed tissue resembling granulation tissue. Miura et al. [43] showed contraction of the floating collagen lattice with the cells as a model of contraction of fibrous tissue. Anchored FPCL, a model of fibrosis tissue as well as granulation tissue, could also contract in the direction of top and bottom. We use these models to determine whether cell contraction actually occurs during FPCL contraction, and we show that TGF-β-induced contraction is generated by activation of the Rho kinase pathway, leading to the activation of myosin ATPase.

Materials and methods

Materials Materials and chemicals were purchased as follows: type I collagen solution (Nitta Gelatin, Osaka, Japan), recombinant human TGF-β1 (R&D Systems, Minnesota, USA), cytochalasin D (Sigma Chemical, Missouri, USA), the specific Rho kinase inhibitor Y27632 (CalBiochem, California, USA), and hydroxyfasudil, a potent and selective Rho kinase inhibitor, and 2,3-butanedione monoxime (BDM), an inhibitor of myosin ATPase activity (Sigma-Aldrich, Missouri, USA). Rabbit polyclonal antibody against phospho-MLC (Ser19) was purchased from Santa Cruz Biotechnology (California, USA). Rhodamine-conjugated phalloidin (Cytoskeleton, Colorado, USA) was used to stain actin filaments.

Cell culture Normal rat kidney fibroblasts (NRK 49F cells) were obtained from the RIKEN Cell Bank (Tsukuba, Japan). Cells were maintained in Dulbecco's Minimal Essential Medium (D-MEM; Nissui Pharmaceutical, Tokyo, Japan) that contained 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 10% fetal bovine serum (FBS; JRH Biosciences, Kansas, USA) at 37°C in a humidified, 5% CO_2 atmosphere. Cells from passage 3 to passage 8 were used in the experiments described below.

FPCL contraction assay A collagen solution (7 ml of 3 mg ml^{-1} stock) was gently mixed with 2 ml of fivefold-concentrated D-MEM on ice. The mixture was adjusted to pH 7.4 with 1 ml of 200 mM hydroxyethyl piperazineethanesulfonic acid plus 2.2% $NaHCO_3$ in distilled water. This collagen mixture was blended with 1 ml of NRK 49F cell suspension (2.5×10^6 cells ml^{-1}). The resultant mixture (0.4 ml) was added to each well of a 24-multiwell plate (Becton-Dickinson Labware, New Jersey, USA), and the formation of the collagen gel (FPCL) was induced by incubation for 30 min at 37°C. After gelatinization, 1 ml of D-MEM that contained 10% FBS was added to each well. The FPCLs were then detached from the lateral wall and bottom of each well with a microspatula and used for the experiments. The diameter of the FPCL was measured with a ruler at the indicated time points, to assess FPCL contraction after treatment with reagents.

Fluorescence microscopy After the contraction experiments, the FPCLs were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) for 10 min. After rinsing, the cells in the collagen lattices were permeabilized with 0.5% Triton X-100 in PBS for 20 min, washed with PBS, and stained with rhodamine-phalloidin for 30 min. After staining, the lattices were rinsed with PBS and observed and photographed using an Olympus BX 60 microscope equipped with epifluorescence optics. FPCLs anchored to the bottoms of two-well Labtek chamber slides (AS ONE, Osaka, Japan) were also subjected to the experimental procedure mentioned above. To determine the phosphorylation level of MLC in NRK 49F cells after treatment with TGF-β1, a monolayer culture of the cells (1×10^4 cells ml^{-1}) was grown in a two-well Labtek chamber slide. After stimulation of the monolayer culture, the cells were fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 10 min. After rinsing, the cells were permeabilized with 0.5% Triton X-100 in PBS for 20 min and washed with PBS. The cells were then incubated with the primary antibody against phospho-MLC for 2 h at room temperature (RT). After washing, they were incubated with goat anti-rabbit fluorescein isothiocyanate-conjugated secondary antibody (Wako Pure Chemical, Osaka, Japan) for 1 h at RT. After staining, the cells were rinsed with PBS, observed, and photographed. As a negative control, the primary antibody was replaced with mouse or rabbit nonimmune serum.

Transmission electron microscopy After the contraction experiments, the FPCLs were cut into pieces. The pieces were fixed in twofold-diluted Karnovsky's fixative [35] for 10 min at 4°C and then washed with 0.1 M cacodylate buffer and postfixed in 2% osmium tetroxide–0.05% potassium ferrocyanide for 30 min at 4°C. The specimens were dehydrated in an ascending series of ethanol and embedded in epoxy resin. Ultrathin sections were cut on an ultramicrotome (LKB 2088 Ultrotome V; LKB, Bromma, Sweden), mounted on copper grids, and stained with methanolic uranyl acetate and lead citrate. The sections were observed and photographed under a transmission electron microscope (TEM-1200 EX II; JEOL, Tokyo, Japan) at 80 kV.

Analyses of cell projection movements FPCLs were anchored to the bottoms of glass-bottomed dishes (Matsunami, Osaka, Japan). Under phase-contrast microscopy, cell contraction, which was exhibited as expansion and contraction of the projections of NRK 49F cells in the anchored collagen lattice, was monitored with time lapse video microscopy for 24 h from 16 h after treatment. The video camera linked to the TE-2000-E microscope (Nikon, Tokyo, Japan) was controlled using the Luminavision software (O-kumashokai, Fukuoka, Japan). Images were acquired at 5-min intervals, stored on a computer, and analyzed by Dipp-Motion 2D (DITECT, Tokyo, Japan).

Statistical analysis The experimental data are presented as mean ± SD. Statistical analyses were performed with the Student's *t* test. Differences were considered to be statistically significant at $p<0.05$.

Results

TGF-β1 enhances collagen lattice contraction by NRK 49F cells

Under control conditions, although the FPCLs did not contract fully on day 1 (Fig. 1a), they contracted progressively over the 5-day culture period. FPCL contraction was

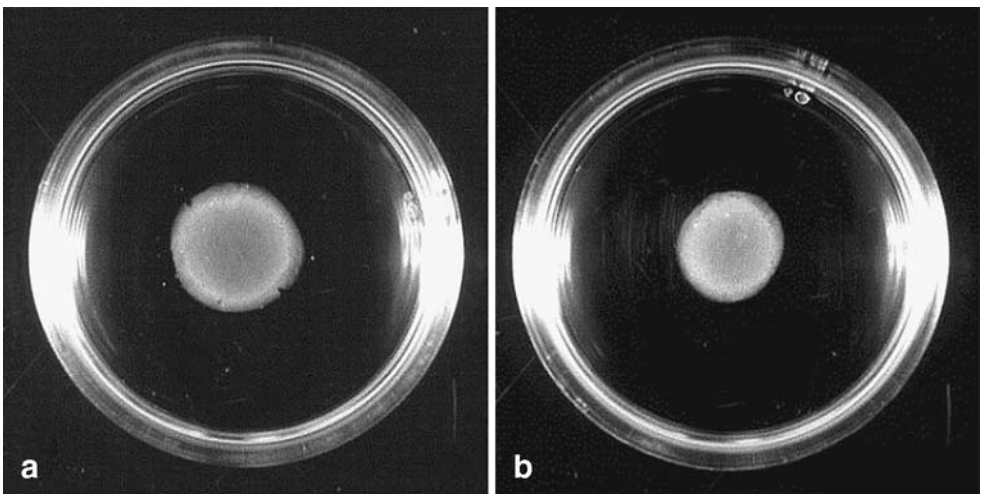

Fig. 1 FPCL contraction produced by TGF-β1. FPCLs were cultured for 1 day in 10% FBS/D-MEM (control, **a**) or 10% FBS/D-MEM that contained 5 ng/ml TGF-β1 (**b**). Photographs show FPCL contraction induced by the addition of TGF-β1 (**b**) but not by the control medium (**a**)

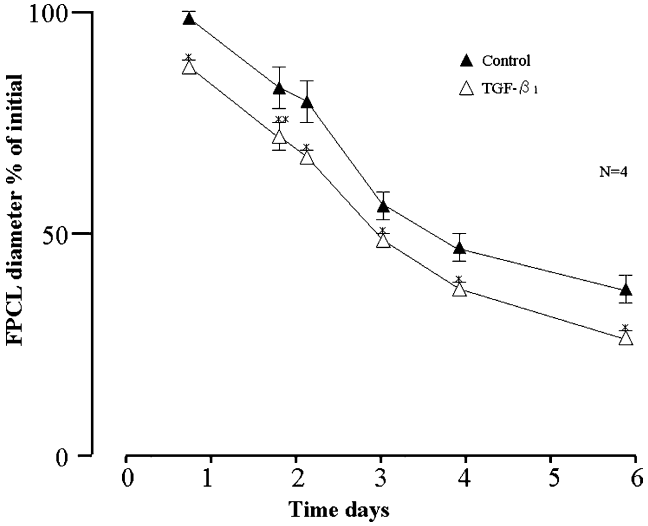

Fig. 2 Time course of FPCL contraction caused by TGF-β1. The FPCL was cultured in 10% FBS/D-MEM in the absence (*closed triangles*) or presence (*open triangles*) of TGF-β1. TGF-β1 significantly enhances FPCL contraction compared to the control over the entire 6-day incubation period. Results are presented as mean ± SD. *Asterisk*, $p<0.001$ and *double asterisk*, $p<0.01$ vs control at each time point

significantly enhanced by the addition of 5 ng ml^{-1} TGF-β1 over the entire 6-day incubation period starting on day 0 (Figs. 1 and 2). On day 1, the diameter of a FPCL treated with or without TGF-β1 was 86.3±1.1 or 99.3±1.1% of the initial FPCL diameter, respectively ($p<0.001$).

TGF-β1 does not affect the formation of stress fibers during FPCL contraction

Electron microscopy identified bundles of actin microfilaments, the so-called stress fibers, in the cytoplasm of NRK 49F cells in the collagen lattices from both the TGF-β1-treated group and the control group. However, there were no differences in the numbers of bundles between the two groups on day 1 (Fig. 3). Under fluorescence microscopy, rhodamine-phalloidin staining of filamentous actins was detected in the cytoplasm of the cells in the FPCL on day 1 (Fig. 4). Microfilaments were present with similar numbers and distributions in cells with and without TGF-β1 treatment.

Inhibition of actin polymerization by cytochalasin D relaxes TGF-β1-induced FPCL contraction

To confirm the participation of intracellular actin microfilaments in FPCL contraction, cytochalasin D, which is an inhibitor of actin polymerization, was used. The FPCL contracted to 74.1±1.7% of its initial diameter after treatment with TGF-β1 for 2 days and relaxed significantly to 79.7±1.2% of its initial diameter 5 h after the addition of 6 μM cytochalasin D ($p<0.001$) (Fig. 5). On the other hand, the FPCL contracted to 71.1±1.1% of its initial diameter without cytochalasin D in the same time period. FPCL recontracted after the removal of cytochalasin D and retreatment with TGF-β1 after FPCL relaxation. Rhodamine-phalloidin staining demonstrated that the microfilament

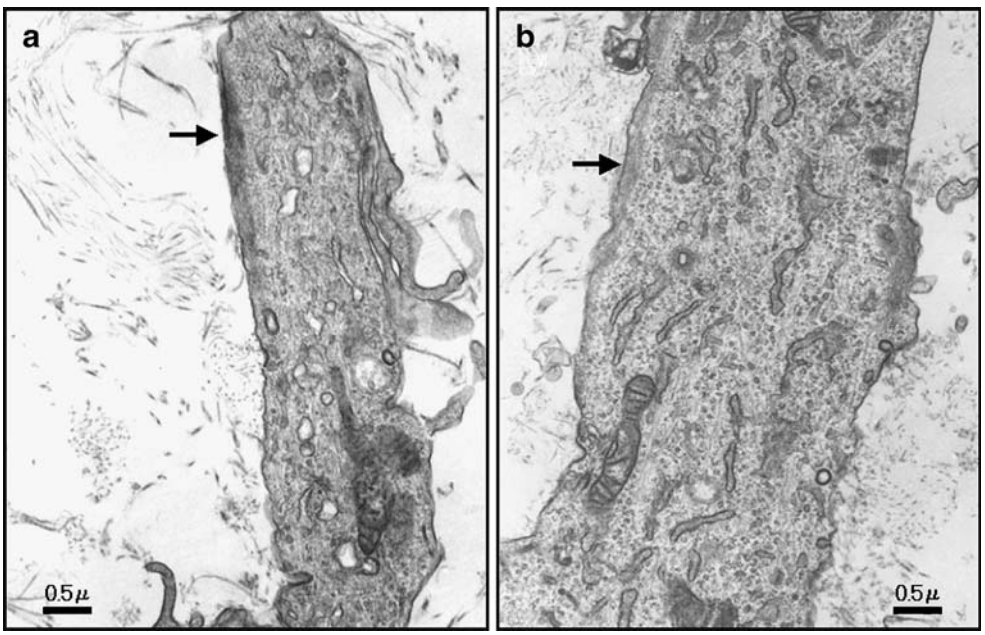

Fig. 3 TEM of bundles of cellular actin microfilaments. The FPCL was cultured in medium with (**b**) or without (**a**) TGF-β1 for 1 day. Bundles of actin microfilaments are present with similar numbers and distributions in cells with and without TGF-β1 treatment. *Arrows* show bundles of actin microfilaments. *Bar*, 0.5 μm

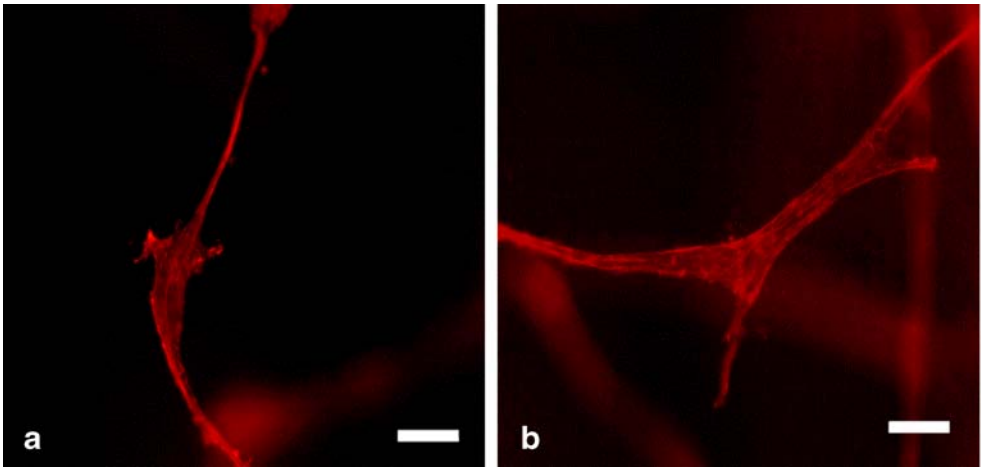

Fig. 4 Effect of TGF-β1 on the fluorescence of rhodamine-phalloidin. Filamentous actin is visualized by staining with rhodamine-labeled phalloidin. **a** Control, **b** TGF-β1-treated FPCL on day 1. There are no differences in the numbers and distributions of actin bundles between the two groups. *Bar*, 20 μm

structures were disrupted in the cells of the FPCL that were relaxed by cytochalasin D and that the cells were assuming a rounded shape (Fig. 6). After the removal of cytochalasin D, cell shape, as well as contractile activity, was restored (data not shown).

Contribution of Rho kinase pathway to TGF-β1-induced FPCL contraction

To determine whether Rho kinase activation is required for TGF-β1-induced FPCL contraction, two structurally different Rho kinase inhibitors, Y27632 and hydroxyfasudil, were used. FPCL contraction was assayed after culture in medium with or without 5 ng ml^{-1} TGF-β1 in the presence or absence of 10 μM Y27632 or 20 μM hydroxyfasudil for 1 day. The stimulatory effect of TGF-β1 on FPCL contraction

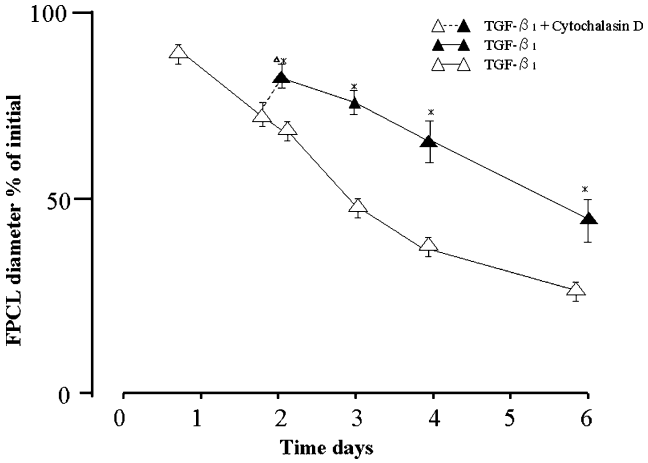

Fig. 5 Effect of cytochalasin D on TGF-β1-induced FPCL contraction. Cytochalasin D (6 μM) was added to medium that contained TGF-β1 on day 2. FPCL diameter was measured 5 h after the addition of cytochalasin D. Cytochalasin D induces the relaxation of FPCL that contracted with TGF-β1 treatment. After FPCL relaxation, the removal of cytochalasin D and retreatment with TGF-β1 leads to FPCL recontraction. Results are presented as mean ± SD. *small open triangle*, $p<0.001$ vs FPCL just before the addition of cytochalasin D; *asterisk*, $p<0.001$ vs FPCL with TGF-β1 alone at each time point

was completely blocked by Y27632 or hydroxyfasudil (Fig. 7). On the other hand, there were no differences in cell shape and rhodamine-phalloidin staining between the control, TGF-β1, and TGF-β1 plus Y27632 groups in floating FPCLs (Fig. 8). In anchored FPCLs, the major part undergoing contraction also revealed no differences in cell shape and phalloidin staining between the three groups. Those morphological features were similar to those in floating FPCLs. Cells with conspicuous stress fibers were sparsely observed in the control group as well as the TGF-β1 group near the part of FPCL attaching to the bottom of the culture dish (data not shown).

TGF-β1 activates Rho kinase leading to the phosphorylation of MLC

To examine whether phosphorylation of MLC is involved in TGF-β1-induced FPCL contraction, immunostaining for phospho-MLC was performed. On day 1, TGF-β1 augmented phospho-MLC immunostaining in the cytoplasm of NRK 49F cells in monolayer culture. Furthermore, Y27632 downregulated phospho-MLC immunostaining that was enhanced by TGF-β1 (Fig. 9).

Actin–myosin interaction via myosin ATPase activation in TGF-β1-induced FPCL contraction

The myosin ATPase inhibitor BDM was used to investigate the contribution of myosin ATPase activation as the final step leading to cell contraction by actin–myosin interaction. BDM suppressed TGF-β1-stimulated FPCL contraction in a dose-dependent manner after 4 h of incubation (Fig. 10).

Accelerated cell contraction during collagen lattice contraction

In the filmed study, anchored FPCL was attached exclusively to the bottoms of the dishes. Because FPCL was not

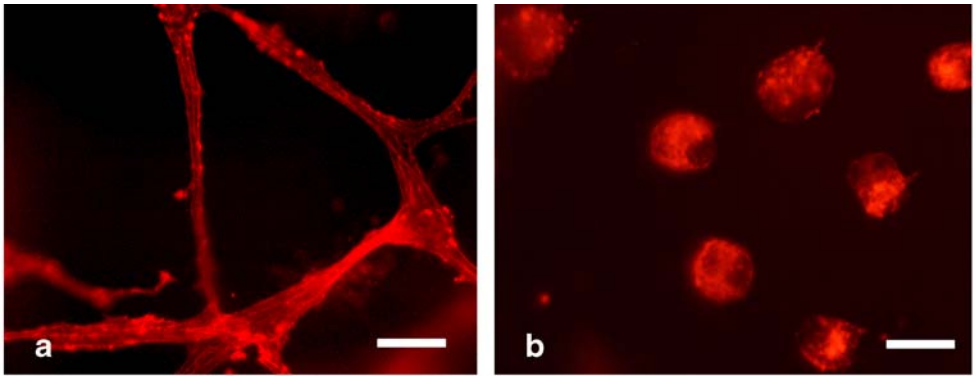

Fig. 6 Effect of cytochalasin D on the fluorescence of rhodamine-phalloidin. Rhodamine-phalloidin staining was performed 5 h after the addition of cytochalasin D. **a** TGF-β1 alone, **b** TGF-β1 plus cytochalasin D. Cytochalasin D disrupts the microfilament structures and makes the cells assume a rounded shape (**b**). *Bar*, 20 μm

attached to the lateral walls of the dishes, the FPCL could only contract in the directions of top and bottom. FPCL contracted in response to TGF-β1 on day 1, with the cells that were in focus at the beginning of the movie becoming gradually more blurred. The cells in the FPCL that was incubated with control medium remained in focus from the beginning to the end of the culture period. The cell motility movie sequence revealed that cell projections extended in every direction and contracted in turn. In the control FPCL, a projection (arrow) of the cell was observed to elongate from 0 to 390 min (Fig. 11a). Another projection (arrowhead) of the cell extended from 50 to 185 min and contracted at the rate of 0.49 μm min^{-1} until 390 min (Fig. 11a). On the other hand, two projections (single arrow and double arrow) contracted more rapidly at 1.41 (single arrow) and 1.38 μm min^{-1} (double arrow) in the TGF-β1-stimulated FPCL than in the control FPCL (Fig. 11b). Another projection of the cell (closed arrowhead) both branched and extended (Fig. 11b). One (open arrowhead) of the branches elongated and subsequently underwent intensive contraction at 2.00 μm min^{-1} from 340 to 390 min (Fig. 11b). Thus, the expansion and contraction of cell projections were repeated in the collagen lattice for a 24-h period from 16 h after the beginning of the culture (Fig. 11). The total distance by which the cell projections contracted for 17 h was significantly longer in the collagen lattice treated with TGF-β1 than in the control lattice, i.e., 267±61 and 131±51 μm, respectively ($p<0.01$; Fig. 12). Collagen fibers in the background were observed to move concomitant with the extension and contraction of the cell projections, although this movement could not be measured in detail.

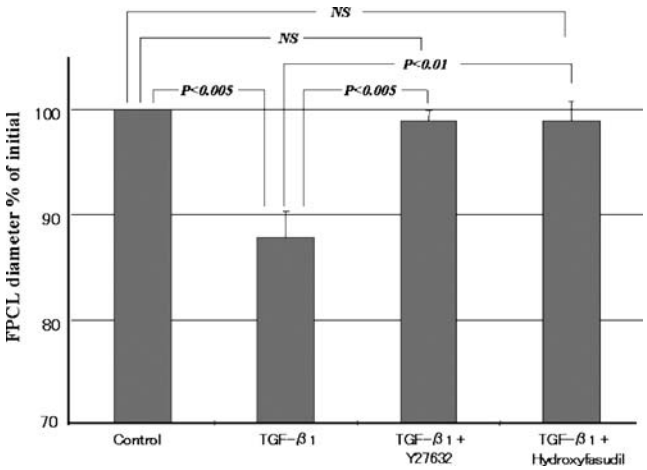

Fig. 7 Effect of Rho kinase inhibitor on TGF-β1-induced FPCL contraction. Y27632 (10 μM) or hydroxyfasudil (20 μM) was added to the FPCL in 10% FBS/D-MEM with TGF-β1 at the beginning of the culture period. FPCL diameters were measured on day 1. TGF-β1-induced FPCL contraction is fully blocked by each of the two Rho kinase inhibitors. Results are presented as mean ± SD

Discussion

TGF-β1 produced greater FPCL contraction than the control treatment during the experimental period. This result is compatible with those of a number of studies reported to date [17, 19, 44, 55]. Although FPCL contraction occurred in response to TGF-β1 on day 1 but not in the control medium, there was no difference in the morphology of the bundles of actin microfilaments between the TGF-β1-treated and control groups, as shown by transmission electron microscopy and fluorescence microscopy. The number of cells in the FPCL is known to affect FPCL contraction [4, 23, 24]. There were no differences in cell numbers between the TGF-β1-treated and control groups on day 1 (data not shown). The FPCL contracted by TGF-β1 expanded in response to cytochalasin D. Fluorescence microscopy revealed few bundles of actin microfilaments, and the cells in the FPCL became rounded. The change in actin microfilaments was reversible. These

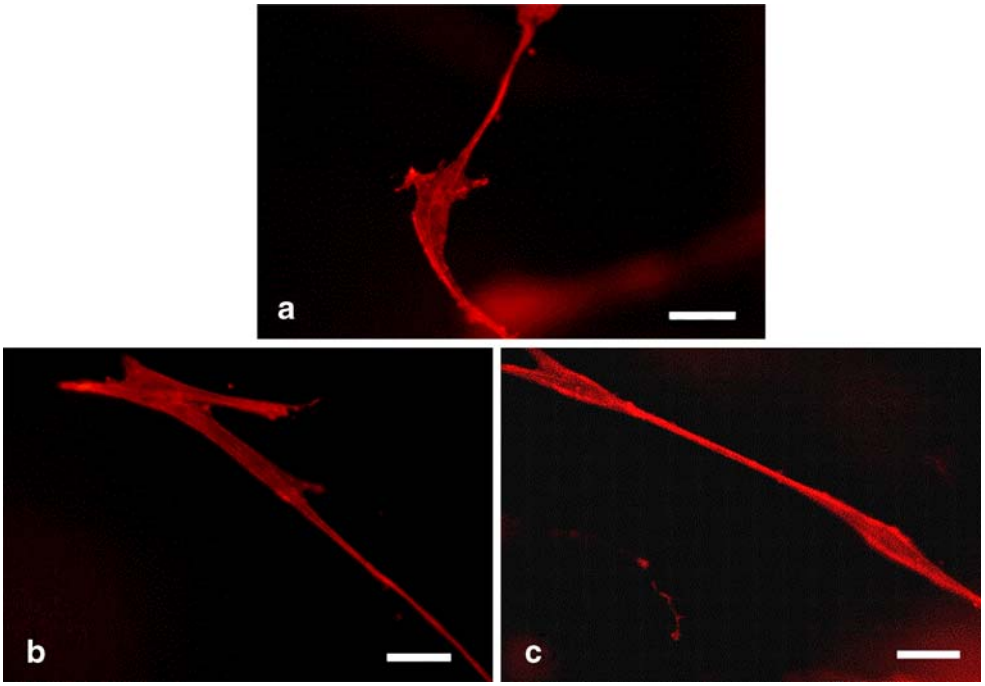

Fig. 8 Effect of Y27632 on the fluorescence of rhodamine-phalloidin. Y27632 was added to the medium that contained TGF-β1 at the beginning of the culture period. Rhodamine-phalloidin staining was performed on day 1. **a** Control; **b** TGF-β1 alone; **c** TGF-β1 plus Y27632. There are no differences of rhodamine-phalloidin staining between the three groups. *Bar*, 20 μm

results suggest that the formation of filamentous actin is necessary for FPCL contraction.

We investigated whether TGF-β1-induced FPCL contraction was mediated by the Rho kinase pathway. The addition of two structurally different Rho kinase inhibitors, Y27632 and hydroxyfasudil, completely blocked FPCL contraction induced by TGF-β1 on day 1. However, in contrast to cytochalasin D treatment, the addition of the Rho kinase inhibitor did not affect the formation of actin microfilament bundles. There was no difference in actin bundle formation between the control and TGF-β1-treated groups and the TGF-β1 plus Rho kinase inhibitor groups. Furthermore, there was no difference in cell numbers between the three groups (data not shown). The inhibitory

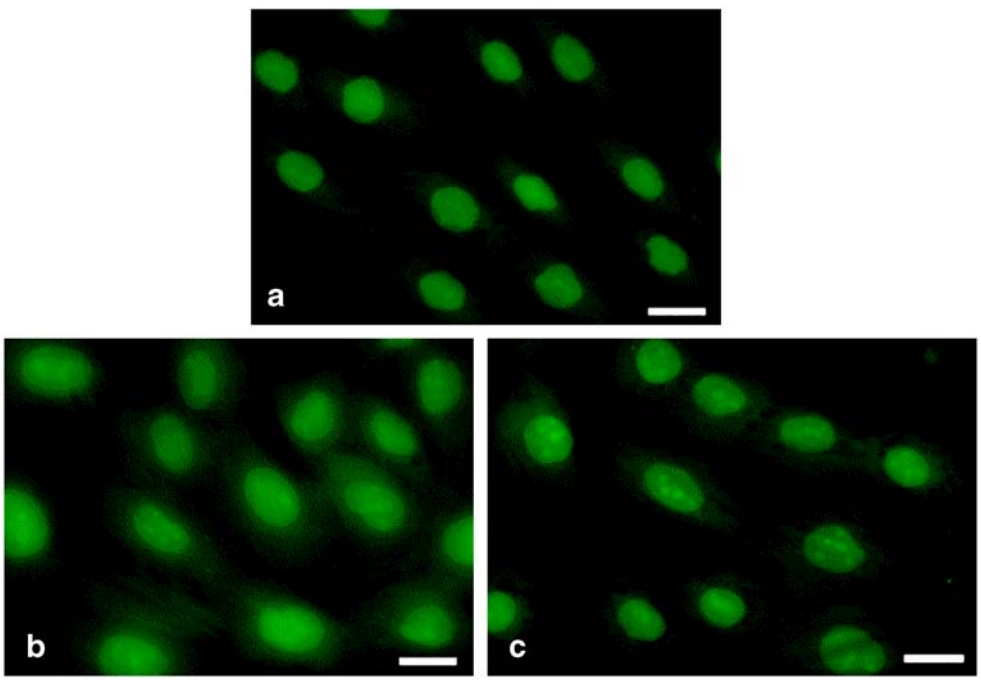

Fig. 9 Effect of TGF-β1 on the immunofluorescence of phospho-MLC. Immunostaining for phospho-MLC was performed on monolayers on day 1. **a** Control; **b** TGF-β1 alone; **c** TGF-β1 plus Y27632. Phospho-MLC immunostaining is enhanced in the cytoplasm by TGF-β1 (**b**) and downregulated by the addition of Y27632 (**c**). *Bar*, 10 μm

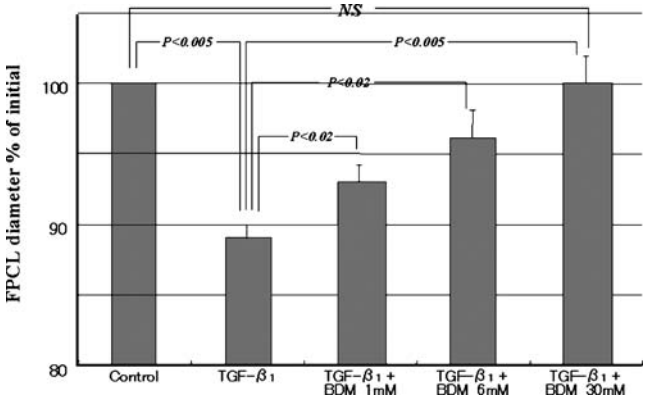

Fig. 10 Effect of BDM on TGF-β1-induced FPCL contraction. The myosin ATPase inhibitor BDM (1, 6, and 30 mM) was added to medium that contained TGF-β1 on day 1. FPCL diameter was measured 4 h after the addition of BDM. BDM represses TGF-β1-induced FPCL contraction in a dose-dependant manner. Results are presented as mean ± SD

effect of Y27632 has been reported to be specific for Rho kinase at 10 μM [31]. TGF-β1 causes FPCL contraction via Rho kinase pathway in NRK 49F cells. The Rho kinase pathway has been demonstrated to play a pivotal role in the contraction of granulation tissues [19, 44] and fibrous tissues [28]. In renal tissues, Rho kinase has been reported to contribute to the development of renal tubulointerstitial fibrosis in various disorders [10, 36, 45, 48]. With regard to FPCL contraction and renal cells, TGF-β1 has been shown to produce contraction of a mesangial cell-populated collagen lattice [38]. However, that report did not include any information regarding the intracellular signaling pathway of TGF-β1-induced contraction. To the best of our knowledge, the present study is the first to demonstrate that TGF-β1 causes the contraction of a renal FPCL via the Rho kinase pathway. It is well known that TGF-β1 transdifferentiates fibroblasts and epithelial cells into myofibroblasts,

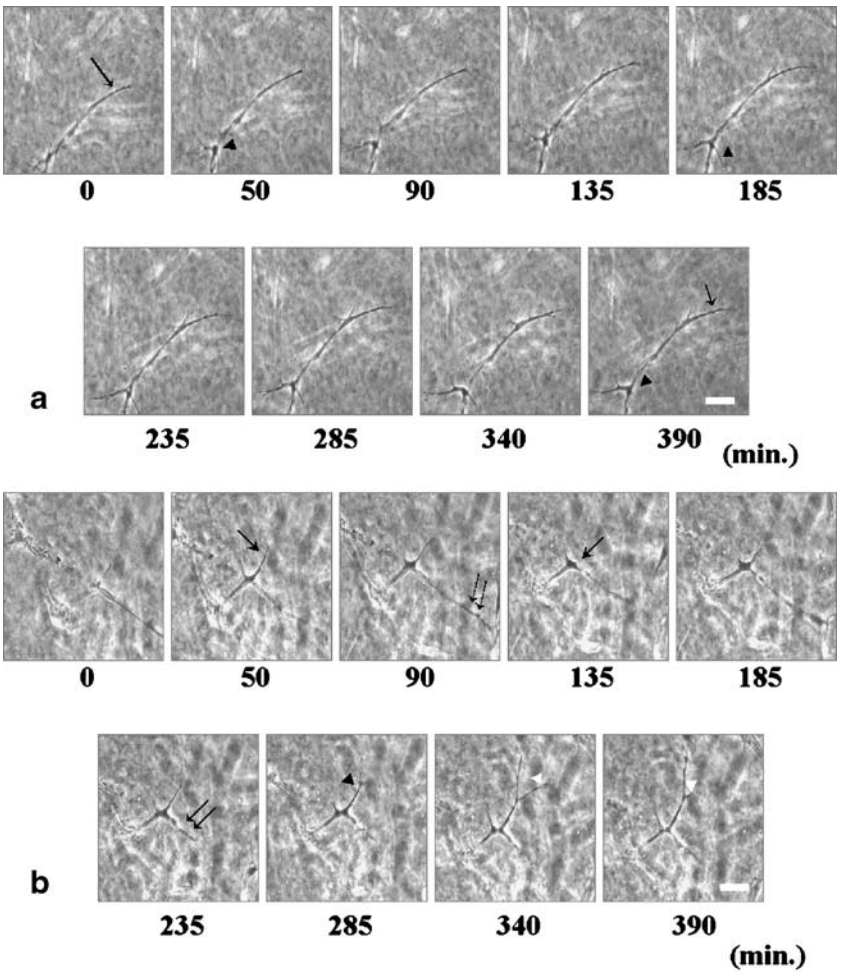

Fig. 11 Motilities of cell projections in the FPCL. Under phase-contrast microscopy, the movie shows the expansion and contraction of cell projections in the FPCL for 390 min from 16 h after initiation of the culture. **a** Control; **b** TGF-β1-treated FPCL. The *arrow* in **a** shows a projection that is elongating slowly. The *arrowhead* in **a** indicates a projection that is elongating and that subsequently contracts. The *single arrow* and *double arrow* in **b** show contracting projections. The *closed arrowhead* in **b** reveals a branching projection. The *open arrowhead* in **b** shows the rapid extension and contraction of a branched projection. *Bar*, 100 μm

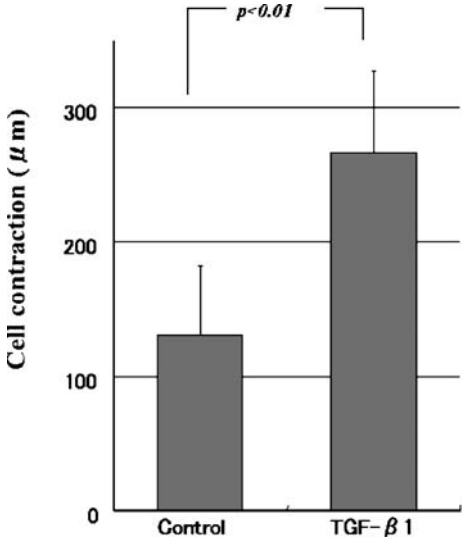

Fig. 12 Contraction distance of cell projections. The total contraction distances of five cell projections were measured for 17 h from 16 h after the initiation of culture. TGF-β1 enhances the contraction of the cell projections. Results are presented as mean ± SD

which are characterized by the expression of α-smooth muscle actin [16, 29]. Myofibroblasts contribute to the progression of fibrosis. In addition, it has been reported that this response to TGF-β1 is mediated by the Rho kinase pathway [26]. However, whether or not myofibroblast transdifferentiation contributes to TGF-β1-induced FPCL contraction remains a controversial issue [3, 15]. Furthermore, TGF-β1 increased phosphorylated MLC in the cytoplasm of NRK 49F cells, as shown by immunohistochemistry on day 1. Y27632 downregulated MLC phosphorylation induced by TGF-β1. These results suggest that Rho kinase evokes the phosphorylation of MLC, after the activation of Rho kinase by TGF-β1. TGF-β1-induced FPCL contraction could have taken place via Rho kinase-dependent MLC phosphorylation. Furthermore, we examined whether myosin-based contractility is essential for FPCL contraction. BDM, which is an inhibitor of myosin ATPase activity, repressed FPCL contraction induced by TGF-β1. It seems likely that phosphorylated MLC activates myosin ATPase, leading to actin–myosin interaction and subsequent cell contraction.

In the filmed sequence, the relationship between collagen lattice contraction and cell motility was examined. The expansion and contraction of cell projections were repeated in a collagen lattice. The rate of cell projection contraction per hour was significantly higher in the FPCL contracted by stimulation with TGF-β1 than in the noncontracted control FPCL. This result suggests a close relationship between the FPCL and cell motility. Cell–matrix interactions would be required for FPCL contraction after cell contraction. Cell–matrix interactions are thought to include cell extension, the formation of focal adhesions at the tip of the cell, intracellular force generation through actin–myosin interaction, and the breakdown of the focal adhesions. In the current study, cell extension and actin–myosin interaction were associated with FPCL contraction. In our preliminary study, focal adhesions were observed to be enlarged to a greater extent in response to TGF-β1 in the monolayer culture on a plastic plate compared to the control (data not shown). The significance of this finding remains to be defined. It may reflect the fact that stronger cell–matrix adhesion is required for more intensive contraction of the cell–matrix complex by TGF-β1 than by the control. Thus, cell contraction is considered to lead to FPCL contraction. In the present study, it appears that DN results in the contraction of fibrotic tissues through fibroblast contraction.

In a previous study, it was reported that collagen lattices contraction was prevented by the formation of advanced glycation endproducts (AGEs), which are produced through the Amadori arrangement from nonenzymatic glycation in the diabetic milieu [27] and occur in diabetic complications [18]. In that study, collagen lattices were exposed to the reducing glucose glucose-6-phosphate at doses greater than or equal to 50 mM for 5 days. High concentrations of glucose evoked cross-linking of collagen fibers by nonenzymatic glycation, as reported by Brownlee et al. [7]. The cross-linked collagen fibers stabilized the collagen lattices and resulted in decreased collagen lattices contraction. Our results are incompatible with this previous report. The discrepancy may be a function of different diabetic milieus. We used TGF-β1 instead of AGEs as a factor that leads to diabetic complications. Our preliminary study showed that TGF-β1-induced FPCL contraction on day 6 was reversible, as evidenced by the results of cytochalasin D treatment. The prevention of contraction by AGE was irreversible. It is likely that FPCL is reversibly contracted at the early stage, while FPCL contraction becomes irreversible at the later stage.

In conclusion, our results suggest that TGF-β1-induced FPCL contraction is the consequence of Rho kinase activation and subsequent phosphorylation of MLC, leading to the activation of myosin ATPase. This mechanism is considered to account for renal atrophy and renal failure in DN, although there is a difference in time required for the limit of tissue contraction between FPCL and diabetic kidney, more than several days and more than several years, respectively. Hydroxyfasudil, a Rho kinase inhibitor, is already in clinical use as a treatment for cerebral vasospasm. It may be of great benefit to patients with DN. Further studies are required to elucidate the detailed mechanism of actin–myosin interaction in fibroblasts.

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ORIGINAL ARTICLE

Myxoinflammatory fibroblastic sarcoma: investigations by comparative genomic hybridization of two cases and review of the literature

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Abstract Myxoinflammatory fibroblastic sarcoma (MIFS) is a rare low-grade sarcoma of the distal extremities characterized by a myxohyaline stroma, a dense inflammatory infiltrate and virocyte- and lipoblast-like giant cells. Up to now, only two cases have been investigated cytogenetically, showing complex and heterogeneous karyotypes, in part with supernumerary ring chromosomes. We characterized two further cases of MIFS immunohistochemically and performed comparative genomic hybridization as well as DNA image cytometry analyses. Both tumors showed the characteristic histomorphological pattern of MIFS and were positive for Vimentin and CD68. Moreover, both cases presented aberrant karyotypes including distinct DNA copy number changes involving chromosome 7 and disclosed DNA aneuploidy.

Keywords Myxoinflammatory fibroblastic sarcoma · Comparative genomic hybridization · CGH · DNA image cytometry · Review

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Introduction

In 1998, Montgomery et al. [14] first described inflammatory myxohyaline tumors of the distal extremities with virocyte or Reed-Sternberg-like cells as a distinct tumor entity with unique histologic features. In the same year, Meis-Kindblom et al. coined the shorter term acral myxoinflammatory fibroblastic sarcoma (MIFS) for the same lesion, usually presenting as poorly circumscribed multinodular mass of the distal extremities ranging from less than 1 to 8 cm in size [2, 12]. Patients typically report a history of slowly growing and painless tumors that only occasionally are associated with pain or decreased mobility [2]. Histologically, MIFS exhibit a dense inflammatory infiltrate, predominantly consisting of lymphocytes and plasma cells, embedded in an alternating myxoid and hyaline stroma. Three main types of neoplastic cells can be distinguished, namely spindle-shaped cells, bizarre virocyte, or ganglion-like cells with enlarged nuclei and often huge, inclusion-like nucleoli and multinucleated lipoblast-like cells of variable size. Tumor cells are

dispersed through the myxohyaline stroma as single cells or form coherent nodules [2, 9, 12–14, 17, 18]. The rates of local recurrences range between 22 and 67%, probably depending on differences in surgical treatment, whereas distant metastases are exceedingly rare (<2% of all reported cases) [2, 12, 14, 17].

Cytogenetic data on chromosomal aberrations of MIFS are scarce, and up to now, only two cases have been investigated so far. One of them revealed a complex karyotype with a reciprocal translocation t(1;10)(p22;q24) in addition to the loss of chromosome 3 and 13, while the other exhibited supernumerary ring chromosomes and a derivate chromosome 13 with additional material on the short arm [9, 11]. In this paper, we report two further cases of MIFS investigated by immunohistochemistry, comparative genomic hybridization (CGH), and DNA image cytometry.

Materials and methods

Immunohistochemistry

The biopsy specimens were formalin-fixed, routinely processed, paraffin-embedded, and stained with hematoxylin and eosin. The panel of applied primary antibodies, dilutions, and different methods of tissue preparation and pretreatment, respectively, are listed in Table 1. All primary antibodies were diluted in a 1% solution of bovine serum albumin in phosphate-buffered saline (pH 7.4) and incubated for 30 min at room temperature. After incubation with the biotinylated secondary antibody, the avidin–biotin–peroxidase complex was applied for 30 min, and color development was performed using diamino-benzidine (DAB). The slides were counterstained with hemalaun, dehydrated, and mounted.

Comparative genomic hybridization

For CGH, tumor DNA was isolated from formalin-fixed and deparaffinized tumor tissue by proteinase K digestion (2 mg/ml final concentration; Roche, Mannheim, Germany) followed by spin column purification (Qiagen, Hilden, Germany). Labeling of tumor DNA with biotin-16-dUTP (Roche, Mannheim, Germany) and normal reference DNA with digoxigenin-11-dUTP (Roche) was performed by standard nick translation. The denatured DNA probe containing 2 μg of tumor DNA, 1.5 μg of reference DNA, and 80 μg of COT-1 DNA was hybridized for 3 days to normal metaphase spreads on glass slides (15×15 mm cover glass area). The slides were then washed, blocked with bovine serum albumin solution, and incubated with fluorescein-conjugated avidin (Vector Laboratories, Burlingame, CA) and rhodamine-conjugated antidigoxigenin (Roche). Finally, the slides were washed and mounted in antifade solution (Vector Laboratories) containing 2.5 μg/ml of 4,6-diamino-2-phenylindole (DAPI) counterstain. Image acquisition was performed on a Zeiss Axioskop fluorescence microscope (Zeiss, Göttingen, Germany) equipped with three separate bandpass filters (DAPI bandpass, Green single bandpass, Red single bandpass) and a high sensitive monochrome charge coupled device camera (Photometrics, Tuscon, AZ). For each analysis, the averaged chromosome-specific green to red fluorescence ratios and their 95% confidence intervals (CI) from at least ten well-selected metaphases were plotted using the Quips CGH software (distributed through Applied Imaging, Newcastle, UK). Relative copy number changes were interpreted as gains when the average green-to-red ratio exceeded 1.2 or 2 (high-level amplification), and

Table 1 Antibodies used in the study

Antibody specificity	Source	Type	Dilution	Pretreatment
Vimentin	Novocastra (Nunningen, Switzerland)	mm	1:50	Microwave
CD15	Dako Cytomation (Baar, Switzerland)	mm	1:100	Microwave
CD30	Dako Cytomation (Baar, Switzerland)	mm	Prediluted	Microwave
CD34	Dako Cytomation (Baar, Switzerland)	mm	1:200	Microwave
CD68	Dako Cytomation (Baar, Switzerland)	mm	1:1,600	Microwave
CK22	Biomeda (Foster City, USA)	mm	1:200	Microwave
EMA	Dako Cytomation (Baar, Switzerland)	mm	1:1600	Microwave
Desmin	Dako Cytomation (Baar, Switzerland)	mm	1:4,000	Microwave
Actin	NeoMarkers (Fremont, USA)	mm	1:25	Microwave
SMA	Dako Cytomation (Baar, Switzerland)	mm	Prediluted	None
S100 Protein	Medite (Nunningen, Switzerland)	rp	1:1,600	Microwave

CD Cluster of differentiation, *CK* cytokeratin, *EMA* epithelial membrane antigen, *SMA* smooth muscle actin, *mm* mouse monoclonal, *rp* rabbit polyclonal

as losses when the corresponding ratio was less than 0.8. Exceptionally, in cases with only trends not reaching the aforementioned thresholds, deviations from normal were classified as gains or losses when the 95% CI varied beyond the ratio of 1.0. An event was defined as a gain or a loss of (part of) a chromosomal arm. Chromosomal regions 1p32-pter, 13p, 14p, 15p, 19, 21p, 22p, telomeres and constitutive heterochromatic regions at 1q, 9q, 16q, and Yq reported to produce false results by CGH were excluded from all analyses [7].

DNA image cytometry

The specimens were evaluated with the semiautomatic AUTOCYTETM cell analytical system (Carl Zeiss AG, CH 8706 Feldmeilen) using a Zeiss AxioplanTM microscope. On average, 135 (104–153) of the most abnormally looking tumor cells were selected as test cells, whereas 50 nuclei of lymphocytes were measured as internal standard.

The histograms were analyzed following the recommendations of the European Society for Analytical and Cellular Pathology (ESACP) [3, 4]. Both stemlines and single events were considered. The key data of DNA cytometry, i.e., integrated optical density and nuclear area, were transferred to the server EUROQUANTTM (http://euroquant.med.tu-dresden.de) at the Institute of Pathology, Technical University/Dresden, which is available free of charge to any user for quality assurance [5]. There, the data were standardized and evaluated on the basis of statistic models, by which, the number of stemlines, their position on the ploidy axis, and their coefficient of variation are determined. EUROQUANT delivers the modal DNA values of peaks and the coefficient of variation (CV) value of stemlines and calculates several quality parameters defined by the ESACP (e.g., parameters for a control of measured glare factors and of the homogeneity of measurement) and compares them to established limit values. Single events were directly analyzed on the basis of the AUTOCYTE data. Ploidy classes were defined as follows: euploid/diploid: 1.8–2.2 c; tetraploid/polyploid: 3.6–4.4 c; octaploid: 7.2–8.8 c; aneuploid: stemline outside of a euploid region (2 c, 4 c, 8 c, etc.), single events >8.8 c.

Results

Clinical findings

Clinical features of the two cases are summarized in Table 2. One patient noticed a slowly growing painless mass in the right index finger and was treated surgically by local excision (case 1). The other patient suffered from pain in the right popliteal fossa and observed a slowly growing tumor that turned out to be located inside the tibial nerve (case 2). After histologic diagnosis, treatment consisted in isolated limb perfusion with tumor necrosis factor alpha (TNF-α) and Melphalan followed by neoadjuvant percutaneous radiotherapy and local excision without destruction of major fascicles. On histologic examination, no viable tumor was detectable anymore, and the patient did not sustain sensory or motor deficits (Fig. 1). At initial surgery, both tumors showed a myxoid aspect with no signs of necrosis or hemorrhage on cut section. Patient 1 developed local recurrence 5 months after initial resection and underwent re-excision followed by another recurrence 16 months later. The second patient is still free of disease (follow-up 14 months).

Microscopical and immunohistochemical findings

Light microscopy showed cell-rich tumors containing myxoid and hyaline areas and zones of fibrosis (Fig. 2). Tumor cells revealed pronounced nuclear polymorphism, including differences in nuclear size and form. Some cells were rather small and spindle-shaped, while others revealed big vesicular nuclei and prominent nucleoli, focally resembling ganglion cells or virocytes (Fig. 2f). Additionally, multinucleated cells and lipoblast-like cells with multiple intracytoplasmatic mucin-containing vacuoles were present in all cases (Fig. 2a–c). The inflammatory infiltrates were dense and particularly prominent in the fibrotic and myxoid areas of the tumors. Immunohistochemically all tumors were strongly positive for vimentin and focally positive for CD68 (not shown). Expression of CK22, CD15, CD30, epithelial membrane antigen (EMA), S100 protein, or desmin could not be detected in any of the two cases.

Table 2 Clinical features

Case	Sex/age	Location	Size	Follow-up
1	F/51	Right index finger	2×1.5×1 cm	Recurrences after 5 and 16 months
2	F/75	Right popliteal fossa, tibial nerve	4×2×1.7 cm	14 months NED

NED No evidence of disease, *M* male, *F* female

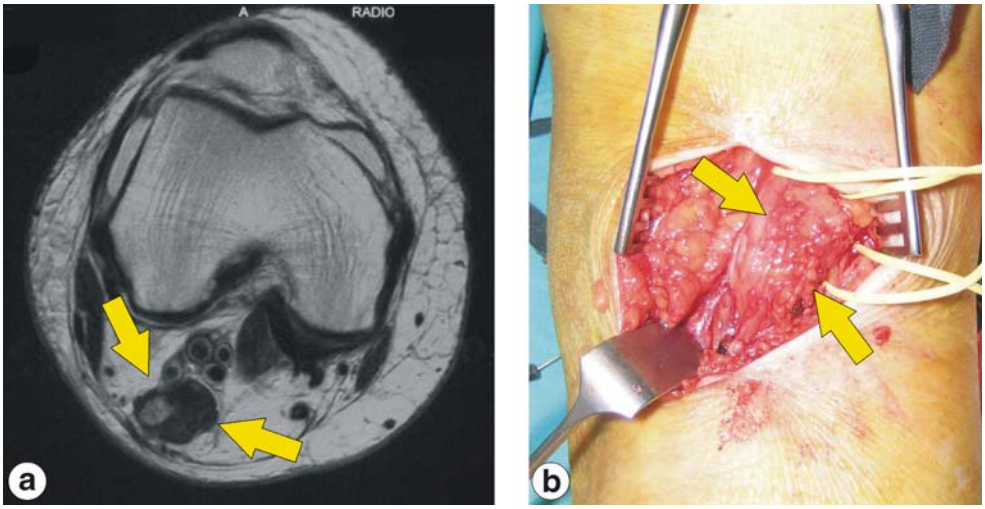

Fig. 1 **a** Case 2: T1-weighted MR showing a hypointense tumor surrounding the tibial nerve (*yellow arrows*). **b** Case 2: Intraoperative situation with isolated nerve before tumor resection (*yellow arrows*)

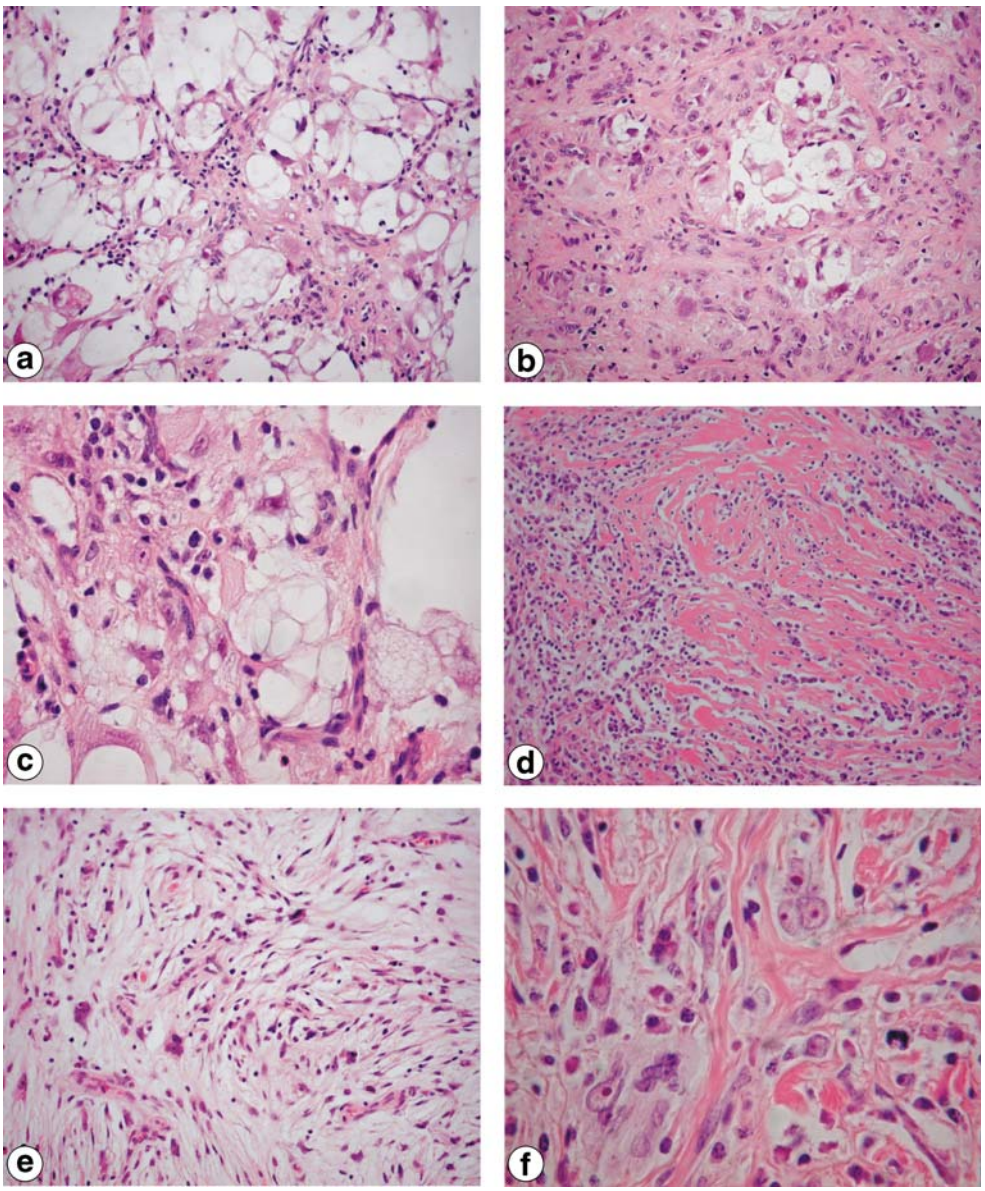

Fig. 2 **a** Case 1—HE×400: multivacuolated lipoblast-like tumor cells admixed with a loose inflammatory infiltrate. **b** Case 1—HE×400: tumor cells are embedded in a myxohyaline stroma. **c** Case 1—HE×640: lipoblast-like tumor cells in close contact to lymphocytes and some eosinophils. **d** Case 2—HE×200: spindle-shaped tumor cells admixed with a dense inflammatory infiltrate and embedded in a collagenous hyalinized stroma. **e** Case 2—HE×200: spindle-shaped tumor cells and lymphocytes in a myxoid stroma. **f** Case 2—HE×640: Ganglion-like tumor cells with large eosinophilic nucleoli accompanied by lymphocytes and plasma cells

Table 3 CGH analysis

Case	Results
1	0.20: rev ish enh(3p11p12, 7q31q32) trend enh(1p, 18), dim(6q14qter)
2	0,20: rev ish enh(5pterq32, 7p15p21, 14q12q21)

Comparative genomic hybridization

DNA copy number changes were identified in both cases (Table 3, Fig. 3). The number of changes ranged from two (case 1) to three (case 2). There were no specific common gains or losses, but both investigated cases showed gains at chromosome 7.

DNA image cytometry

All tumors revealed aneuploid stemlines and additional aneuploid single events. Modal DNA value of peaks ranged between 2.54 c and 2.72 c (mean 2.63 c). The CV varied between 7.31 and 7.36% (mean 7.34%).

Discussion

Myxoinflammatory fibroblastic sarcomas are rare low-grade tumors typically presenting as painless and slowly growing masses of the distal extremities. According to the Medline database, 133 cases have been reported up to now, including

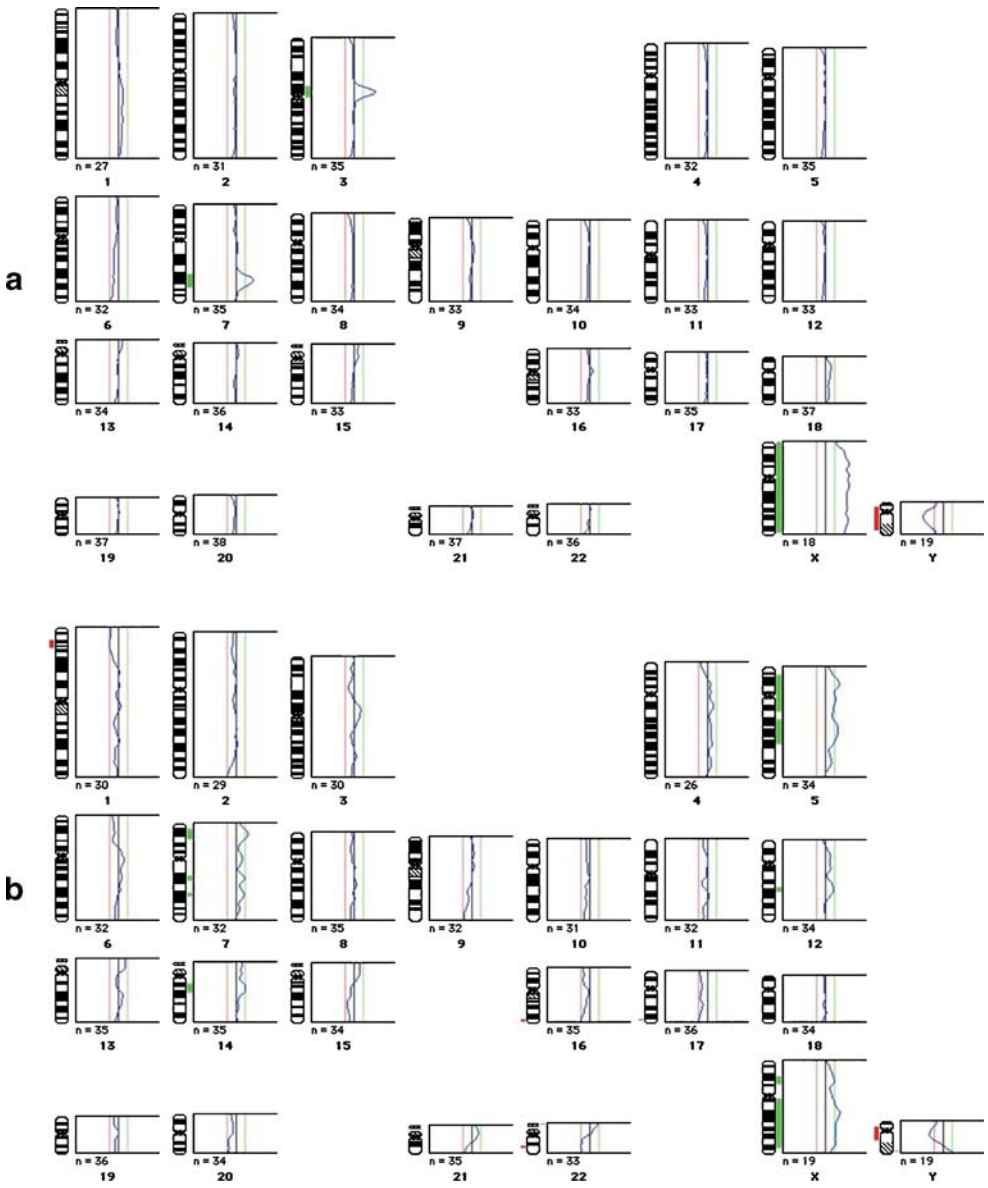

Fig. 3 a–b Comparative genomic analyses of cases 1–2

several tumors of the proximal extremities as well (upper arm, thigh) [1, 6, 8–10, 12–17]. Both investigated cases revealed the characteristic histomorphological and immunohistochemical pattern of MIFS. Besides the alternating and confluencing myxoid and hyaline stroma containing a predominantly lymphoplasmocytic inflammatory infiltrate, all tumors exhibited intermixed spindle cells, large polygonal and partially bizarre ganglion-like cells with huge inclusion-like nucleoli and bubbly multinucleated cells resembling lipoblasts (Fig. 2). Immunohistochemically, the tumor cells stained positive for Vimentin and CD68.

Cytogenetic data on chromosomal aberrations on MIFS are limited. To our knowledge, only two cases have been investigated by conventional cytogenetic and fluorescence in situ hybridization analyses so far. One case showed a derivate chromosome 13 with additional material on the short arm and supernumerary ring chromosomes, while the other exhibited a complex karyotype with a reciprocal translocation t(1;10)(p22;q24) and losses of chromosomes 3 and 13 [9, 11]. The two cases of the present study also revealed aberrant karyotypes involving gains at several different chromosomal regions. Although no specific common aberration was observed, both cases shared gains at chromosome 7. Losses of chromosomal regions were not detected. Furthermore, no chromosomal alterations were shared with the previously investigated two MIFS [9, 11]. DNA image cytometry revealed both tumors of our series to be DNA aneuploid, which has never been investigated so far.

In conclusion, the two MIFS of the present study show considerable genetic diversity, which is in keeping with the two previously investigated cases. However, no single marker or profile of DNA copy number changes characterizing all tumors could be detected. Further, cytogenetic or CGH analyses have to be awaited to define specific cytogenetic profiles of MIFS and possible separate subtypes of MIFS, respectively.

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ORIGINAL ARTICLE

Supplemental utility of nested PCR for the pathological diagnosis of disseminated trichosporonosis

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Abstract Disseminated trichosporonosis is known to be a severe opportunistic mycosis and has a high mortality rate. In autopsy cases, it is often difficult to diagnose as trichosporonosis because the causative *Trichosporon* species are pathologically similar to other fungi, especially the *Candida* species. Immunohistochemical analysis is essential for the differential diagnosis, but an antibody to *Trichosporon* is not available commercially. In the present study, we investigated the supplemental utility of nested polymerase chain reaction (PCR) for the pathological diagnosis of trichosporonosis from formalin-fixed and paraffin-embedded tissues. Total DNA was purified from 30 major organs in three autopsy cases, and *Trichosporon* DNA was specifically amplified by nested PCR using three sets of primers. Of 22 organs in which Grocott's stain was positive for fungal infection, 170- and 259-bp PCR products were detected in 20 (91%) and 12 (55%) organs, respectively. In short-term fixation (about 1 day), these bands were highly detected in ten (100%) and nine (90%) organs, whereas the detection efficiency tended to decrease after long-term fixation and decalcification. No PCR product of 412 bp was detected in any organs. These findings suggest that nested PCR from short-term-fixed tissues is useful for supportive pathological diagnosis of disseminated trichosporonosis.

Keywords *Trichosporon* · Fungemia · Pathological diagnosis · Nested PCR

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Introduction

Trichosporon species are recognized as significant peccant fungi in opportunistic mycoses. These are currently reclassified into more than 30 individual species by DNA sequence analysis [26]. *Trichosporon asahii*, *T. dermatis*, *T. montevideense*, *T. mucoides*, and *T. ovoides* are related with summer-type hypersensitivity pneumonitis [21, 26, 28], whereas *T. asahii*, *T. asteroids*, *T. cutaneum*, *T. inkin*, *T. loubieri*, *T. mucoides*, and *T. ovoides* are associated with superficial and disseminated infections to the human organs [16, 18]. Of these species, *T. asahii* and *T. mucoides* are major causative species for acute-onset disseminated trichosporonosis.

Acute trichosporonosis generally disseminates to multiple organs and is highly fatal in immunocompromised hosts. Most of the patients had received chemotherapy for hematological malignancies or solid malignant neoplasm, as well as treatment with immunosuppressants after bone

marrow transplantation or organ plantation [1, 5, 8, 12, 14, 15]. The attributable mortality rate is higher than that of other fungemia [14]. Thus, it is important to identify *Trichosporon* species from patients' blood at an early stage. Unfortunately, however, patients occasionally rapidly die before detection of *Trichosporon*, and an autopsy is often performed to determine the causative pathogen.

In autopsy cases, the pathological diagnosis of trichosporonosis is usually derived from the characteristic structures of species; (1) oval or square spores in the core of the fungal colonies; (2) slender or occasional curled hyphae in the periphery of the colonies; and (3) presence of both separate hyphae and pseudohyphae. However, *Trichosporon* lacking these features is occasionally confused with other fungi such as the *Candida* species. Some reports of disseminated trichosporonosis are diagnosed by immunohistochemical examination using in-house antibodies to *Trichosporon* [7, 10, 11, 20], but these antibodies are now limited to a few laboratories. Therefore, we tried to investigate the usefulness of nested polymerase chain reaction (PCR) for the pathological diagnosis of disseminated trichosporonosis in formalin-fixed and paraffin-embedded tissues.

Materials and methods

Samples and pathological investigation

Three autopsy cases in which *Trichosporon asahii* was routinely identified premortem by blood culture and sequent VITEK® 2 compact system (Japan BioMérieux, Tokyo, Japan) were used for this investigation. All autopsy samples were fixed in 20% formalin. Bone marrow samples were treated with Plank-Rychlo solution [25] (7 g of $AlCl_3 \cdot 6H_2O$, 5 ml of 86% formic acid, 8.5 ml of 12N HCl) for 15 h, and they were neutralized with 5% Na_2SO_4 for 12 h. Paraffin-embedded sections (4 μm) were performed with hematoxylin and eosin (HE) and Grocott's stains. Positive results with these stains microscopically determined fungal infection.

DNA extraction and nested PCR

Three 8-μm tissue sections were deparaffinized in xylene and rinsed in ethanol. Tissues were suspended with 200 μl of DNA lysis buffer [100 mM Tris–HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA, 0.5% sodium dodecyl sulfate, and 1 mg/ml proteinase K] and incubated at 55°C for 16 h. After addition of 100 μl of phenol and 100 μl of chloroform-isoamyl alcohol (24:1), the solution was mixed well with vortex and centrifuged at 12,500×*g* for 2 min. The supernatant was mixed with 100 μl of 7.5 M ammonium acetate, 0.05 mg/ml glycogen, and 400 μl of ethanol, and the resulting suspension was incubated at −20° C for 2 h. DNA was trapped in the glycogen by centrifugation at 12,500×*g* for 20 min at 4°C and finally resuspended with 10 μl of TE buffer.

Specific PCR primers for the *Trichosporon* species were used for amplification of different product sizes as shown in Table 1 [19, 27, 29]. All primers were synthesized by Tsukuba Oligo Service (Tsukuba, Japan). The amplification

Table 1 Oligonucleotides used for nested PCR

Group	Primer	Sequence of oligonucleotide (5′-3′)	Products (bp)	Species	Ref.
1	1st and 2nd PCR				
	TRF	AGAGGCCTACCATGGTATCA	170	*T. species*	[29]
	TRR	TAAGACCCAATAGAGCCCTA			
2	1st PCR				
	TB26-1	AAAGATGAAAAGCACTTTGG	287	*T. asahii*, *T. mucoides*, *T. montevideense*, *Cryptococcus neoformans*, *Malassezia furfur*, *Malassezia pachydermatis*, *Sporobolomyces roseus*, *Rhodotorula acheniorum*, *Rhodotorula minuta*	[19]
	TB26-2	AAGCCATTATGTCAACATCC			
	2nd PCR				
	TB26-9	AGCACTTTGGAAAGAGAG	259	*T. asahii*, *T. mucoides*	
	TB26-10	CCTAAGCTCGAACGTGCC			
3	1st PCR				
	TAAF	GGATCATTAGTGATTGCCTTTATA	529	*T. asahii, T. coremiiforme, T. faecale*	[27]
	ITS4R	TCCTCCGCTTATTGATATG			
	2nd PCR				
	TAAF	GGATCATTAGTGATTGCCTTTATA	412	*T. asahii*, *T. coremiiforme*, *T. faecale*	
	S2-2	GGACCCAGTGAAACTTATTACAC			

reaction was performed in a PCR buffer containing 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1 mM $MgCl_2$, 2.5 μM dNTP, 2 mM primers, 0.5 U Taq DNA polymerase (Ampli Taq Gold™; Applied Biosystems, Tokyo, Japan), and 2 μl of extracted DNA template. The reaction mixture was amplified in an automated thermal cycler (ASTEC PC808; ASTEC, Fukuoka, Japan). An initial denaturation at 95°C for 5 min was followed by 40 cycles consisting of denaturation at 95°C for 1 min; annealing at 55°C for 2 min (TB26-1 and TB26-2), 56°C for 2 min (TRF and TRR) or 57°C for 1 min (TAAF and ITS4R); and extension at 72°C for 2 min. Final extension was performed at 72°C for 7 min. In the nested PCR step, 2 μl of the first amplification product was added to a new reaction mixture with inner primers and reamplified with 40 cycles as described above, except for the annealing step at 60°C for 2 min (TB26-9 and TB26-10) and 65°C for 1 min (TAAF and S2-2). Amplified PCR products were separated by electrophoresis on 2% agarose gel and visualized by staining with SYBR Green I (Molecular Probes, Eugene, OR, USA).

Table 2 Detection of *Trichosporon* DNA from paraffin-embedded tissues

Case	Organs	Fixation	Pathological investigation		Nested PCR		
		Time	HE	Grocott	170 bp	259 bp	412 bp
1	Ileum	23 h	+	+	+	+	−
	Spleen	23 h	+	+	+	+	−
	Liver	23 h	+	+	+	+	−
	Lung	23 h	+	+	+	+	−
	Bone marrow	9 days	+	+	−	−	−
	Pancreas	9 days	+	+	+[a]	−	−
	Kidney	9 days	+	+	+	−	−
	Urinary bladder	9 days	+	+	+[a]	−	−
	Heart	9 days	+	+	+	−	−
	Lung	9 days	+	+	+	+[a]	−
2	Liver	1 days 5 h	+	+	+	+	−
	Spleen	1 days 5 h	+	+	+	+	−
	Heart	1 days 5 h	+	+	+	+	−
	Lungs	1 days 5 h	+	+	+	+	−
	Bone marrow	1 days 5 h	+	+	+	−[b]	−
	Urinary bladder	21 days	+	+	+	−	−
	Kidney	21 days	+	+	+	+	−
	Brain	21 days	+	+	+	+	−
	Adrenal gland	21 days	+	+	+	−	−
	Pancreas	21 days	+	+	+[a]	−	−
3	Lung	1 days 5 h	−	+	+	+	−
	Liver	1 days 5 h	−	−	−	−	−
	Spleen	6 days	−	−	−	−	−
	Bone marrow	6 days	−	−	−	−	−
	Kidney	6 days	−	−	−	−	−
	Urinary bladder	6 days	−	−	+[a]	+[a]	−
	Colon	6 days	−	−	−	−	−
	Heart	6 days	−	−	−	−	−
	Thyroid gland	6 days	−	+	−	−	−
	Adrenal gland	6 days	−	−	−	−	−

[a] Slight bands were detected.
[b] Slightly longer band than 259 bp was observed.

Immunohistochemistry

Immunohistochemical analysis was performed by Dr. Hotchi using specific antibodies to *Trichosporon*, *Candida*, and *Aspergillus*. These in-house-produced antiserum and control specimens were as previously reported [7, 10].

Results

We detected *Trichosporon* by both conventional pathological examination and nested PCR analysis to elucidate the utility of nested PCR for the diagnosis of disseminated trichosporonosis. In a total of 30 organs, fungi were observed in 20 (67%) and 22 (73%) organs under HE and Grocott's stains, respectively (Table 2). HE staining showed numerous fungal colonies composed of basophilic and segment-like fungal bodies, but detailed fungal structures were unclear (Fig. 1). On the other hand, Grocott's staining revealed clearly dark brownish or blackish fungal hyphae (2 to 4 μm in diameter). In a few colonies, careful observation revealed slightly large and oval spores (up to 10 μm in diameter), as well as terminal slender hyphae. Despite the presence of these fungal structures being highly suspicious of *Trichosporon* infection, we could not definitively conclude that these fungi were *Trichosporon* species. In nested PCR using specific primers, 170- and 259-bp *Trichosporon* DNA was amplified in a total of 21 (70%) and 13 (43%) samples, respectively (Fig. 2). In the 22 organs that were positive for Grocott's staining, 170-bp PCR products were highly detected in 20 (91%) samples, while 259-bp bands were observed in 12 (55%) samples (Table 3). In the total of eight samples that were negative for Grocott's staining, only one sample (13%) was positive for both 170- and 259-bp bands. In ten samples that were positive for Grocott's staining in the short-term fixation (about 1 day), both 170- and 259-bp bands were highly amplified in ten (100%) and nine (90%) samples, respectively. However, in 12 samples which were Grocott's stain-positive in long-term fixation (6 to 21 days), these bands were decreasingly detected in ten (83%) and two (17%) samples. In two bone marrow samples positive for fungal colonies with Grocott's staining, only the170-bp band was detected in one sample (50%). On the other hand, in no sample was the band of 412 bp detected.

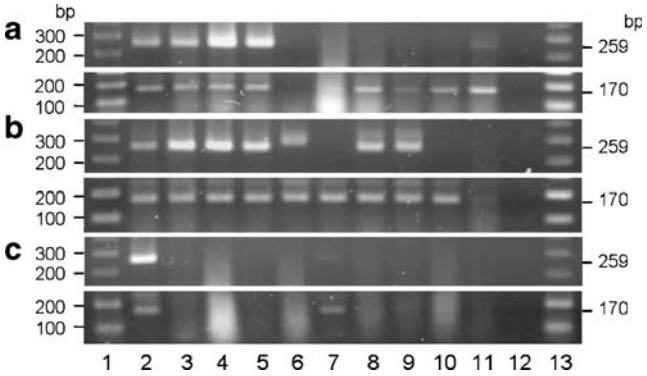

Fig. 2 Detection of *Trichosporon* DNA by nested PCR. The specific 259- and 170-bp bands for *Trichosporon* are detected in multiple organs (**a**–**c**, cases 1–3). **a** *Lanes 2* through *11* are shown as follows: ileum, spleen, liver, lung (1-day fixation), bone marrow, pancreas, kidney, urinary bladder, heart, and lung (9-day fixation). **b** *Lanes 2* through *11*: liver, spleen, heart, lung, bone marrow, urinary bladder, kidney, brain, adrenal gland, and pancreas. **c** *Lanes 2* through *11*: lung, liver, spleen, bone marrow, kidney, urinary bladder, colon, heart, thyroid gland, and adrenal gland. Negative controls (no template) are indicated in *lane 12*. Molecular weight markers are shown in *lanes 1* and *13*

To confirm whether the infected fungi were *Trichosporon* species, an immunohistochemical analysis using a specific anti-*Trichosporon* antibody was performed (Fig. 3). The fungi were positive for anti-*Trichosporon* antiserum, but no reaction occurred with antibodies to either *Candida* or *Aspergillus*. In the control specimens for *Candida*, hyphae and round spores were strongly positive for the anti-*Candida* antibody. Meanwhile, the antibody to *Aspergillus* revealed the characteristic 45° angle branch of *Aspergillus*. Thus, the disseminated fungi were immunohistochemically identified as being of the *Trichosporon* species.

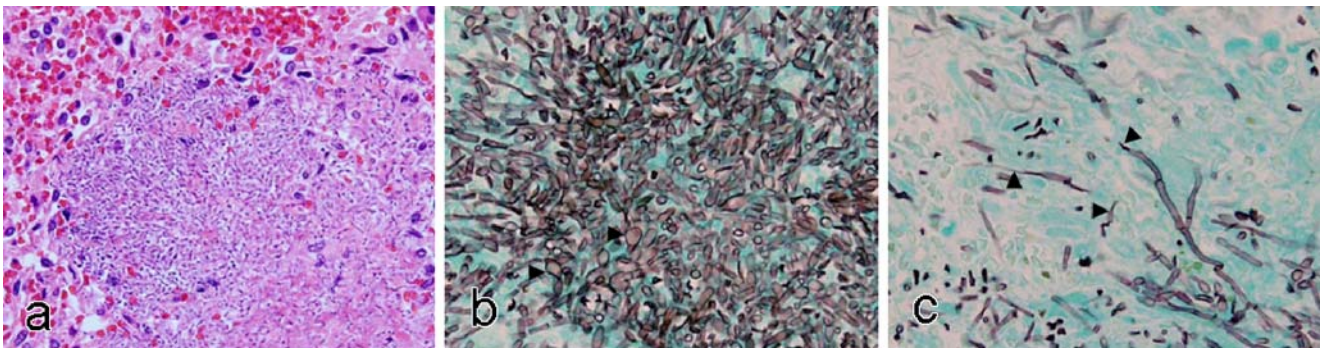

Fig. 1 Histopathological findings of disseminated trichosporonosis. Fungal colonies composed of numerous filamentous hyphae are observed (**a**, HE stain). High-power view under Grocott's stain showing the fungi have slightly large oval spores in the core of the colony (*arrowheads*, **b**) and slender hyphae in the periphery (*arrowheads*, **c**)

Table 3 Detection of *Trichosporon* using Grocott's staining and nested PCR

Samples	Grocott's stain, *n* (%)		Positive for nested PCR[a]		
			170 bp, *n* (%)	259 bp, *n* (%)	412 bp, *n* (%)
Total samples	Positive	22 (73)	20/22 (91)	12/22 (55)	0/22 (0)
n=30	Negative	8 (27)	1/8 (13)	1/8 (13)	0/8 (0)
Fixation for about 1 day	Positive	10 (91)	10/10 (100)	9/10 (90)	0/10 (0)
n=11	Negative	1 (9)	0/1 (0)	0/1 (0)	0/1 (0)
Fixation for 6 to 21 days	Positive	12 (63)	10/12 (83)	2/12 (17)	0/12 (0)
n=19	Negative	7 (37)	1/7 (14)	1/7 (14)	0/7 (0)
Decalcification (bone marrow)	Positive	2 (67)	1/2 (50)	0/2 (0)	0/2 (0)
n=3	Negative	1 (33)	0/1 (0)	0/1 (0)	0/1 (0)

[a] Of Grocott's stain-positive or -negative samples, nested PCR-positive samples are shown.

Discussion

Although disseminated trichosporonosis is a lethal mycosis, the causative *Trichosporon* species are often pathologically confused with other fungi. In this paper, we demonstrated that nested PCR analysis was useful for pathological diagnosis of disseminated trichosporonosis even in formalin-fixed and paraffin-embedded tissues. However, we should consider the possibility of false positive results due to contamination by superficial and resident *Trichosporon* species. In some reports, *Trichosporon* species are isolated from skin, sputum, urine, and stools [1, 2, 9, 18, 30], but it is difficult to identify the causative species of disseminated trichosporonosis from these materials. In our analysis, *Trichosporon* DNA was amplified in the urinary bladder and gastrointestinal tract, but we should refrain from pathological diagnosis of disseminated trichosporonosis based on the results of skin, urinary bladder, and gastrointestinal tract, in addition to the trachea and bronchi. Meanwhile, identification of *Trichosporon* species from blood culture and sequent biochemical analysis is routinely essential for clinical diagnosis of disseminated trichosporonosis. Sugita et al. [27] reported on the usefulness of nested PCR analysis from patients' sera. In autopsy cases, however, sera are probably unsuitable for nested PCR due to possible fungal translocation after the destruction of the

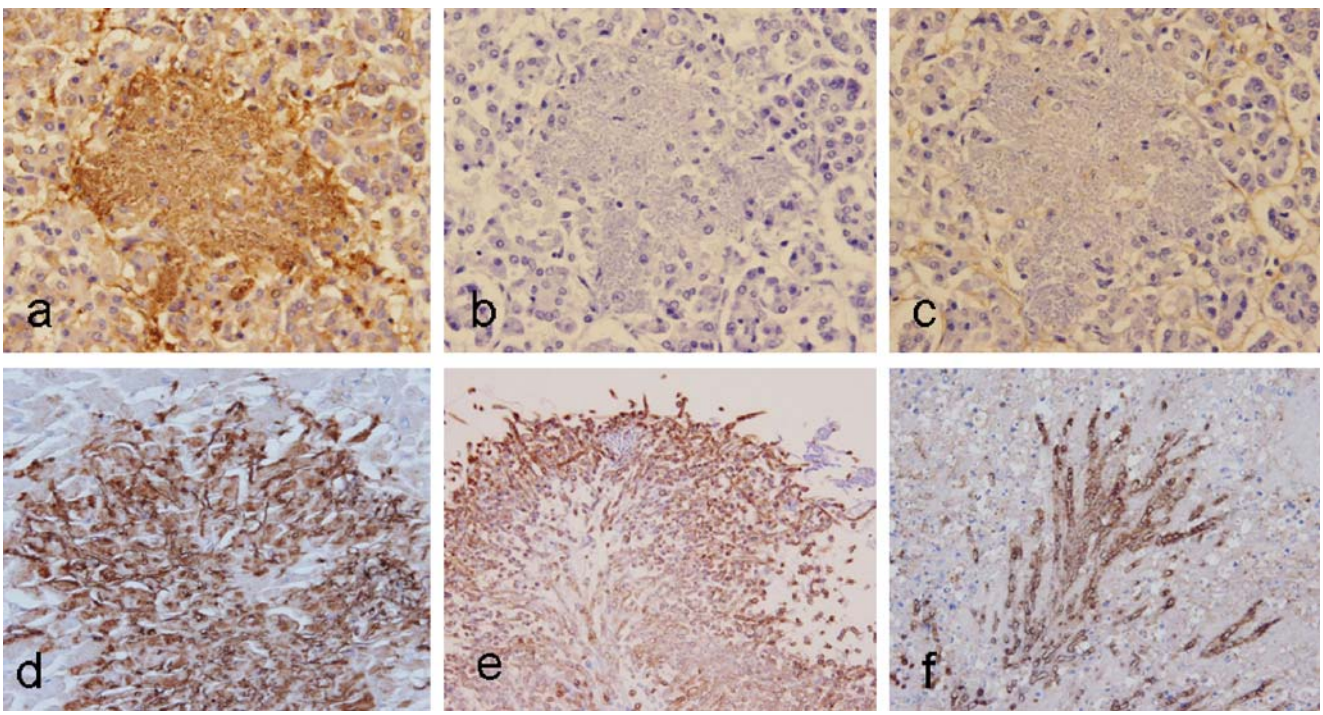

Fig. 3 Immunohistochemical confirmation of trichosporonosis. Fungi are positive for anti-*Trichosporon* antibody (**a**), but are negative for antibodies to *Candida* (**b**) and *Aspergillus* (**c**). Control specimens for *Trichosporon* (**d**), *Candida* (**e**), and *Aspergillus* (**f**) are detected with these antibodies

gut mucosal barrier after death. Thus, nested PCR should be performed on specimens from visceral organs with the exception of some materials as described above.

We should also pay attention to false negatives in nested PCR results that have been caused by DNA degradation. Generally speaking, DNA is known to be unstable for formalin and acidic solutions. In our investigation, the detection efficiency of nested PCR tended to decrease after long-term fixation in formalin and decalcification with a solution containing formic acid and hydrochloric acid. In addition, fungal hyphae were observed under Grocott's stain in the thyroid gland of case 3, but nested PCR revealed no reaction. HE staining showed disappearance of basophilic fungal structures with a massive abscess. It is possible that the fungal DNA degraded after administration of antifungal agents or long-term fixation. Therefore, appropriate sampling from short-term-fixed materials is significant for effective and accurate nested PCR analysis. In addition, the pathological diagnosis of disseminated trichosporonosis should be determined carefully with consideration of the results of nested PCR in combination with the histopathological findings under HE and Grocott's stains.

Disseminated trichosporonosis cases have recently been increasing and are associated with a poor prognosis [8, 12, 13, 17]. Although some reports suggest that an antifungal agent voriconazole is effective for *Trichosporon* compared to other fungi in vitro [6, 22] and in vivo [3, 4], *Trichosporon* is generally less susceptible to the usual antifungal agents such as amphotericin B or micafungin [17, 23, 31]. In addition, the possible air contamination of *Trichosporon* by removal of the catheter has been reported [24]. Thus, an accurate pathological diagnosis of disseminated trichosporonosis even in postmortem patients is significant as a warning sign to prevent hospital-acquired infection.

Trichosporon species have not been detected with nested PCR from formalin-fixed and paraffin-embedded tissues. Therefore, the methods in our present study would be helpful for the pathological diagnosis of trichosporonosis in both autopsy and biopsy materials such as the cutaneous white piedra or cystitis caused by *Trichosporon* infection, thereby leading to determination of antifungal agents.

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ORIGINAL ARTICLE

Epithelial–mesenchymal transition (EMT) of renal tubular cells in canine glomerulonephritis

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Abstract Tubulo-interstitial fibrosis in dogs may result from primary injury to the interstitium or develop secondary to other renal diseases. As in human renal pathology, tubular epithelial cells (TEC) are believed to actively participate in the mechanisms of renal fibrosis. In this study, we examined the changes in the tubular epithelial component in two specific canine diseases. Immunohistochemistry showed the expression of the epithelial marker cytokeratin, the smooth muscle marker α-SMA, the mesenchymal marker vimentin and PCNA in 20 dogs with membranous glomerulonephritis and membrano-proliferative glomerulonephritis. Results showed that the loss of the epithelial marker in TEC was directly correlated to the grade of tubulo-interstitial disease present and independent of the type of glomerulonephritis. Varying degrees of vimentin positivity were detected in tubular epithelium in areas of inflammation, and low numbers of scattered α-SMA-positive cells were also observed. Immunohistochemistry showed that epithelial tubular cells lose their cytokeratin staining characteristics and transdifferentiate into cells exhibiting key mesenchymal immunophenotypic feature of vimentin-positive staining in both diseases investigated. The integrity of the tubular basement membrane is likely to be fundamental in maintaining the epithelial phenotype of TEC. Animal models provide opportunities for investigating the pathogenesis of renal fibrosis in humans.

Keywords Cytokeratin · Dog · Interstitial fibrosis · Tubular epithelial cells · Vimentin

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Introduction

In dogs, membranous and membrano-proliferative glomerulonephritis are acknowledged to have a variety of aetiologies. Protozoal, bacterial and viral infections are the best-described causes, whilst yet other cases are considered idiopathic. As the glomerular lesions progress, the tubulo-interstitial (TI) compartment becomes involved, and TI damage (TID) is a common outcome of all chronic progressive renal diseases regardless of aetiology or site of primary injury [12, 14]. Histologically, these end stage lesions are characterized by interstitial fibrosis with inflammatory cell infiltrates and loss of peritubular capillaries, whilst tubules undergo concurrent degenerative, atrophic and regenerative changes [4]. Decline in renal function (reduced glomerular filtration rate) in chronic renal disease appears to be correlated more closely with

interstitial lesions than with glomerular damage [8, 15]. Interstitial fibrosis is linked to a pathological imbalance between extra-cellular matrix deposition and degradation, which is stimulated by a variety of cytokines and growth factors [13]. The fibrogenic response and the inflammatory infiltrates progressively reduce the number of functional nephrons leading to renal failure [9].

Previous studies carried out on kidneys from humans, rats and cattle have shown that, during renal injury, tubular epithelial cells (TEC) are able to acquire features typical of mesenchymal cells [16, 20, 22]. This process has been named epithelial–mesenchymal transition (EMT). EMT has been demonstrated in lung fibrosis as well as in some type of tumours, and it is considered a physiologic process in embryogenesis [21]. Renal tubular EMT, by definition, is a process in which tubular cells lose their epithelial phenotype and acquire characteristic features of mesenchyme [7] such as the expression of vimentin, the characteristic cytoskeleton protein of mesenchymal cells. Proteinuria and chronic hypoxia both appear to play significant roles in EMT, and several authors have suggested the importance of reduced local oxygen tension, resulting from peritubular capillary destruction, in development of the process [5, 11].

This current study aims to use immunofluorescence and immunohistochemical techniques to detect and quantify phenotypic changes in TEC undergoing EMT during the course of two specific canine glomerular renal diseases.

Materials and methods

Tissue samples

Twenty cases exhibiting renal lesions of one of two specific glomerular diseases were selected from archives of the Department of Animal Pathology (University of Turin) from a period spanning 4 years (2003–2007). The glomerular diseases chosen were membranous glomerulonephritis and membrano-proliferative glomerulonephritis as defined in the WHO classification of glomerular diseases. Kidney tissue samples were obtained by renal biopsy (Trucut method) in routine diagnostic cases (eight biopsies), whilst the remaining tissue was collected from dogs subjected to post-mortem examination (12 dogs). Controls were five young dogs (age range 2–6 years). These dogs were strays from dog shelters that were euthanised for aggressive behaviour. No significant lesions in systemic organs were found. Renal clinical examinations were normal. Signalment and clinical data from the dogs considered in the study are shown in Table 1.

Table 1 Clinical features and TID grade

Case number	Age (years)	Sex	Glomerular lesion	Cause	TID grade
1	8	M	MPGN[b]	*Leishmania spp.*	2
2	7	M	MPGN[b]	Idiopathic	1
3	5	F	MPGN[b]	Canine adenovirus 1	1
4	6	M	MPGN[b]	Idiopathic	1
5	4	F	MPGN[b]	Canine adenovirus 1	2
6	8	M	MPGN[b]	*Borrelia burgdorferi*	2
7	8	M	MPGN[b]	*Leishmania spp.*	2
8	3	F	MPGN[b]	*Leishmania spp.*	3
9	10	M	MPGN[a]	Chronic pancreatitis	3
10	11	M	MPGN[a]	*Dirofilaria immitis*	2
11	12	M	MPGN[a]	Neoplasm	2
12	16	F	MPGN[a]	Neoplasm	2
13	5	F	MGN[b]	*Leishmania spp.*	1
14	6	F	MGN[b]	*Dirofilaria immitis*	2
15	5	M	MGN[b]	*Dirofilaria immitis*	1
16	7	M	MGN[b]	Idiopathic	1
17	5	M	MGN[a]	Neoplasm	1
18	2	M	MGN[a]	Idiopathic	3
19	11	M	MGN[a]	Pyometra	2
20	12	F	MGN[a]	*Dirofilaria immitis*	2
21	5	M	Control	–	0
22	6	M	Control	–	0
23	2	F	Control	–	0
24	3	M	Control	–	0
25	2	M	Control	–	0

[a] Autopsy
[b] Biopsy

Histology

Renal samples for histological examination were fixed in 10% buffered formalin and embedded in paraffin. Sections were cut at 4 μm in thickness and stained with: haematoxylin–eosin, periodic acid-Schiff (PAS), acid–fuchsin orange G, Masson's trichrome and phosphotungstic acid haematoxylin.

To quantify the interstitial changes in the two forms of glomerulonephritis, the severity of TID was graded. Interstitial fibrosis and inflammation were assessed at ×200 magnification and scored as follows: normal tubulointerstitium (score=0), mild tubular atrophy and interstitial oedema or fibrosis affecting up to 25% of the field of view (score=1), moderate TI fibrosis affecting 25–50% of a given field (score=2), severe TI fibrosis >50% of a field

(score=3). Score data are shown in Table 1. The samples were scored by a veterinary pathologist and a human renal pathologist in a blinded fashion.

Immunofluorescence

For immunofluorescence staining, unfixed renal tissue was OCT-embedded, snap-frozen in liquid nitrogen and stored at −80°C. Six-micrometer-thick sections were fixed with acetone for 15 min. After washing with PBS (two passages), slides were incubated with fluorescein isothiocyanate-labelled anti-goat IgA, IgG, IgM and complement C3 antibodies specific for the dog (Bethyl Laboratories, Montgomery, AL, USA). Primary antibodies were omitted as negative controls.

Immunohistochemistry

Immunoperoxidase staining was performed on renal sections of formalin-fixed, paraffin-embedded renal samples. An immunohistochemical panel was performed to assess changes to the epithelial tubular cells. Antibody against cytokeratin (CK) AE1/AE3 (Dako, Glostrup, Denmark) was used to detect cells expressing an epithelial phenotype. Mesenchymal markers were stained with antibodies against vimentin (Dako) and α-smooth muscle actin (α-SMA, Dako). Proliferation cell nuclear antigen (PCNA; Clone PC10, Dako) was used as index of cellular proliferation.

Double immunohistochemical staining

On selected representative cases, CK AE1/AE3 and vimentin double antigen-immunoperoxidase labelling was performed using a commercial kit (Vector Laboratories, Burlingame, CA, USA), according to manufacturer's instructions. Peroxidase activity was demonstrated firstly by DAB+Ni^{2+} (grey to black chromogen reaction) for Vimentin and secondly by 3-amino-9-ethylcarbazole (AEC, red chromogen reaction) for CK AE1/AE3.

Quantification of immunohistochemistry and statistical analysis

Quantification of results in each case was achieved by counting the numbers of TEC per ten high power fields (HPFs, ×200) that expressed cytoplasmic staining for the mesenchymal marker. Loss of cytoplasmic staining for the epithelial marker CK was evaluated by counting the number of negative-stained cells per HPFs. Data were analysed using Minitab 14, and results were expressed as means, standard deviations and the numbers of TEC for each fields. To demonstrate that CK and vimentin expression were statistically correlated to the TID grade, a number of paired comparisons were performed.

Results

Tubular-interstitial damage was a common finding in membranous and membrano-proliferative glomerulonephritis (Fig. 1d). All three different grades of TID were present amongst the samples examined. The quantitative analysis data corresponding to the grade of TID are summarized in Table 1. Renal samples, independent of the diagnosis, showed variable intensity of positive immunostaining in the TEC for all markers.

A diffuse and statistically significant increase (p=0.001) in the number of PCNA-positive cells was noted in nuclei of tubules within foci of nephritis (3.5±1.5 TEC/field in TID grade 3; Fig. 1c).

In control kidneys, TEC exhibited strong and uniform cytoplasmic expression of the epithelial marker CK (98.7±1.3 TEC/field in controls; Fig. 1a). In contrast, expression of CK was reduced in tubules associated with mild or severe interstitial fibrosis and completely lost in atrophic regions (54.5±4.8 TEC/field in TID grade 3; Fig. 1b).

The mesenchymal markers (vimentin and α-SMA) showed no staining in TEC from control kidneys. However, expression of vimentin was demonstrated in TEC of degenerate, atrophic tubules and tubules within areas of inflammation (39.5±13.9 TEC/field in TID grade 3; Fig. 2a,d). Some α-SMA-positive myofibroblasts were found in contact with intact tubules both in areas with no interstitial inflammation and in areas of severe TID (0.5± 0.1 TEC/field in TID grade 3). In fibrotic regions, within areas of inflammation, there was occasional positive staining of α-SMA in cells considered to be peritubular myofibroblasts. Both the size and the number of capillaries, next to the zone of cortical interstitial fibrosis and tubular atrophy, were subjectively reduced. Vimentin-PAS immunohistochemical staining showed thickening of the tubular basement membrane (TBM) with occasional multifocal membrane rupture. A high percentage of the tubules with vimentin positivity exhibited preservation of TBM integrity (Fig. 2c).

Statistical results

A linear regression model was generated using the TID grade as predictors and CK and vimentin as dependent variables (Fig. 3). The Pearson correlation coefficient confirmed the existence of a linear relationship. The two

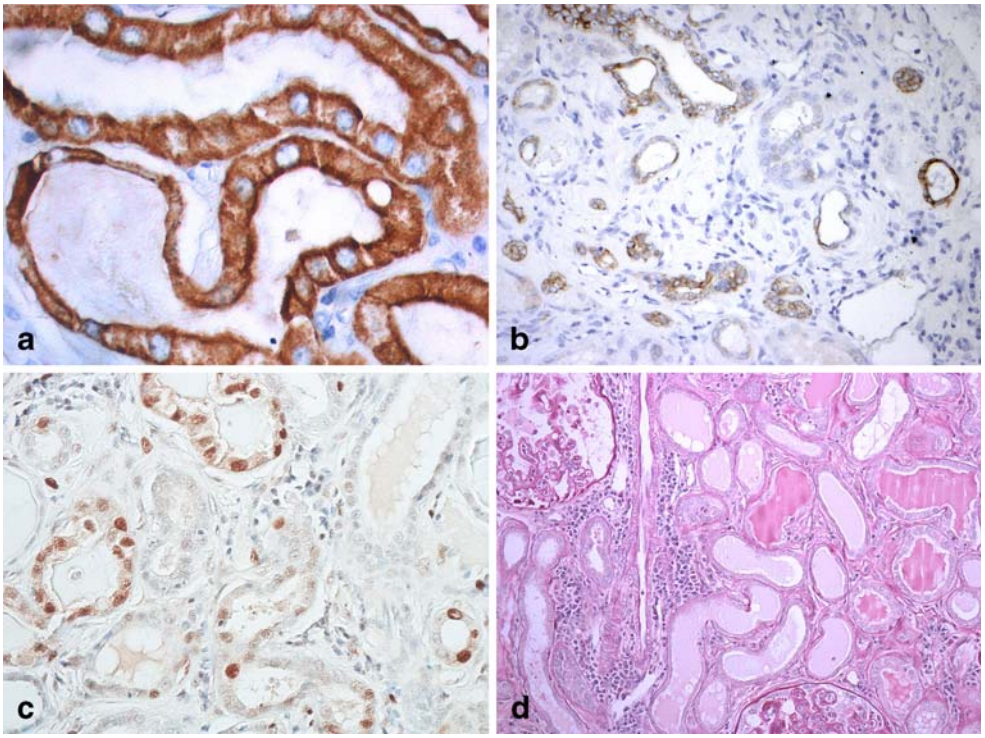

Fig. 1 **a** Normal CK expression in the cytoplasm of TEC in control kidney (×500). **b** Loss of CK expression in most of tubular cross sections inside the inflammation, TID grade 2 (×200). **c** Positive expression of scattered nuclei in epithelial tubular cells inside the inflammation, TID grade 2 (×300). **d** Area of interstitial nephritis, characterized by inflammatory cells (lymphocytes and plasma cells), tubular atrophy and minimum extent of fibrosis, TID grade 3 (PAS, ×200)

lines, with the confidence band at the level 95%, exhibited the behaviour between the indices: as the TID grade increases, the proportion of CK-positive TEC reduces (in fact, the slope is significantly negative); as the TID grade increases, the proportion of vimentin-positive TEC increases (the slope is significantly positive).

Discussion

The present results suggest that TEC are able to lose the typical cytoskeletal intermediate filament of epithelial cells (CK) and transdifferentiate into cells exhibiting the typical cytoskeletal intermediate filament of mesenchymal cells

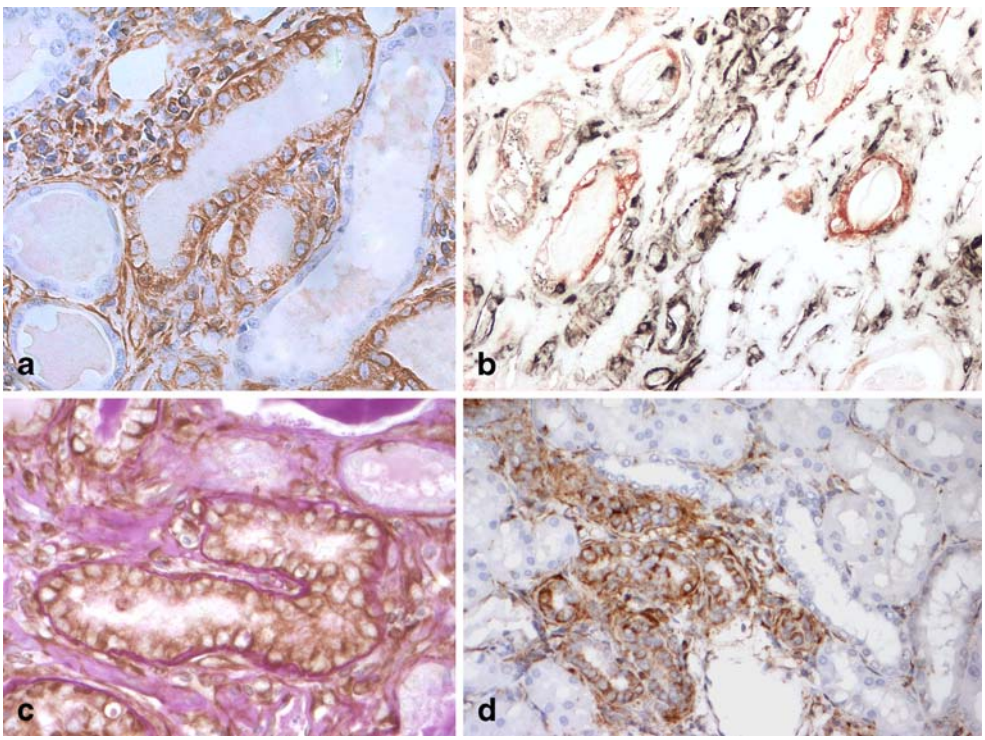

Fig. 2 **a** Diffuse expression of vimentin marker in TEC inside the inflammation, TID grade 2 (×400). **b** Atrophic-degenerating TEC in area of severe TID predominantly express vimentin (black chromogen reaction) and only in few tubules co-localization with CK (red chromogen reaction) is still present (×200). **c** Vimentin expression in tubules associated with thickening of PAS-positive TBM, TID grade 2 (×500). **d** Positive vimentin expression in TEC inside the area of inflammation and negative vimentin expression in tubules not involved, TID grade 1 (×200)

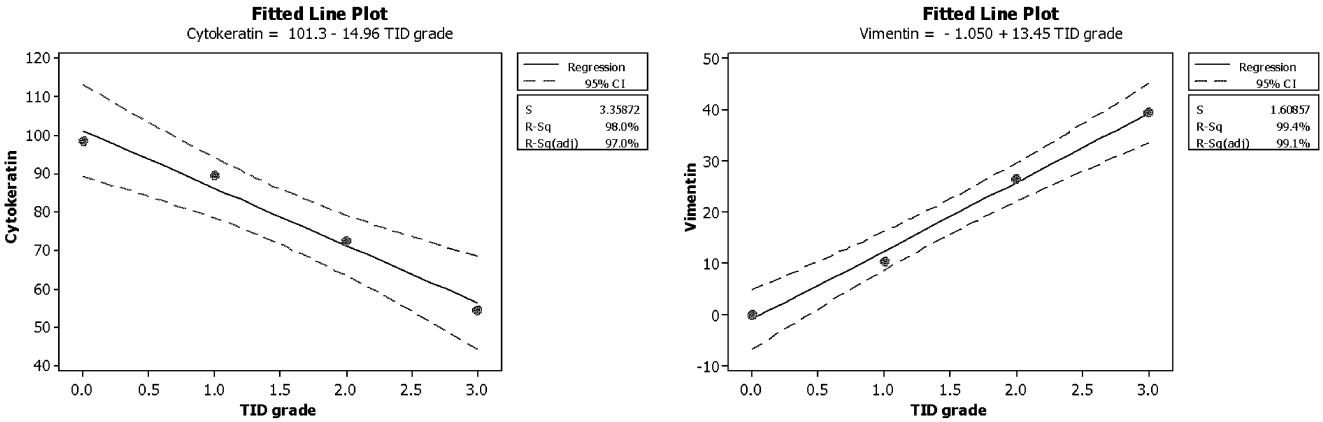

Fig. 3 Linear relationship between TID grade and CK loss (*left*) and vimentin expression (*right*)

(vimentin). This change in the cytoskeletal components named EMT was observed in both the canine renal conditions considered in this study. The process of cell shifting between epithelial and mesenchymal phenotype has long been known to play an important role in cellular transdifferentiation during organ fibrosis and tumour progression in human pathology [21] as well as in cases of interstitial nephritis in animals [16]. During TID, TEC are probably capable of these radical changes in cytoskeletal phenotype expression because of their embryonic metanephrogenic mesenchymal origin [13].

Studies carried out in human renal pathology and in experimental models in laboratory animals have confirmed that TEC actively participate in the mechanisms of injury within the interstitial compartment. These modifications act at different steps including proliferation and phenotypic changes, to eventually lead to synthesis of extracellular matrix. Furthermore, in humans as well as in dogs, the expression of class II major histocompatibility complex molecules in TEC in interstitial nephritis has been demonstrated, drawing attention to the possible role of TEC in the development of TI disease [19]. The present work focuses on the modification of TEC in the two most common canine renal diseases, membranous glomerulonephritis and membrano-proliferative glomerulonephritis. The association between the thickening of the TBM (depicted by the PAS staining) and vimentin expression by TEC suggests that direct interactions between the TEC and the extracellular matrix of the interstitium could be important in the induction of the transdifferentiation process. Other researchers have hypothesised that the critical features of EMT are the ability of epithelial cells to lose polarity, to disassemble cell adhesion systems and their strict relation with the basement membrane [1, 2, 17, 21]. The integrity of the TBM is thus likely to be fundamental in maintaining the epithelial phenotype of TECs [18].

In this study, we have described the first steps in the pathogenesis of renal fibrosis in two of the most common disease resulting in renal failure in dog. Renal tubular EMT is a phenotypic alteration that occurs after chronic injury and results in increased numbers of interstitial mesenchymal cells. As such, EMT may be involved in the initiation of renal scar formation. However, activation of resident interstitial fibroblasts would also be expected to occur after injury as part of normal wound healing by which the injured kidney attempts to repair and to recover [10]. The variable intensity of vimentin-positive staining in TEC suggests the existence of different steps in phenotype transition with the proportion of TECs undergoing transition related to the grade of TI inflammation (see Table 2). Double immunostaining demonstrated the co-expression of CK and vimentin markers within TEC in TID (Fig. 2b).

Table 2 Summary of immunohistochemical results

	Control		TID grade 1		TID grade 2		TID grade 3		*p* value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Cytokeratin	98.7	1.3	89.6	2.3	72.6	4.8	54.5	4.8	0.0012
Vimentin	0.0	0.0	10.5	3.2	26.5	8.3	39.5	13.9	0.0010
α-SMA	0.0	0.0	0.3	0.1	0.5	0.1	0.8	0.2	0.0084
PCNA	0.7	0.3	1.2	0.6	2.6	0.9	3.5	1.5	0.0010
Number of cases	5		7		10		3		

Results are reported as percentage of positive TEC.

Vimentin, the intermediate filament protein specific for mesenchymal cells [6], from our work appears to be the most suitable marker for the defining the process of EMT in the kidney. In our study, PCNA staining results showed an increase in the proliferation index of TEC within the TI compartment directly correlated with the severity of TID. This could be considered further evidence of the suggested multi-potential capacity of tubular cells in repairing damage [23]. EMT features were totally absent in the control kidneys, suggesting a link between TI inflammation and phenotypic transition of epithelial tubular cells. Histological studies of human kidneys and animal models have also shown that TI injury is associated with damage to renal arterioles and loss of peritubular capillaries [3, 5]. In our work, the mild increase in number of α-SMA-positive cells recorded in TID correlated with a minimal myofibroblasts proliferation. Glomerular inflammation and scarring have the potential to compromise blood flow in downstream peritubular capillaries and thereby result in ischemic injury to tubule cells. This mechanism may be intimately involved with EMT, with hypoxia activating fibroblasts and altering the extra-cellular matrix metabolism of resident renal cells. Within areas of fibrosis, small arteries may remain constricted causing anoxia and perpetuating the vicious cycle of TI disease, through further fibroblasts activation [11].

In summary, the crucial loss of the epithelial phenotype in EMT in dogs can be demonstrated by CK and vimentin co-expression in double immunostaining, whilst the process of EMT may contribute to the pathogenesis of fibrosis in the canine kidney. The importance of EMT in canine TI disease remains to be fully elucidated. The canine model can be used to define the evolution of the lesions, and sequential screening of the progression of the renal fibrosis at different times would be helpful in understanding the mechanism of transition.

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ORIGINAL ARTICLE

The pathological response to neoadjuvant chemotherapy with FOLFOX-4 for colorectal liver metastases: a comparative study

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Abstract FOLFOX-4 (*fol*inic acid/5-*f*luorouracil/*ox*aliplatin) chemotherapy is used to treat patients with colorectal liver metastases. We aimed to assess hepatic histopathological responses to neoadjuvant FOLFOX-4 chemotherapy in patients with colorectal liver metastases. We selected all patients (n=54) treated with FOLFOX-4 for colorectal liver metastases between June 2002 and June 2005. Only 25 underwent hepatectomy and formed the study group. Histological responses were assessed in the study group and a matched control group (n=25) that did not receive neoadjuvant chemotherapy. The median (IQR) body mass index in the study and control groups was 24 (22–26) and 24 (23–25) kg/m^2, respectively, (P = NS). Complete histological resolution of tumour occurred in six (24%) patients in the study group. Median residual tumour cellularity was less (35 vs 70%) and fibrosis greater (50 vs 5%) in patients in the study group when compared with controls (P<0.001). The liver surrounding the tumour was steatotic in 17 (68%) patients in the study group and five (20%) controls (P= 0.001). Hepatic sinusoidal dilatation was more pronounced in patients in the study group than in controls (P<0.001). The response to FOLFOX-4 was associated with tumour necrosis, fibrosis and inflammation. More than two thirds of patients undergoing hepatectomy after FOLFOX-4 had steatosis despite being non-obese.

Keywords Chemotherapy · Colorectal liver metastases · Complications · FOLFOX-4 · Hepatectomy · Neoadjuvant · Outcome · Oxaliplatin · Steatosis

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Introduction

Oxaliplatin (trans-l-diaminocyclohexane oxalatoplatinum), is a platinum derivative with a high level of in vitro and in vivo activity against colorectal cancer cells [9]. Oxaliplatin-based chemotherapy alters the natural history of unresectable colorectal liver metastases by downstaging the disease, allowing operability and prolonging survival in some patients [1–3, 5, 11].

Hepatic sinusoidal abnormalities have been shown to be present in up to 78% of patients receiving oxaliplatin [12]. Changes range from sinusoidal dilatation to sinusoidal obstruction and perisinusoidal fibrosis leading to veno-occlusive disease of the liver. Patients with colorectal liver metastases treated with oxaliplatin or irinotecan-based

neoadjuvant chemotherapy can also develop what is termed chemotherapy associated steatohepatitis [7, 14]. It has also been shown that steatosis is associated with neoadjuvant irinotecan chemotherapy (20% of patients) and sinusoidal dilatation with neoadjuvant oxaliplatin chemotherapy (19% of patients) [13]. Patients who developed steatosis in this study had a significantly higher 90-day postoperative mortality compared with those who had liver resection without chemotherapy [13]. In contrast, two studies have shown no significant increase in risk of steatosis in patients receiving oxaliplatin-based chemotherapy compared to patients receiving no chemotherapy [4, 8].

The aim of this study was to evaluate the histological responses in patients undergoing hepatectomy for colorectal liver metastases after neoadjuvant chemotherapy exclusively with FOLFOX-4 (*fol*inic acid/5-*f*luorouracil/*ox*aliplatin) and to compare these responses with those in patients who did not receive neoadjuvant chemotherapy.

Materials and methods

Study design

The study was a retrospective comparative one set in a university teaching hospital.

Study groups

All patients who received neoadjuvant FOLFOX-4 chemotherapy for colorectal liver metastases between June 2002 and June 2005 were identified from a prospectively maintained oncology database. The study group comprised patients who underwent hepatectomy after treatment with FOLFOX-4. A control group of patients who underwent hepatectomy without neoadjuvant chemotherapy was selected from our Liver Unit database by matching for age, sex, Dukes' stage of the primary tumour and body mass index (BMI). While selecting controls, the investigators were blind to the operative outcome and histological findings.

Interventions

Study group The group was composed of patients undergoing neoadjuvant FOLFOX-4 chemotherapy and hepatectomy. On the first day of each cycle, patients received folinic acid (200 mg/m^2) and oxaliplatin (85 mg/m^2) as intravenous infusions over 2 h. They also received an intravenous injection of 5-fluorouracil (400 mg/m^2), followed by an intravenous infusion of 600 mg/m^2 over 22 h. The same doses of folinic acid and 5-fluorouracil were repeated on day 2. There was no treatment from days 3–14 and the next cycle was started on day 15. Hepatectomy was performed 5–7 weeks after the last cycle of chemotherapy.

Control group This group was composed of patients who did not receive neoadjuvant chemotherapy but underwent hepatectomy for colorectal metastases.

Assessment of response

Clinical case notes were reviewed along with computed tomography scans before and after chemotherapy. Histology was reviewed by one pathologist (AMZ) in all those who underwent hepatic resection, and histological responses were graded objectively.

Three sections of tumour and non-tumoural liver from one large block of the resected specimen were sampled to evaluate pathological changes. Tumour regression, fibrosis, vascular changes, inflammatory response and steatosis were recorded.

Tumour regression was graded according to the percentage area of fibrosis in the residual tumour (or tumour area in control group) into five grades according to the method proposed by Dworak et al. [6]: *Grade 0*: no regression, *Grade 1*: dominant tumor mass with obvious fibrosis and/or vasculopathy, *Grade 2*: dominantly fibrotic changes with few tumor cells or groups (easy to find), *Grade 3*: very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance, and *Grade 4*: no tumor cells, only fibrotic mass (total regression or response). The mitotic index was assessed by determining the mean of the number of mitotic figures in the tumour in 20 random high power fields (×40 magnification) per resected specimen.

The degree of inflammatory response was characterised into three main grades, *Grade 0*: no inflammatory response, *Grade 1*: mild or minimal response (characterised by the presence of small percentage of inflammatory cells - <10% in the field) and *Grade 2* moderate or marked response (characterised by the presence of ≥10% inflammatory cells in the surrounding area of the tumour).

Sinusoidal dilatation was graded according to the system described by Rubbia-Brandt et al. [12]: *Grade 0*: absent, *Grade 1*: mild (centrilobular involvement limited to one third of the lobular surface), *Grade 2*: moderate (centrilobular involvement extending to two thirds of the lobular surface) and *Grade 3*: severe (complete lobular involvement). Presence of pericellular/perisinusoidal fibrosis was also noted.

Steatosis was assessed in the peri-tumoural liver, and the presence of macrovesicular and microvesicular steatosis was noted [15]. The degree of steatosis in the liver not involved with tumour was classified into five grades: *Grade 0*: no

steatosis, *Grade 1*: characterised by the presence of steatosis in <10% of the parenchymal area, *Grade 2*: characterised by the presence of steatosis in ≥10% but <30% of the total parenchymal area, *Grade 3*: steatosis present in ≥30% but <60% of the parenchymal area and *Grade 4*: steatosis present in ≥60% of the parenchymal area.

Statistical analysis

The data were analysed with SPSS for Windows™ Release 14.0 software (SPSS, Chicago, USA). The Mann–Whitney *U* test was used to compare the percentage responses, and the χ^2 test was used for categorical data. Differences were considered significant at $p<0.05$.

Ethics

The Ethics Committee of Nottingham University Hospitals approved the study.

Results

Over the study period, 54 patients (Table 1) received a median (IQR) of six (four to eight) cycles of neoadjuvant FOLFOX-4 chemotherapy for colorectal liver metastases. Twenty-nine of the 54 patients (53.7%) did not undergo liver resection because of extrahepatic disease (13), locally advanced disease (2), poor response to chemotherapy (11) and medical reasons (3). The study group consisted of the 25 patients who underwent hepatectomy after FOLFOX-4. The control group was well matched for age, sex, BMI and Dukes' stage of the colonic primary with the study group (Table 1).

Operative morbidity and mortality

Operations performed in the study and control groups, respectively, were right hepatectomy ($n=13$ and 12), right trisectionectomy ($n=2$ and 2), left hepatectomy ($n=2$ and 3) and segmental resection ($n=8$ and 8). Eight patients in the study group developed postoperative complications, which included a bile leak (2), liver failure (2), sepsis (2), bilioma (1) and hepatic duct stricture (1). Four patients in the control group developed postoperative complications—wound infection (1), pulmonary embolism (1), atrial fibrillation (1) and pneumonia (1). There was no statistical difference in the type of operation and rate of complications in the two groups. There were no deaths within 30 days in either group, but one patient in the study group who underwent a left hepatectomy died of fulminant liver failure 3 months later.

Histological response

The histological responses in the resected liver (Figs. 1 and 2) are summarised in Table 2. The interface between the tumour and the surrounding normal liver showed a marked inflammatory response in the FOLFOX-4-treated group compared to control group (Table 2).

Sinusoidal dilatation was more pronounced in the study group than in controls (Table 2, Fig. 2). However, pericellular/perisinusoidal fibrosis was present in only three patients in the study group and two in the control group ($P=0.49$).

Steatosis in the tumour-free liver (Fig. 2) was more frequent and more pronounced in FOLFOX-4-treated patients than controls (Table 3). There was no relation between the development of steatosis and BMI (Table 3).

Table 1 Profile of the study and control groups

	Patients who received FOLFOX-4 ($n=54$)	Hepatectomy after FOLFOX-4: Study group ($n=25$)	Hepatectomy without neoadjuvant chemotherapy: Control group ($n=25$)
Median (IQR) age (years)	65 (61–72)	65 (61–72)	64 (60–70)
Sex ratio (M/F)	33:21	16:9	16:9
Dukes' stage of the primary (A/B/C)	3:2:49	3:0:22	2:4:19
Median (IQR) body mass index (kg/m^2)	25 (23–28)	24 (22–26)	24 (23–25)
Median (IQR) number of metastases	3 (2–4)	2 (1–2)	2 (1–2)
Median (IQR) diameter (cm) of largest metastasis	4.9 (2.7–7.0)	3.5 (2.5–6.5)	4.0 (2.8–7.0)

Differences between the groups were not statistically significant.

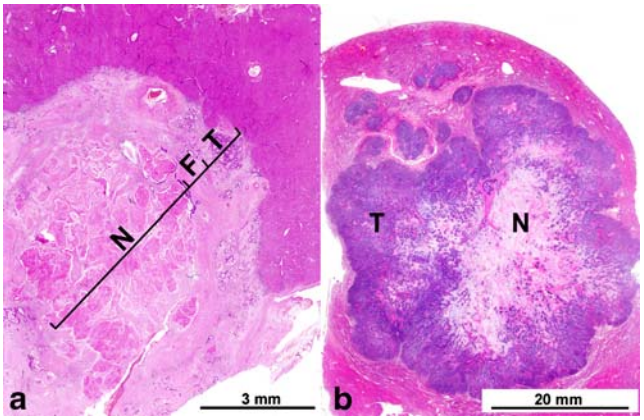

Fig. 1 **a** Haematoxylin & eosin-stained section of liver (×1) demonstrating a moderate response to chemotherapy with three zonal changes: central zone of necrosis (*N*), mid zone of fibrosis (*F*) and outer zone of residual tumour (*T*). **b** Haematoxylin & eosin stain (×1) of a tumour nodule from a patient who did not receive neoadjuvant chemotherapy showing two zones: central zone of necrosis (*N*) and outer zone of tumour (*T*)

Discussion

The present study has shown that the histological response to FOLFOX-4 is through a fibrotic process, replacing the tumour nodule. This desmoplastic reaction was negligible in the control group. There also was a marked inflammatory response within the tumour and in the interface between the tumour and surrounding liver parenchyma. Both the treated and the control groups had central tumour necrosis, but this was much more pronounced in the treated group. Histologically, three zones could be identified in a FOLFOX-4-treated liver nodule—a central zone of necrosis, an intermediate zone of fibrosis and a peripheral zone of inflammation.

The liver not involved with tumour showed steatotic change in 68% of patients treated with FOLFOX-4 compared to 20% of controls. None of these patients with steatosis had a history of alcohol dependence or diabetes mellitus nor were they on medications likely to cause steatosis, implicating FOLFOX-4 therapy as a potential causative agent of steatosis. This finding gains prominence in the context of the more extensive liver resections being carried out after neoadjuvant FOLFOX-4. However, the limiting factor in liver resections is an adequate functional liver volume to prevent postoperative liver failure. Chemotherapy-induced steatosis can potentially compromise function of the remnant liver.

A large series of 325 liver resections in patients with steatosis showed an increased rate of postoperative infective morbidity, but no effect on mortality when compared with 160 matched controls without steatosis [10]. However, these patients developed steatosis from varying aetiologies, with chemotherapy being just one of them [10]. Yedibela et al. [14] studied 64 patients who underwent hepatectomies for colorectal cancer metastases (neoadjuvant chemotherapy, 32; no chemotherapy, 32) and found that chemotherapy can induce a steatotic change in the non-tumour liver in 6% of treated patients compared to 1% of controls. However, the authors did not specify the chemotherapeutic agent used or grade the steatosis. The term "chemotherapy associated steatohepatitis" (CASH) was coined by Fernandez et al. [7] when they demonstrated a frequent occurrence of steatohepatitis in patients receiving oxaliplatin/irinotecan-based chemotherapy for colorectal liver metastases (n=14) compared to those receiving 5-fluorouracil (n=10) and controls (n=13) who did not receive chemotherapy. They also showed that a BMI >30 kg/m^2 was a risk factor [7]. Vauthey et al. [13] have recently demonstrated that steatosis occurred in irinotecan- rather than oxaliplatin-based regimens and was strongly linked to irinotecan-treated patients irrespective of BMI. However, oxaliplatin-treated patients in this study had a stronger association with sinusoidal dilatation than steatosis [13]. Despite the relatively large numbers of patients in the Vauthey study, the treated and control groups were not matched for comparing histology and clinical outcome [13]. Aloia et al. [4] studied a cohort of 92 patients, 75 of whom underwent hepatectomy after different chemotherapy regimes (5-FU + folinic acid or FOLFOX) and 17 after no chemotherapy. Although two thirds of patients in the chemotherapy group received oxaliplatin, they did show an increased rate of steatosis. In a similar study, Karoui et al. [8] demonstrated that although neoadjuvant chemotherapy was associated with sinusoidal dilatation, hepatocyte atrophy or necrosis, there was no significant difference in steatosis, inflammation and fibrosis in the normal liver in the chemotherapy-treated group compared to controls. However, both these studies [4, 8] had similar biases of the study group being treated with different chemotherapeutic regimes and the study and control groups not being matched. In contrast to these, our

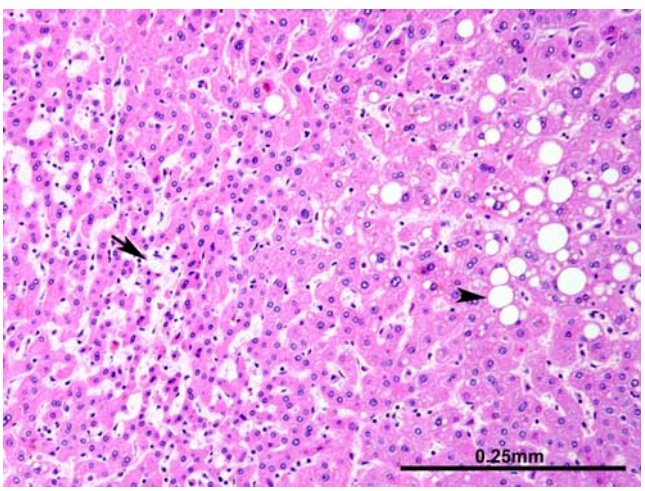

Fig. 2 Haematoxylin & eosin-stained section of liver not involved with tumour (×10): central vein zone showing sinusoidal dilatation (*arrow*) and hepatic steatosis (*arrowhead*)

Table 2 Summary of the histological response to neoadjuvant FOLFOX-4 compared with controls

Tissue	Histology	Resected after FOLFOX-4 (n=25)	Resected without chemotherapy (n=25)	P value
Tumour nodule	Complete resolution of tumour [n (%)]	6 (24%)	0	0.02
	Dworak grading [6]			<0.0001
	Grade 0: no regression [n (%)]	0	16 (64%)	
	Grade 1: dominant tumor mass with obvious fibrosis and/or vasculopathy [n (%)]	4 (16%)	9 (36%)	
	Grade 2: dominantly fibrotic changes with few tumor cells or groups (easy to find) [n (%)]	7 (28%)	0	
	Grade 3: very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance [n (%)]	8 (32%)	0	
	Grade 4: no tumor cells, only fibrotic mass (total regression or response) [n (%)]	6 (24%)	0	
	Inflammatory response			<0.001
	Grade 0: (no inflammation) [n (%)]	5 (20%)	19 (76%)	
	Grade I: (<10% inflammation) [n (%)]	19 (76%)	6 (24%)	
	Grade II: (≥10% inflammation) [n (%)]	1 (4%)	0	
	Mitotic index [median (IQR)]	2.5 (2–3)	4 (3–6)	0.003
Non-tumour liver	Steatosis [n (%)]	17 (68%)	5 (20%)	0.001
	Macrovesicular [n (%)]	5 (20%)	1 (4%)	
	Microvesicular [n (%)]	12 (48%)	4 (16%)	
	Sinusoidal dilatation			<0.001
	Grade 0: absent [n (%)]	0	14 (56%)	
	Grade 1: mild (centrilobular involvement limited to one third of the lobular surface) [n (%)]	14 (56%)	11 (44%)	
	Grade 2: moderate (centrilobular involvement extending to two thirds of the lobular surface) [n (%)]	8 (32%)	0	
	Grade 3: severe (complete lobular involvement) [n (%)]	3 (12%)	0	

comparative study demonstrates a strong link between FOLFOX-4 therapy and steatosis in a series of patients uniformly treated with FOLFOX-4, compared with matched controls. Steatosis occurred more frequently and was more pronounced in FOLFOX-4-treated patients compared to those who did not receive chemotherapy. However, there was no correlation with BMI, suggesting that the development of steatosis may be independent of obesity. Two patients in our study had postoperative liver failure. Both these patients had ≥60% steatotic change in the hepatic parenchyma. Histological examination of other resected specimens clearly demonstrated that liver not involved with tumour was steatotic to varying degrees.

It may appear that steatosis induced by FOLFOX-4 may be a reasonable trade-off for down staging of metastases and operability. On the other hand, we cannot ignore the fact that significant steatosis was responsible for two cases of liver failure (one death) in our series. The risk of steatosis should be carefully assessed before planning a major hepatectomy after neoadjuvant chemotherapy with FOLFOX-4. Biopsy of the liver not involved with tumour may be of value in assessing this risk preoperatively, and

Table 3 Degree of steatosis and body mass index in FOLFOX-4-treated colorectal liver metastases compared with controls

Degree of steatosis	Resected after FOLFOX-4 (n=25)		Resected without chemotherapy (n=25)	
	n*	Median (IQR) BMI (kg/m^2)[a]	n*	Median (IQR) BMI (kg/m^2)[a]
Grade 0 (no steatosis)	8	26 (24–28)	20	24 (23–25)
Grade I (<10%)	4	23 (21–23)	2	23 (23–24)
Grade II (≥10% but <30%)	7	24 (23–26)	2	23 (22–24)
Grade III (≥30% but <60%)	4	23 (23–26)	1	24
Grade IV (≥60%)	2[b]	25 (25–26)	0	–

*p=0.01

[a] No significant differences

[b] Both patients developed postoperative liver failure and one died.

preoperative portal vein embolization may have to be considered in some patients who may have insufficient hepatic reserve after resection.

Sinusoidal dilatation complicated by perisinusoidal fibrosis has been documented after oxaliplatin-based chemotherapy [4, 8, 12]. It has been suggested that these changes may result in impairment of liver function and limit the regenerative capacity of the liver [12]. In contrast to this, we showed only hepatic sinusoidal dilatation without significant perisinusoidal fibrosis in the FOLFOX-4-treated group.

We conclude that FOLFOX-4 therapy is an independent risk factor for the development of hepatic steatosis. Further studies of the development, progression and resolution (if it occurs) of hepatic steatosis and its temporal relationship to the cycles of chemotherapy may help time post-chemotherapy liver resections prudently. The presence of steatosis and the degree of the steatotic change may be critical in assessing the risk of postoperative liver failure. As steatotic livers are more prone to ischaemia-reperfusion injury, avoidance of hypoxia during surgery is advisable.

Conflict of interest None of the authors has a conflict of interest to declare.

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ORIGINAL ARTICLE

Primary and metastatic high-grade pleomorphic sarcoma/ malignant fibrous histiocytoma of the gastrointestinal tract: an approach to the differential diagnosis in a series of five cases with emphasis on myofibroblastic differentiation

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Abstract Primary and metastatic so-called malignant fibrous histiocytoma/undifferentiated high-grade pleomorphic sarcoma (MFH) is rare in the gastrointestinal (GI) tract with ≈50 primary and five metastatic cases reported so far. We evaluated two primary gastric and three metastatic intestinal high-grade pleomorphic sarcomas with features of storiform-pleomorphic MFH. Gastric tumours occurred in a 79-year-old man and a 68-year-old woman. One patient died post-operatively, and the other was disease-free at 6 months. Three patients presented with GI metastasis 24, 60 and 0 months after diagnosis of MFH of the heart (n=1) and the thigh (n=2). Metastases were located in the small (n=1) and large bowel (n=2) and were characteristically pedunculated and polypoid with oedematous haemorrhagic stroma. Concurrent metastases (brain, lung, bone) were present in all three cases. Tumours expressed α-smooth muscle actin (four of five), platelet-derived growth factor receptor (PDGFR) α (three of three) and PDGFRβ (two of three) but were negative for CD117, CD34 and other lineage-specific markers. Ultrastructural examination revealed myo/fibroblastic features. Both gastric MFH were wild type for *KIT* and *PDGFRα*. In conclusion, primary and metastatic MFH of the GI tract commonly express PDGFRα and show a myo/fibroblastic phenotype. They should be distinguished from a variety of primary and metastatic pleomorphic neoplasms, in particular high-grade sarcomatous GI stromal tumours (GIST), pleomorphic leiomyosarcoma, sarcomatoid carcinoma and other mimics.

Keywords MFH · Gastrointestinal · GIST · Peritoneum · PDGFRα · PDGFRβ

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Introduction

Once the most commonly diagnosed soft tissue sarcoma (STS) in adults [43], the existence of 'malignant fibrous histiocytoma' (MFH) as a diagnostic entity has been questioned in the recent years [15, 16]. This dramatic shift in the diagnosis and classification of STS was the result of recent studies showing that the majority of pleomorphic STS formerly called 'MFH' could be classified into one of the well-characterized sarcoma types, if ancillary diagnostic tools are applied [15, 16, 18, 24, 29]. More importantly, the clinical relevance of this sub-classification could be confirmed in several studies [16, 24]. Adult high-grade pleomorphic sarcomas with myogenic differentiation (leiomyosarcoma, rhabdomyosarcoma and myofibrosarcoma) are characterized by a poor outcome compared to other pleomorphic sarcoma types [16, 24]. However, no significant difference in the prognosis was observed among the various sub-types of adult pleomorphic myogenic sarcomas [16, 24]. The term 'undifferentiated high-grade pleomorphic sarcoma/MFH' has been

adopted by the latest World Health Organization (WHO) classification of soft tissue tumours for pleomorphic STS showing no specific line of differentiation [17]. MFH most commonly involves the deep soft tissue of the extremities (mainly the thigh) with a predilection for men [17, 43]. Metastases occur predominantly in the lung (82%), lymph nodes (32%), liver and bone (15% each) and other rare sites [43].

Primary and metastatic MFH is rare in the gastrointestinal (GI) tract with ≈50 primary [33, 41, 42, 44] and five metastatic cases reported from different parts of the GI tract, usually as single case reports [1, 6, 9, 22, 33, 39–42, 44]. In this study, we describe and compare the clinicopathological and immunohistochemical features of two primary gastric MFH and three metastatic MFH involving the small and large bowel and discuss their broad differential diagnosis with a reference to myofibroblastic differentiation.

Materials and methods

Cases were retrieved from routine surgical pathology files of the Nuremberg Medical Center (a large community-based hospital). One case has been reported previously [2]. At least ten paraffin blocks were available in cases with large neoplasms. Immunohistochemical stains were performed by the avidin–biotin-peroxidase complex method on fresh-cut sections using a Dako Envision+ autostainer and an Envision+ kit (K5007, Dako, Lot 29661). The following antibodies were applied: vimentin (Vim 3B4, 1:50, Linaris, Wertheim-Bettingen, Germany), α-smooth muscle actin (α-SMA; 1A4, 1:200, Dako), desmin (D33, 1:250, Dako), *h*-caldesmon (h-CD, 1:75, Dako), S-100 (polyclonal, 1:2,500, Dako), CD117 (anti-Human c-kit proto-oncogene product, polyclonal antibody, 1:200, Dako), CD34 (BI-3C5, 1:200, Zytomed), fibronectin (polyclonal antibody, 1:2,000, Dako), HMB45 (1:50, Dako), pan-keratin (KL-1, 1:300, Immunotech), Ki-67 (MiB-1, 1:150, Dako), myogenin (F5D, 1:50, Dako), platelet-derived growth factor receptor (PDGFR) α (polyclonal, 1:50, New England Biolabs) and PDGFRβ (Clone 28E1, 1:100, New England Biolabs). In brief, tissue sections were mounted on poly-L-lysine-coated slides, incubated at 37°C for 30 min, deparaffinized and washed, followed by microwave antigen retrieval in target buffer (pH 6.0, Dako, no. S1699; Lot 6859) for CD117, α-SMA, desmin, CD34, S100 and Ki-67. Alternatively, for PDGFRα and PDGFRβ slides were incubated in a pressure cooker at 120°C, and primary antibodies were incubated overnight at 4°C. Endogenous peroxidase was blocked by applying a peroxidase-blocking solution (Dako, S2023, Lot 29772) for 8 min at room temperature. Appropriate positive and negative controls were used in each case. At least three paraffin blocks per case were evaluated for CD117, CD34, desmin, α-SMA and *h*-caldesmon.

Mutation analysis of *KIT* and *PDGFRA*

Genomic tumour DNA from both gastric neoplasms was extracted from formalin-fixed paraffin-embedded tissue by microdissection and investigated for presence of *KIT* exons 9, 11 and 13 and *PDGFRα* exons 12 and 18 mutations using polymerase chain reaction amplification and direct sequencing as previously described [4, 20].

Ultrastructural studies

Only formalin-fixed paraffin embedded tissue was available. Tissue samples of 2 mm^2 were punched out from tumour blocks in cases 1, 2 and 3, deparaffinized and processed for electron microscopy as previously described [5].

Results

The five cases represented 2% of all primary and metastatic mesenchymal GI neoplasms in our routine case material and 24% of 21 non-GIST sarcomas primary or metastatic to the GI tract exclusive of retroperitoneal liposarcoma and other intra-abdominal sarcomas secondarily involving the GI tract.

Clinical features

Primary tumours

These were found in a 79-year-old man and a 68-year-old woman. Tumours arose in the gastric fundus and greater curvature. Clinical symptoms were non-specific. Endoscopic biopsy was performed in case 1 and was consistent with MFH. Treatment was total gastrectomy and two-thirds-gastrectomy (Billroth II), respectively. The first patient died 2 weeks post-operatively of pulmonary embolism, but autopsy was not performed. The other was alive and well at 6 months (Table 1).

Metastatic tumours

Patient ages at time of diagnosis of GI metastasis were 30, 54 and 61 years. All patients presented with abdominal pain and/or signs of GI bleeding. Time to GI metastasis was 24, 60 and 0 months from initial diagnosis of the primary sarcoma. Involved GI sites were the small bowel, left colon and rectum (one each). The site of primary sarcoma was the heart (left atrium; $n=1$) and the proximal thigh ($n=2$). Two patients received curative surgery for their primary sarcoma

Table 1 Clinicopathologic features of primary and metastatic MFH in the GI tract (n=5)

Number	Age/sex	Site	Symptoms	Size	Gross features	Other metastasis outcome
Primary MFH						
1	79/M	Stomach fundus	Non-specific, large ulcer	8×7.5×5 cm	Polypoid ulcerated and intramural, whitish	Died 2 weeks post-operatively of pulmonary embolism, no concurrent MTS
2	68/F	Stomach greater curvature	Weight loss, Abdominal mass	12×9×6 cm	Extramural, gastric wall infiltrated, cystic areas, fleshy whitish-yellow	No MTS, NED at 6 months
Metastatic MFH						
3	30/M	Small bowel	Abdominal pain, lower GI bleeding	4 cm	4 cm pedunculated polyp, 1.4 cm intramural nodule	Cerebral MTS 12 months before GI manifestation GI metastasis 24 months from diagnosis of left atrial MFH
4	54/F	Descending colon	Non-specific, +ve haemoccult	3.5 cm	3.5 cm pedunculated polyp	Multiple lung MTS concurrent with GI MTS 60 months from diagnosis of thigh MFH
5	61/M	Rectum, probably Rt. Flexure, cecum	non-specific	3 mm	3 mm polyp, small shallow ulcers in Rt flexure +cecum	thigh MFH with concurrent femur, pleuropulmonary cerebral and GI MTS DOD at 5 weeks

MTS Metastasis, *NED* no evidence of disease, *DOD* died of disease

with no evidence of local recurrence at the time of GI metastasis. The patient with left atrial MFH received, in addition, percutaneous radiotherapy. The third patient had an inoperable 20×14-cm deeply seated intramuscular soft tissue mass in the proximal right thigh with pathological fracture of the femur. Diagnosis was achieved by core biopsy. Synchronous rectocoloscopy revealed multiple shallow ulcers and polypoid lesions measuring up to 5 mm in the cecum, right flexure and the distal rectum. He died of respiratory failure because of extensive pleuropulmonary metastases 5 weeks from initial diagnosis. Multiple further organ metastases were diagnosed before or concurrent to GI metastases in all patients (cerebral metastases in case 3, pulmonary metastases in case 4, pleuropulmonary, cerebral and osseous metastases in case 5; Table 1).

Pathological findings

Primary tumours

Representative gross and histological features are depicted in Fig. 1. Tumours measured 12 and 8 cm in maximum diameter and revealed a fish-flesh-like to grey-whitish focally whorled cut surface (Fig. 1a,b). The deep margins were irregularly infiltrating into the subserosal tissue. Histologically, both tumours consisted of large highly pleomorphic plump spindled to epithelioid cells arranged in intersecting sheets with an occasional storiform pattern (Fig. 1c). In some areas, the tumour cells were reminiscent of myofibroblasts with stellate and wavy nuclei and copious pale eosinophilic to amphophilic cytoplasm that gradually merged with the surrounding stroma (Fig. 1d). Osteoclast-like giant cells were prominent in case 2 (Fig. 1e) and were typically associated with stromal haemorrhage. Mitoses ranged from 17 to 50 in ten high-power fields (average, 31). Scattered entrapped adipocytes were seen near the exterior border of the tumour in case 2 (Fig. 1f). Immunohistochemistry (Table 2) showed a strong reactivity for vimentin in both lesions. α-SMA was expressed in 80 and 20% of tumour cells, respectively (Fig. 1g). Osteoclast-like giant cells were strongly reactive for CD68-KP1 (Fig. 1h). Both cases stained with PDGFRα with a moderate to strong cytoplasmic and membranous staining in ≥70% of the cells (Fig. 1i). In addition, case 1 stained weakly for PDGFRβ. Tumours were consistently negative for CD117, CD34, *h*-caldesmon, protein S100, pan-keratin, HMB45, desmin, myogenin and ALK-1. Case 1 revealed a weak reactivity in scattered cells for MDM2.

Metastatic tumours

The surgical specimen from cases 3 and 4 harboured 4- and 3.5-cm pedunculated polyps with a dark red soft cut surface (Fig. 2a,b). In addition, a 1.4-cm intramural nodule was submitted from case 3 separately. In case 5, minute

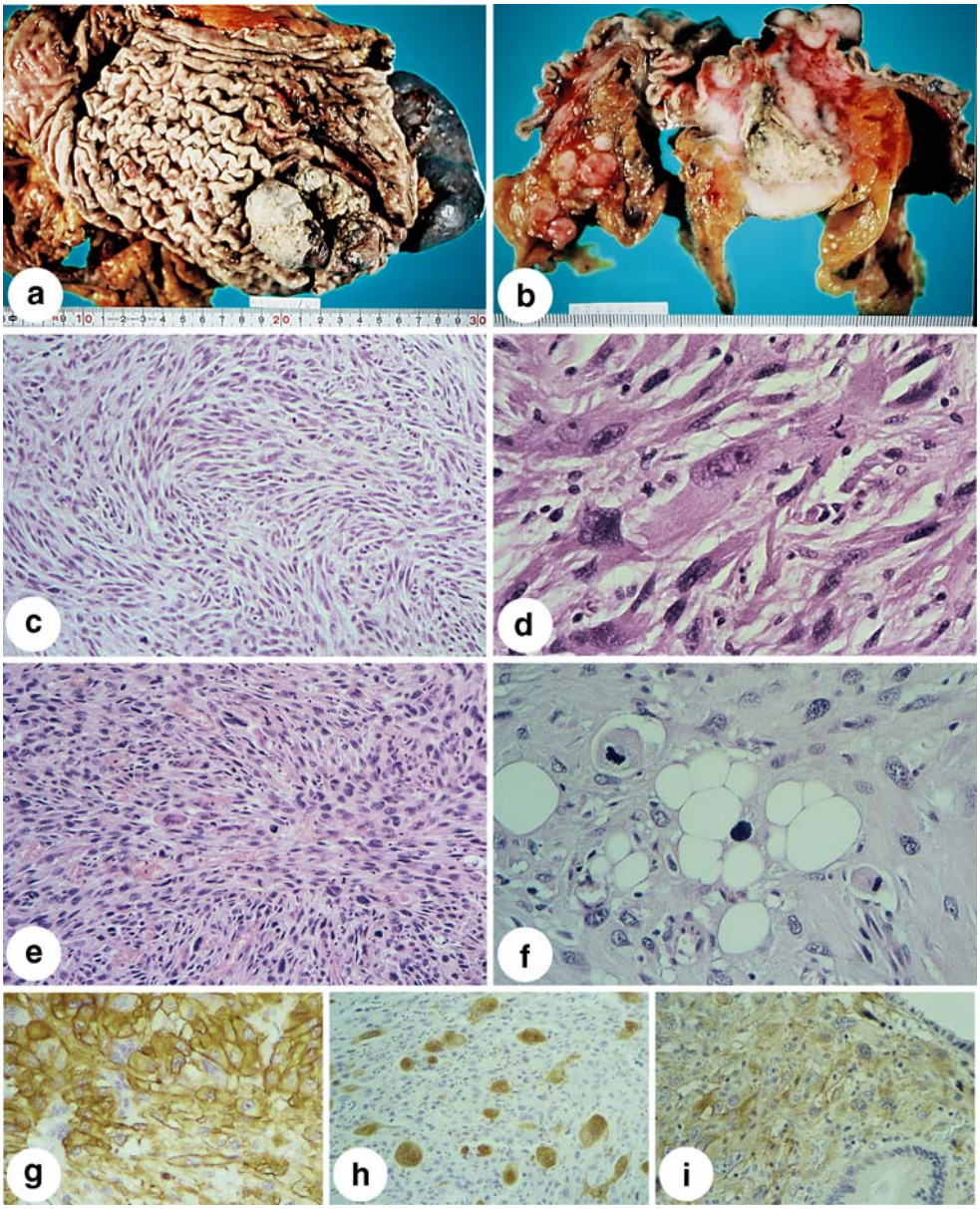

Fig. 1 **a**–**i** Gross and histopathological features of primary gastric MFH from case 1 (**a**–**d**) and case 2 (**e**–**i**). **a** Gastrectomy specimen showing a large ulcerated fundic mass bulging the mucosa. **b** Whitish cut surface with deep ulceration and infiltration into perigastric tissue. **c** Spindle cells arranged in sweeping fascicles. **d** High nuclear features with gradual disappearance of cytoplasm into stroma. **e** Fascicular/storiform pattern with occasional osteoclastic giant cells. **f** Focal entrapment of adipocytes mimicking liposarcoma. **g** Diffuse reactivity for α-SMA with peripheral accentuation. **h** CD68 highlighting osteoclast-like giant cells. **i** Moderate expression of PDGFRα (note non-staining mucosal glands)

endoscopic biopsies from colorectal lesions were submitted. Histological examination showed essentially similar microscopic features with a higher nuclear pleomorphism and a remarkably oedematous and haemorrhagic angiomyxoid stroma (Fig. 2c). Ulceration was more extensive, and no more than minimal involvement of the inner muscularis propria was seen, except for the smaller intramural nodule in case 3. The latter showed focal epithelioid areas with cytoplasmic vacuoles reminiscent of epithelioid GIST (Fig. 2d) but was predominantly highly pleomorphic in other areas. The rectal biopsy from case 5 revealed metastatic sarcoma, but colonic biopsies were negative for tumour. Immunohistochemistry revealed a similar profile as for cases 1 and 2 with a strong expression of α-SMA in cases 3 and 4 (Fig. 2e). Case 3 stained strongly with PDGFRα (Fig. 2f). All other markers were negative. Primary MFH from the thigh and left atrium were all of the storiform-pleomorphic type.

Molecular findings

Both primary gastric tumours (cases 1 and 2) were examined and were wild type for *KIT* exons 9, 11 and 13 and *PDGFRα* exons 12 and 18.

Ultrastructural features

Tissue preservation was generally poor. Tumour cells (cases 1, 2 and 3) revealed features of fibroblastic/myofibroblastic differentiation with spindle to plump vesicular indented nuclei and abundant dilated rough endoplasmic reticulum (Fig. 3). Peripherally located microfilaments with focal

Table 2 Immunohistochemical findings in primary and metastatic gastrointestinal MFH (n=5)

Marker	Case 1	Case 2	Case 3	Case 4	Case 5
Vimentin	+++	+++	+++	+++	+++
α-SMA	+	+++	++	(+)	+++
Desmin	–	–	–	–	–
h-Caldesmon	–	–	–	–	–
S-100	–	–	–	–	–
CD117	–	–	–	–	–
CD34	–	–	–	–	–
PDGFR α	+++	+++	+++	ND	ND
PDGFRβ	+	–	+	ND	ND
HMB45	–	–	–	–	–
Myogenin	–	–	–	–	–
Pan-keratin	–	–	–	–	–
MiB-1	80%	60%	50%	40%	40%

(+) Rare isolated tumour cells positive, + 10–25% tumour cells positive, ++ 25–50% tumour cells positive, +++ >50% tumour cells positive, *ND* not done

densities and rare cell-to-matrix connections were present in case 2 (Fig. 4) (Table 3).

Discussion

This represents the first study combining primary and metastatic MFH of the GI tract from a single institution. Previously, reported primary MFH of the GI tract involved the small and large bowel more frequently and only rarely the stomach [22, 33, 41, 42, 44]. As the majority of them have preceded the era of sub-classifying pleomorphic STS, it is likely that a considerable fraction of previous reports might have represented pleomorphic variants of other specific sarcoma types. The clinical features and the biological course of primary GI MFH are comparable to their soft tissue counterparts [33, 41, 42]. Symptoms were generally non-specific, but signs of GI bleeding are common. Rare cases presented initially with pulmonary

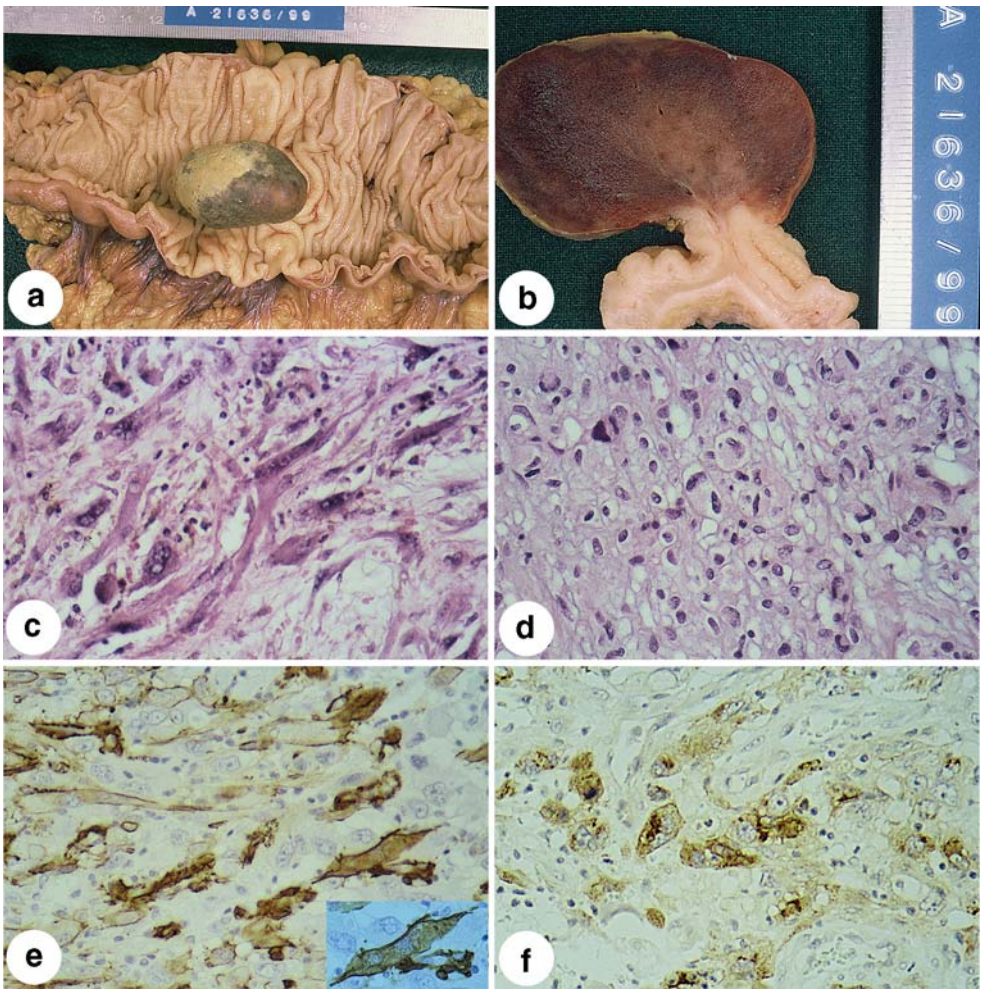

Fig. 2 **a**–**f** Gross and histopathological features of metastatic MFH. **a** Left hemicolectomy specimen (case 4, [2]) showing an ulcerated smooth-surfaced pedunculated polyp with extensive haemorrhage on cut surface (**b**). **c** Highly pleomorphic tumour cells set in an edematous angiomyxoid stroma with haemorrhages. **d** Focal epithelioid areas mimicking GIST. **e** Strong α-SMA staining highlighting cell–stromal connections (*inset*). **f** PDGFRα with a coarsely granular paranuclear cytoplasmic pattern in pleomorphic tumour cells

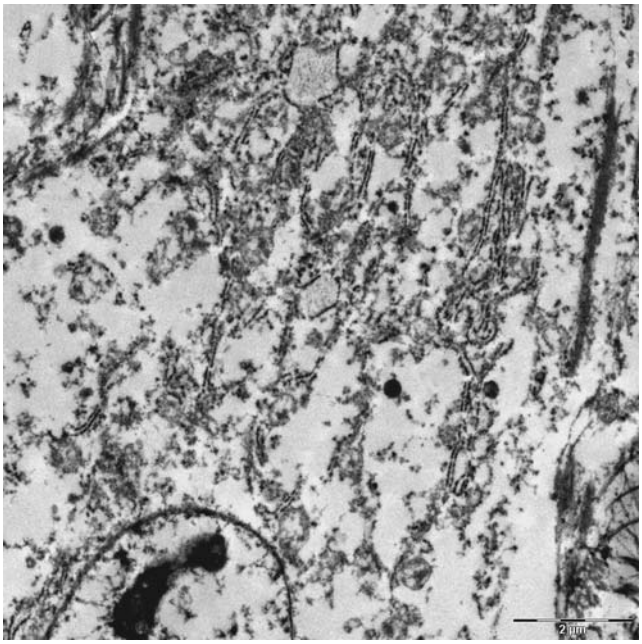

Fig. 3 Ultrastructurally, prominent dilated rough endoplasmic reticulum filling the cytoplasm (case 2, original magnification 5,000×)

metastases [44] and intussusception [22]. In a previous review [41], 24% of patients with a mean follow-up of 15 months died of disease 2–48 months post-operatively (mean, 15). Metastases/local recurrence developed in 35% of cases.

Intra-abdominal metastasis from non-visceral STS is rare and affected only 0.9% of STS patients in one large study [7]. Myxoid liposarcoma was the most frequent histological type, and only 2 of 19 were MFH [7]. GI metastasis from STS usually presents months to years after diagnosis but may be present initially as in the current study. In our cases, GI metastasis occurred with almost equal frequency as primary GI MFH. This contrasts with previous reports (≈50 primary vs 5 metastatic MFH), possibly suggesting under-recognition of GI metastasis from MFH or over-diagnosis of primary GI MFH. It is of interest that one of our cases (case 3) was initially diagnosed as a GI primary because of lacking clinical information. On preparing this study, we discovered that this patient had a storiform-pleomorphic MFH of the left atrium treated by surgery and radiotherapy 2 years ago with subsequent cerebral metastasis. Distinguishing primary and metastatic MFH in the GI tract is mandatory for appropriate patient treatment. An occult or previously resected STS should be excluded by appropriate clinical and imaging procedures before rendering a diagnosis of primary MFH in the GI tract. However, subtle differences exist between primary and metastatic GI MFH. A predominantly polypoid pattern combined with a remarkable stromal oedema and haemorrhage argue for metastatic disease, as does multi-focal occurrence. Primary polypoid GI sarcomas limited to the mucosa/submucosa are exceedingly rare with only three unequivocal examples reported previously [3, 36, 46], where two of them were multi-focal [3, 46].

The differential diagnosis of MFH/undifferentiated high-grade pleomorphic sarcoma encompasses a wide range of pleomorphic neoplasms and is a matter of exclusion [17]. Pleomorphic leiomyosarcomas reveal, at least focally, typical areas that permit diagnosis. Their cells show a brightly eosinophilic cytoplasm and blunt-ended nuclei by light microscopy with a common expression of desmin and *h*-caldesmon [30, 32]. Ultrastructurally, they possess diffusely distributed cytoplasmic myofilaments and a well-developed external lamina [11, 18]. A sub-set of MFH expressing smooth muscle markers represented unusual variants of leiomyosarcoma and could be re-classified as such using ancillary methods [15, 16, 19, 29, 32]. It is interesting to note that a subset of MFH revealed similar cytogenetic features as leiomyosarcoma suggesting a common pathogenetic pathway [38].

'Myofibrosarcomas' show histological and ultrastructural features intermediate between fibrosarcoma and leiomyosarcoma. They are characterized by a pale eosinophilic to amphophilic cytoplasm, fusiform-tapered indented or plump nuclei, prominent dilated rough endoplasmic reticulum, modestly developed peripherally located myofilaments with focal densities, sub-plasmalemmal attachment plaques and specialized cell-to-matrix connecting structures (fibronexus junctions or microtendons) [11, 12, 18, 31]. The latter was considered by some authors essential for

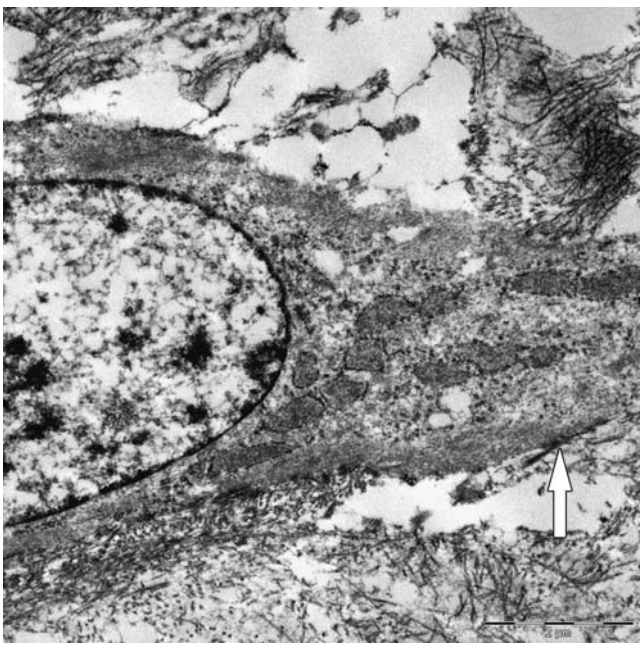

Fig. 4 High-power view in case 2 showing subplasmalemmal filaments with focal densities and a cell-to-matrix connecting structure (arrow, original magnification 5,000x)

Table 3 Ultrastructural features of primary and metastatic MFH in the GI tract (n=3)

Case number	Indented nuclei	Discont. basal lamina	Micropino-cytotic vesicles	Dilated rough ER	Microfilaments with focal densities	FNJ	Predominant cell type
1	+	ND	ND	+	+	ND	Fibroblasts
2	++	ND	ND	+++	+++	?	Myofibroblasts
3	+	ND	ND	+	No	ND	fibroblasts

FNJ Fibronexus junction, *ND* not detected, + focally present, ++ prominent, +++ diffusely present, ? questionable

myofibroblastic differentiation [11, 12]. However, based on the degree of differentiation in individual cases, these features may be less well developed and focal. The strong and peripherally accentuated expression of α-SMA in some of our cases (in particular in case 2) together with the cytomorphological and ultrastructural features and the lack of *h*-caldesmon all argue for a myofibroblastic phenotype. Admittedly, ultrastructural evaluation on formalin-fixed paraffin embedded tumour tissue was largely limited by poor tissue preservation. Thus, a diagnosis of 'pleomorphic myofibrosarcoma' could not be definitively confirmed. Ultrastructural myofibroblastic differentiation in MFH has been documented in several studies but carried no prognostic significance [31]. Although the occurrence of low-grade myofibroblastic sarcoma was recognized by the current WHO classification of soft tissue tumours [28], the concept of 'pleomorphic myofibrosarcoma' is not generally accepted. The nature and significance of the myofibroblast-like phenotype seen in MFH and other pleomorphic neoplasms (specific line of differentiation vs a common final pathway for undifferentiation) is still controversial [13, 31]. Likewise, cutoffs for minimal criteria for diagnosing 'pleomorphic myofibrosarcoma' and the potential clinical relevance of such a diagnosis remain to be defined.

Dedifferentiated liposarcoma (DDL) follows a more favourable clinical course and should be separated from MFH [25]. Intra-abdominal DDL is typically a retroperitoneal neoplasm with a well-differentiated lipogenic component or a history of atypical lipomatous tumour. However, rare cases may present initially as DDL, and a minor lipogenic component may be missed on sectioning. A majority of retroperitoneal MFH represented DDL in a recent retrospective study [10]. Nuclear staining for MDM2 and CKD4 is sensitive in detecting DDL, but both markers lack absolute specificity and may be rarely seen in a subset of high-grade sarcomas [8]. The presence of pleomorphic lipoblasts in an 'MFH-like' background is characteristic of pleomorphic liposarcoma. Entrapment of fatty tissue in MFH, as in the current study, may mimic pleomorphic liposarcoma.

KIT-negative GISTs are generally cellular and monotonous throughout and have a prominent epithelioid component, and a majority of them harbour mutations in PDGFRα exons 18 and 12 [26]. PDGFRα and PDGFRβ have emerged as additional immunomarkers in identifying KIT-negative GIST [34, 35, 37], but results have been contradictory. While one study reported a strong reactivity for PDGFRα in KIT-negative epithelioid GISTs but in none of KIT-expressing GISTs [37], another reported co-expression of both proteins in KIT-mutated GISTs [34]. Furthermore, 25% of non-GIST neoplasms [35] and 27% of desmoid tumours [37] stained with PDGFRα. None of four sarcomas tested expressed PDGFRα [35]. A remarkable pleomorphism comparable to MFH is exceptional in GISTs and may indicate dedifferentiation, but this rare phenomenon is usually focal, and a conventional component is evident (own unpublished data). Conversely, focal epithelioid areas in MFH may mimic GIST, but more typical areas are usually easily detectable. *h*-Caldesmon is commonly expressed in GISTs and in leiomyosarcoma but not in MFH or myo/fibroblastic sarcomas [14, 30]. The biological significance of PDGFRα/β staining in MFH in the current study is not clear. This might indicate a non-specific neoexpression, but a potential role for these receptor tyrosine kinases in the pathogenesis and progression of a subset of MFH, similar to desmoid tumours [23], cannot be excluded, meriting future investigation of more cases.

Sarcomatoid carcinoma usually shows evidence of origin from a mucosal surface with a detectable epithelial component in most cases (at least dysplastic or carcinoma in situ changes). Expression of keratins may be helpful in equivocal cases. The polypoid pattern adopted by many sarcomatoid carcinomas may mimic metastatic pleomorphic sarcoma.

Osteoclast-like giant cells (as in one of the current cases) have been described as a rare non-specific finding in a variety of neoplasms [21, 27] and as an integral component of a recently described clear cell sarcoma-like GI neoplasm [45]. The latter rare entity reveals a strong reactivity for S100 combined with absent expression of melanocytic markers and KIT. Some of the cases harboured the characteristic t(11;22)(q13;q12) translocation [45].

Other more common pleomorphic intra-abdominal and retroperitoneal sarcomas, metastatic genital tract sarcomas,

pleomorphic rhabdomyosarcoma, pleomorphic epithelioid angiomyolipoma/malignant PEComa with high-grade features, follicular dendritic cell sarcoma, sarcomatoid mesothelioma, melanoma and other miscellaneous primary and metastatic GI sarcomas may mimic and be mistaken for MFH, if not thought of and should be excluded by careful analysis of architectural and cytomorphological features, assisted by appropriate use of ancillary methods.

In summary, we documented five cases of primary and metastatic MFH in the GI tract demonstrating myofibroblast-like features and expressing PDGFRα. Before rendering a diagnosis of primary MFH in the GI tract, a variety of primary and metastatic 'MFH-like' neoplasms and a concurrent or previously excised STS should be excluded by appropriate morphological and clinical evaluation. Similarly, extensive sectioning to detect or exclude a better differentiated minor component would help establish a correct diagnosis in liposarcoma, pleomorphic leiomyosarcoma, dedifferentiated pleomorphic GISTs and others.

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ORIGINAL ARTICLE

Altered expression of desmocollin 3, desmoglein 3, and β-catenin in oral squamous cell carcinoma: correlation with lymph node metastasis and cell proliferation

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Abstract Desmocollin 3 (Dsc3) and desmoglein 3 (Dsg3) are both transmembrane glycoproteins that belong to the cadherin family of calcium-dependent cell adhesion molecules. β-Catenin is a member of the cadherin–catenin complex that mediates homotypic cell–cell adhesion and is also an important molecule in the wnt signaling pathway. In this study, we examined the simultaneous expression level of Dsc3, Dsg3, and β-catenin in oral squamous cell carcinomas (OSCCs) and normal oral epithelia using immunohistochemistry. There was a significant correlation ($p<0.05$) among the following variables in OSCCs: reduced or loss of expression of Dsc3, Dsg3, and β-catenin compared to normal oral epithelium, reduced or loss of expression of Dsc3 and histological grade (moderately or poorly differentiated), and reduced or loss of expression of β-catenin and lymph node metastasis. Furthermore, a positive correlation was found between reduced or loss of β-catenin staining and reduced or loss of Dsc3 staining in lymph node metastatic cancer tissue ($r=0.734$, $p<0.05$). These results suggest an abnormal expression of Dsc3, Dsg3, and β-catenin induced in the progression of oral carcinomas and that the Dsc3 expression level might be related to the regulation of β-catenin in lymph node metastasis and cell proliferation in OSCCs.

Keywords Oral squamous cell carcinoma · Desmosomal cadherins · β-Catenin · Lymph node metastasis · Cell proliferation

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Introduction

Desmosomes are intercellular junctional apparatuses that connect intracellular intermediate filaments to the cell surface and mediate strong cell–cell adhesion [5]. Malignant transformation is often characterized by major changes in the organization of the cytoskeleton, decreased adhesive strength, and aberrant adhesion-mediated signaling [5, 6]. Disruption of normal cell–cell adhesion in transformed cells may contribute to enhancement of tumor cell migration and proliferation, leading to invasion and metastasis [6].

The main components of desmosomes consist of the products of three gene superfamilies: The desmosomal

cadherins, the armadillo family of proteins, and the plakins family of cytolinkers [11, 12, 38].

Desmosomal cadherins are divided into two subfamilies: desmogleins (Dsg) and desmocollins (Dsc). There are four main types of epidermis-specific Dsg proteins, Dsg1, Dsg2, Dsg3, and Dsg4, and three major Dsc proteins, Dsc1, Dsc2, and Dsc3, each cadherin subtype being encoded by a unique gene [12]. They belong to the cadherin family of calcium-dependent cell adhesion molecules. Plakoglobin belongs to the armadillo family and binds directly to the cytoplasmic tail of both Dsg and Dsc [27, 26] and is closely related to the adherens junction molecule β-catenin [39].

β-Catenin interacts with the cytoplasmic tail of the calcium-dependent cell–cell adhesion molecule and through the association with plakoglobin creates a link between cadherin and the actin cytoskeleton [39]. More specifically, β-catenin is involved in the Wingless/Wnt signaling pathway [2] and interacts with epidermal growth factor receptor [16], the adenomatous polyposis coli protein product [35], and proto-oncogene c-ErbB2 [25]. β-Catenin also interacts with the two nuclear transcription factors, lymphoid-enhancing binding factor-1/T cell factor-4 [24].

Many studies have demonstrated a reduction in or loss of cell surface desmosomal cadherin expression, β-catenin membranous expression, and β-catenin mutation, which correlated with esophageal carcinoma [23], oral carcinoma [37, 18], and other carcinomas in systemic organs [29, 19, 1]. It was also shown that downregulation of desmosomal protein and abnormal β-catenin expression was associated with invasion and cell proliferation in pharyngeal [9], oral [9, 34], hepatocellular [7], and pancreatic [31] carcinomas.

In this study, we examined the simultaneous expression levels of Dsc3, Dsg3, and β-catenin in both oral squamous cell carcinomas (OSCCs) and normal oral epithelia using immunohistochemistry. We analyzed the relationship between clinicopathological findings, especially metastasis or cell proliferation and Dsc3, Dsg3, and β-catenin expression levels. We also examined the intracellular localization of Dsc3, Dsg3, and β-catenin expression.

Materials and methods

Human samples and clinicopathologic data

Between January 2001 to December 2003, we collected 48 formalin-fixed, paraffin-embedded human primary OSCC biopsy specimens (without previously treated), which included 24 cases without lymph node metastases and 24 cases with lymph node metastases, and 26 normal oral epithelia from the patients admitted to Tokyo Medical and Dental University Hospital, Japan. The patients with OSCC consisted of 20 women and 28 men with an age range of 28–82 years and an average age at the diagnosis of 59 years. Tumors were located in the tongue in 20 cases (41.7%), in the gingiva in 12 cases (25%), in the floor of the mouth in nine cases (18.8%), and in the buccal mucosa in seven cases (14.6%). Clinical details of each patient included age, gender, tumor size, clinical stage, lymph node status, and histological grade of tumor differentiation. Histological grading was done according to the World Health Organization (WHO) classification [8, 28].

Serial sections of 4 μm thick were cut from the paraffin-embedded tissue blocks and mounted on silanized slides. One section was stained with hematoxylin–eosin and examined to confirm the original diagnosis and tumor grade, and the other sections were submitted to immunohistochemical staining.

Immunohistochemical analysis

Anti-β-catenin (1:100, E-5, mouse monoclonal, Santa Cruz Biotechnology, Santa Cruz, CA) antibody, Dsc3 (U-114, mouse monoclonal, ready to use, Progen Biotechnik, Germany) antibody, and Dsg3 (1:25, 5G11, mouse monoclonal, Zymed, Berlin, Germany) antibody were used for all specimens. After deparaffinization and rehydration, endogenous peroxidase activity was blocked by immersing the sections into methanol containing 0.3% hydrogen peroxide at room temperature for 20 min. For antigen retrieval, histological sections were heated twice in 10 Mm citrate buffer (pH 6.0) for 10 min each time in a 500-W microwave oven, and sections were subsequently incubated with 10% normal rabbit serum to block nonspecific immunological reactions for 30 min. Each section was incubated with primary antibody overnight in a moist chamber, followed by incubation with biotinylated anti-mouse IgA+G+M secondary antibody and streptavidin–peroxidase complex (Nichirei, Tokyo, Japan). Finally, 3,3'-diaminobenzidine was used as a chromogen to visualize labeling. Nuclei were counterstained with methyl green. Negative controls were prepared by substituting the primary antibody with phosphate-buffered saline.

Scoring of immunostaining

The adjacent normal oral epithelium was used as an immunopositive control for Dsc3, Dsg3, and β-catenin staining that is normally seen at cell–cell junctions. A grading system was devised to obtain a semiquantitative evaluation of the distribution of each protein in the tissue slides. Scores were awarded according to Alazawi et al. [1] as follows: 0, negative staining throughout the epithelium, I, minimal membrane staining, II, patchy nonpericellular membrane staining, III, pericellular staining in one third of

Table 1 Comparison of immunohistochemical staining score of β-catenin, Dsc3, and Dsg 3 in normal oral mucosa and OSCCs

	Dsc3							Dsg3							β-Catenin						
	0	I	II	III	IV	V	Total	0	I	II	III	IV	V	Total	0	I	II	III	IV	V	Total
NOM[a]	0	0	0	1	10	15	26	0	0	0	3	8	15	26	0	0	0	4	11	11	26
OSCC[b]	6	10	14	10	8	0	48	4	13	15	6	8	2	48	4	10	18	12	4	0	48
P	<0.01							<0.01							<0.01						

[a] *NOM* Normal oral mucosa
[b] *OSCC* Oral squamous cell carcinoma

the epithelium, IV, pericellular staining in two thirds of the epithelium, and V, pericellular staining in all three thirds of the epithelium.

Statistical analysis

The statistical data was stored and analyzed by SPSS 13.0 for Windows software (SPSS). The Chi-squared test was used to analyze the association between clinical parameters (age, gender, T status, clinical stage, regional lymph node status, and histopathological grade) and immunohistochemical staining results. The relationship between the expression of β-catenin and Dsc3/Dsg3 were analyzed using Pearson's coefficient of correlation method. A p value less than 0.05 was accepted as statistically significant.

Results

Immunohistochemical examination showed Dsc3, Dsg3, and β-catenin expressions in all samples as follows.

Expression of Dsc 3

As shown in Table 1, Dsc3 membranous immunoreactivity was confined to the basal and spinous cells of normal oral epithelium (15 cases scored V points, ten cases scored IV points, and only one case scored III points, Fig. 1a), and intracytoplasmic expression was not observed. On the other hand, the reduced or absent membranous expression of Dsc3 was found in the OSCC cases (30 of 48 cases showing 0–II scores) with only 18 cases showing III–IV

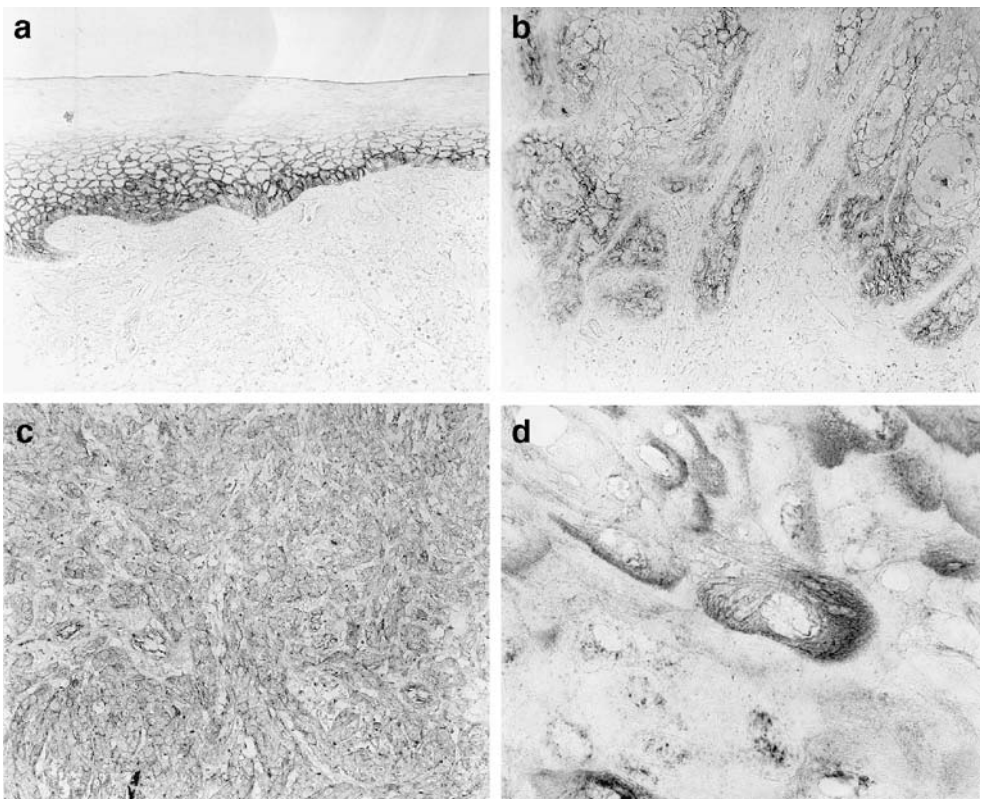

Fig. 1 **a** Dsc3 immunohistochemical staining in normal oral epithelium showing strong linear membranous expression (150×). **b** Dsc3 immunohistochemical staining showing partially reduced membranous and cytoplasmic expression in moderately differentiated OSCC (150×). **c** Dsc3 staining is completely absent in poorly differentiated OSCC (150×). **d** Abnormal Dsc3 immunohistochemical localization in the cytoplasm of tumor cells (750×)

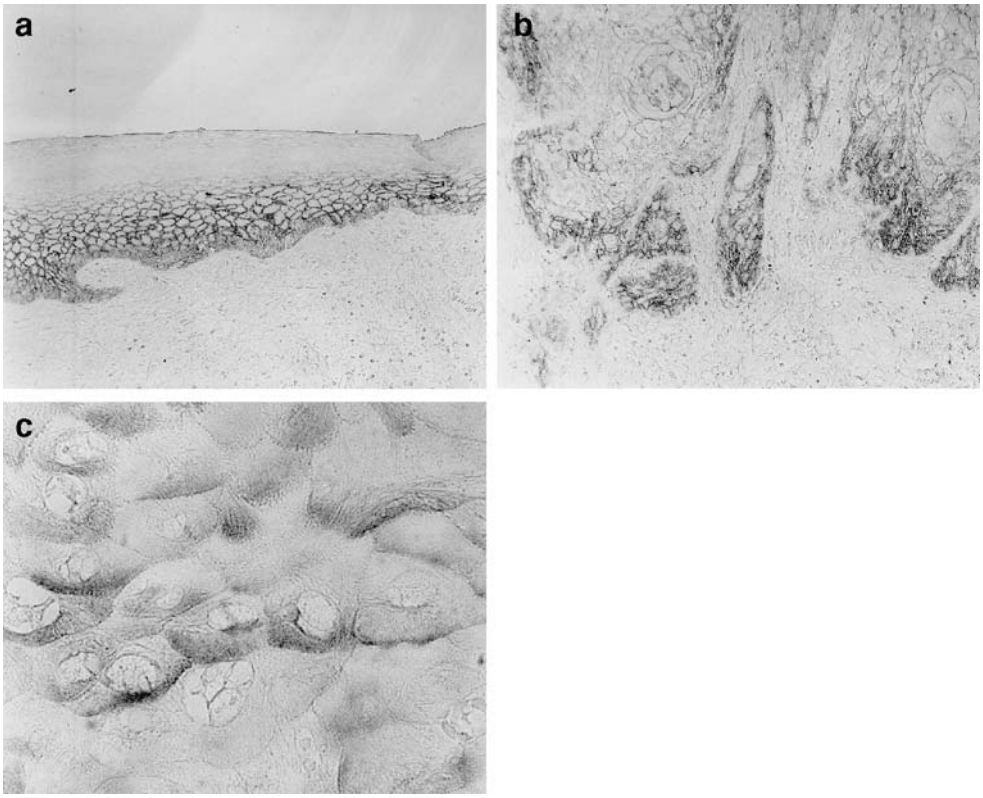

Fig. 2 **a** In normal oral epithelium, immunohistochemical staining of Dsg3 shows strong linear membranous expression (150×). **b** Dsg3 immunohistochemical staining showing partially reduced membranous and cytoplasmic expression in moderately differentiated OSCC (150×). **c** Abnormal Dsg3 localization in the cytoplasm of tumor cells (750×)

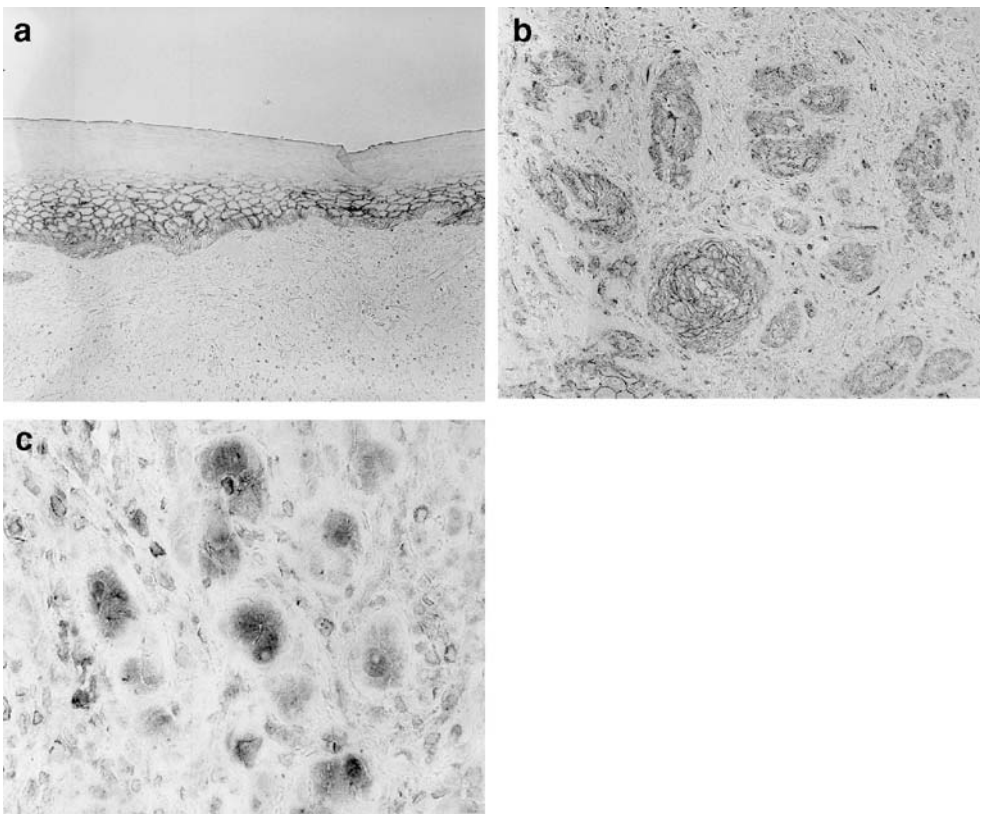

Fig. 3 **a** In normal oral epithelium, β-catenin shows normal membranous expression (150×). **b** Peripheral loss of membranous of β-catenin in well differentiated OSCC (150×). **c** Nuclear β-catenin expression in tumor cells of OSCCs (750×)

scores (Fig. 1b,c). The scores of Dsc3 expression were significantly lower in OSCCs than in normal oral epithelium ($p<0.01$). In addition, Dsc3 exhibited diffuse intracytoplasmic immunostaining, and nuclear staining was not observed in OSCC tumor cells (Fig. 1d).

Expression of Dsg 3

The staining results of Dsg3 are summarized in Table 1. Dsg3 membranous staining was located in the basal and spinous cells of normal oral epithelium (15 cases scored V points, eight cases scored IV points, and only three cases scored III points, Fig. 2a), and cytoplasmic expression was not observed, whereas reduced or absent membranous staining of Dsg3 was found in OSCCs (32 of 48 showing 0–II scores, 16 of 48 showing III–V scores, Fig. 2b). The staining score of Dsg3 was significantly lower in OSCCs than in normal oral epithelium ($p<0.01$). In addition, intracytoplasmic accumulation of Dsg3 was observed in OSCC tumor cells (Fig. 2c).

Expression of β-catenin

The expression of β-catenin in normal oral epithelia and OSCCs is presented in Table 1. Among 26 specimens of normal oral epithelium, β-catenin expression was showed as a strong, linear membranous pattern (11 cases scored V points, 11 cases scored IV points, and four cases scored III points, Fig. 3a). Faint staining was detected in the cytoplasm, but no nuclear staining was found, whereas reduced or absent membranous expression of β-catenin was recognized in OSCCs (32 of 48 showing 0–II scores) with only 16 cases with score III or IV (Fig. 3b). There was a statistically significant difference in low expression level of β-catenin between OSCCs and normal oral epithelium ($p<0.01$). In addition, β-catenin exhibited

Table 2 Correlation between β-catenin, Dsc3, and Dsg 3 immunohistochemical staining score and clinicopathological parameters in OSCCs

	Dsc3			Dsg3			β-Catenin		
	0~II[a]	III~V[b]	Total	0~II[a]	III~V[b]	Total	0~II[a]	III~V[b]	Total
Age									
≥60	16	10	26	17	9	26	18	8	26
<60	14	8	22	15	7	22	14	8	22
p value	>0.05			>0.05			>0.05		
Gender									
Male	20	8	28	19	9	28	20	8	28
Female	10	10	20	13	7	20	12	8	20
p value	>0.05			>0.05			>0.05		
T status									
T1+T2	18	8	26	16	10	26	19	7	26
T3+T4	12	10	22	16	6	22	13	9	22
p value	>0.05			>0.05			>0.05		
Clinical staging									
Stage 1–2	16	6	22	13	9	22	15	7	22
Stage 3–4	14	12	26	19	7	26	17	9	26
p value	>0.05			>0.05			>0.05		
Histological differentiation									
Grade 1	6	16	22	13	9	22	17	5	22
Grade 2	18	1	19	18	1	19	12	7	19
Grade 3	6	1	7	3	4	7	3	4	7
p value	<0.05			>0.05			>0.05		
N status									
N^{-}[c]	14	10	24	14	10	24	10	14	24
N^{+}[d]	16	8	24	18	6	24	22	2	24
p value	>0.05			>0.05			<0.05		

[a] *0~II* Score 0, I, and II
[b] *III~V* score III, IV, and V
[c] N^{-} Negative lymph node metastasis
[d] N^{+} Positive lymph node metastasis

Table 3 Correlation between expression levels of β-catenin and Dsc3/Dsg3 in lymph node metastasis

correlation	LN[a] positive for metastasis		LN negative for metastasis	
	Pearson's correlation (coefficient, *r*)	*p* value	Pearson's correlation (coefficient, *r*)	*p* value
0–II[b]				
β-Catenin/Dsc3	0.734	<0.05	0.219	>0.05
β-Catenin/Dsg3	0.428	>0.05	0.317	>0.05
Dsc3/Dsg3	0.348	>0.05	0.289	>0.05
III–V[c]				
β-Catenin/Dsc3	0.216	>0.05	0.209	>0.05
β-Catenin/Dsg3	0.308	>0.05	0.231	>0.05
Dsc3/Dsg3	0.231	>0.05	0.199	>0.05

[a] Lymph node
[b] Score 0, I, and II
[c] Score III, IV, and V

cytoplasmic and nuclear immunoreactivity in OSCC tumor cells (Fig. 3c).

Relationship between clinicopathological features and expression of Dsc3 Dsg3 and β-catenin in OSCCs

The variety of Dsc3, Dsg3, and β-catenin expression in relation to clinicopathological features are presented in Table 2. No correlation was found between the reduced or loss of membranous staining of Dsc3, Dsg3, β-catenin and clinical parameters such as age, sex, T status, and clinical stage ($p>0.05$, respectively). Whereas, the statistical analyses showed a positive correlation between reduced or loss of membranous staining of β-catenin and lymph node metastasis ($p<0.05$) and also between reduced or loss of membranous staining of Dsc3 and histological grade (moderately or poorly differentiated; $p<0.05$).

β-Catenin expression levels correlate with decrease in Dsc3 and Dsg3 expression levels in lymph node metastasis in OSCC

The reduced or absent expression of β-catenin had a positive correlation with reduced or absent expression of Dsc3 showing scores 0, I, and II in 24 cases with lymph node metastasis ($p<0.05$, Table 3). There was no significant correlation between the expression of Dsc3/Dsg3 and β-catenin/Dsg3 revealing scores 0, I, and II in 24 cases with lymph node metastasis ($p>0.05$, respectively, Table 3). However, there were no correlations among the decrease in β-catenin/Dsc3, β-catenin/Dsg3, and Dsc3/Dsg3 expression levels exhibiting scores III, IV, and V in 24 cases with lymph node metastasis ($p>0.05$, respectively, Table 3). It is also showed in Table 3 that no correlation among the decrease in β-catenin/Dsc3, β-catenin/Dsg3, and Dsc3/Dsg3 expression levels exhibiting scores 0, I, and II and scores III, IV, and V in 24 cases without lymph node metastasis ($p>0.05$, respectively).

Discussion

Desmosomes are intercellular junctional apparatuses that provide membranous anchors for intermediate filament cytoskeleton. A reduced number of desmosomes would represent reduced intercellular adhesiveness, which, in turn, would be expected to promote invasive and metastatic ability [34]. In this study, we used immunohistochemistry to examine the cellular expression of Dsc3, Dsg3, and β-catenin in OSCCs and normal oral epithelium. We analyzed the relationship between clinicopathological features, especially metastasis or cell proliferation and Dsc3, Dsg3, and β-catenin expression levels. We also assessed intracellular localization of Dsc3, Dsg3, and β-catenin.

Desmosomal proteins are well established markers of epithelial differentiation [10]. Our results showed that Dsc3 and Dsg3 are strongly expressed in the basal and lower spinous layers in normal oral epithelium. Moreover, our results also demonstrated that a reduction in or absence of expression of Dsc3 and Dsg3 was significantly higher (30 of 48 and 32 of 48 cases showing 0–II scores, respectively) in OSCCs than in normal oral epithelium. The results are generally in agreement with previous studies [1, 34, 15, 22].

Hynes [17] reported that adhesion molecules transduce signals across the cell membrane both inward and outward and that such signals can modulate cell behavior, proliferation, and gene expression. A relationship between suprabasal misexpression of Dsc3 and cell proliferation/early terminal differentiation is suggested by Hardman et al. [14]. Dsc and Dsg misexpression has been examined and founded in actinic keratoses, Bowen's disease, and squamous cell carcinoma (SCC) of the skin and oral cavity formerly [22, 34], this study furthers our understanding by showing that there was a definite relation between the reduction in or absence of expression of Dsc3 and histological differentiation (moderately or poorly differentiation) in OSCCs. The results of the present study suggest that desmosomes may have a possible role in suppression of cell proliferation in OSCCs.

It was reported that normal oral epithelium showed only membranous staining for β-catenin and membranous staining was lost or reduced with an increase in cytoplasmic staining in oral carcinoma [18]. In basal cell carcinomas of the skin, oral SCC and breast cancer, nuclear and diffuse intracytoplasmic staining of β-catenin was observed without membrane signal reinforcement [18, 19, 32]. Thus, membranous staining may be lost or markedly reduced according to the cytoplasmic internalization/nuclear staining of β-catenin. Our results showed that a reduction in or absence of membranous β-catenin staining was significantly different in OSCCs (0–II scores in 32 cases) from normal oral epithelium (III–V scores in 26 cases). These results are supported by other studies showing a similar correlation between β-catenin and the progression of oral cancer [36] and skin tumor [3].

Hiraki et al. [15] and Kurzen et al. [22] had recently reported an association between the abnormal expression of β-catenin at the time of invasion/metastasis and progression in oral cancer and skin tumor. The current study promotes our understanding by showing that β-catenin is also significantly associated with lymph node metastasis in OSCCs, but no correlation was found between the expression or absence of β-catenin and clinicopathological parameters such as age, gender, T status, clinical stage, as well as histological differentiation.

Dsc also plays an important role in epidermal integrity and differentiation. Dsc3 expression might be more compatible with keratinocytic proliferation and dynamic cell–cell adhesion. Plakophilin 2 binds β-catenin in vitro, and experimental upregulation enhances β-catenin signaling; at the same time, plakophilin 2 is also expressed in the basal cell layer of epidermis, where it should interact with Dsc3 [4]. Aberrant expression of Dsc3 in the epidermal suprabasal layers regulated epidermal differentiation and affected β-catenin expression [14, 13]. Hardman et al. [14] have reported a correlation between Dsc3 and β-catenin stability, providing further evidence for the integration of desmosomal cadherins and cell-signaling pathways fundamental to epidermal proliferation and differentiation programs. A study regarding the relationship between β-catenin, Dsc3, and Dsg3 expression in 24 cases with lymph node metastases showed that while there was a significant positive correlation between the reduction in or loss of β-catenin and the reduction in or loss of Dsc3 (Pearson's correlation coefficient $r=0.734$, $p<0.05$), no correlation was found between β-catenin and Dsg3 and between Dsc3 and Dsg3 expression showing scores 0, I, and II. In other words, our results showed that the decrease in Dsc3 expression levels positively regulates β-catenin expression levels in lymph node metastasis in OSCCs. The research on relation between desmosomal components and β-catenin signaling have been carried out, where it was shown that Dsc3 regulated β-catenin in suprabasal keratinocytes [14] and human odontoblasts [33], by inducing β-catenin stabilization and altering signaling.

Additionally, our results also showed that a diffuse perinuclear cytoplasmic internalization of Dsc3 and Dsg3 and a cytoplasmic and nuclear staining pattern of β-catenin were found in OSCC tumor cells. This change of distribution was accompanied by a reduction in membranous expression in most tumors and significantly correlated with disease progression in accordance with other studies [18, 20, 21, 32, 30]. Thus, a reduction in or absence of membranous pattern of cadherin proteins and β-catenin, together with internalization to the cytoplasm, may be associated with tumor progression.

Conclusion

We have shown that there was a significant correlation between loss of or reduction in β-catenin staining and loss of or reduction in Dsc3 staining in lymph node metastasis in OSCCs. Our observations suggest that the abnormal expression of Dsc3, Dsg3, and β-catenin were correlated with the progression of oral carcinomas. Dsc3 expression levels might be related to the regulation of β-catenin in metastatic activities and cell proliferation in OSCCs, and internalization of Dsc3, Dsg3, and β-catenin may also be associated with progression of OSCCs.

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ORIGINAL ARTICLE

Expression and distribution of MUC18 in human uveal melanoma

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Abstract The immunoglobulin superfamily protein MUC18 is involved in transendothelial migration and signal transduction, and is expressed in malignancies including cutaneous melanoma. Recent in vitro studies showed evidence of increased MUC18 protein in some uveal melanoma cell lines with an increased potential for invasion. We assessed seven uveal and three metastasis-derived melanoma cell lines for the expression of MUC18 mRNA and protein by RT-PCR, and immunoblotting and immunocytochemistry, respectively. We also examined the expression and distribution of MUC18 in paraffin sections of primary uveal melanomas (n=23; 5/23 spindle; 18/23 mixed and epithelioid) and normal eyes (n=3) using a polyclonal goat anti-human antibody to MUC18 visualized with peroxidase and Vector NovaRED. Distribution and intensity of immunostaining was graded semi-quantitatively (grade 0 to 3) by 2 independent observers. All cell lines expressed MUC18 mRNA and protein (~130 kDa), and showed punctate cell membrane MUC18 immunostaining. Primary melanomas displayed heterogeneous cell membrane and cytoplasmic MUC18, with moderate to strong immunolabelling (≥grade 2) in ~70% of tumours. Vasculature in tumours and in retina and choroid of all melanoma-affected and normal eyes showed intense MUC18 immunostaining. These observations further suggest a role for MUC18 in uveal melanoma growth; moreover, interactions between MUC18-positive melanoma cells and vasculature may be important for the hematogenous spread of cells during metastases.

Keywords Immunohistochemistry · Oncology · Uveal melanoma · Vascular endothelium

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Introduction

Uveal melanoma, affecting iris (3%), ciliary body (5–10%) and choroid (~90%), is the most common primary adult eye cancer with an incidence of 8 per million per year in Caucasians [43, 53, 55]. Metastatic disease can develop after a prolonged disease-free period, does not respond well to therapy and advances rapidly to be often fatal within 12 to 15 months after diagnosis [15, 16, 24, 41]. More aggressive uveal melanomas display a predominantly epithelioid histopathology, with vasculature and a complex extravascular matrix pattern (vasculogenic mimicry) clearly seen using periodic acid Schiff (PAS) stain [14]. PAS+ closed loops and networks are seen in more aggressive choroidal and ciliary body melanomas, in liver metastases and secondary metastatic sites [14], and in other tumors including cutaneous melanoma, and prostate and breast carcinomas [11, 12, 19, 31].

Mucin isotype 18 (MUC18/CD146), melanoma cell adhesion molecule (MelCAM/MCAM) or S-Endo 1-associated antigen is a transmembrane immunoglobulin (Ig) superfamily cell adhesion molecule normally expressed in vascular endothelium, activated T-lymphocytes, myofibroblasts, smooth muscle, Schwann cells and ganglion cells [6,

49]. Cell–cell adhesion *via* MUC18 occurs through an as yet unidentified heterophilic ligand [50]. MUC18 expression is also strongly up-regulated in malignant cutaneous melanoma compared to benign nevi, particularly in metastatic lesions [21, 27, 28]. In tissue microarrays of cutaneous melanomas, MUC18 immunostaining is reported to be a highly significant independent prognostic marker inversely correlated with survival of patients [37]. In vitro and in vivo studies in cutaneous melanoma also indicate that MUC18 antibody can target both MUC18-positive melanoma cells and vascular endothelium, with possible inhibitory therapeutic effects on cutaneous melanoma growth and metastases [30, 32, 34].

Recent studies using 2D gel electrophoresis also found MUC18 protein expression in several uveal melanoma cell lines [38, 39]. Cell lines with increased levels of MUC18 protein generally displayed an increased potential for in vitro invasiveness, suggesting an association between MUC18 and tumor growth [39]. Pre-conditioning of collagen I matrix by aggressive metastatic (MUM2B) uveal melanoma cells also induced expression of MUC18 (and other genes) in poorly aggressive (OCM-1A) uveal melanoma cells, suggesting a role for MUC18 in uveal melanoma growth [45]. In the present study, we extend these in vitro observations to a series of uveal and metastasis-derived cell lines, and describe the distribution of MUC18 protein expression in primary human uveal melanomas and normal eyes.

Materials and methods

Uveal melanoma and metastasis-derived cell lines

Cell lines

Uveal melanoma (OCM-1, OCM-3, OCM-8, 92.1, Mel202, C918, OCM-1A) and metastasis-derived (OMM-1, MUM2B, MUM2C) cell lines were grown in either RPMI-1640 medium (OCM-3 and OCM-8, Mel202, 92-1, C918, MUM2C, MUM2B) or Dulbecco's Modified Eagle's Medium (DMEM; OCM-1A, OCM-1 and OMM-1) supplemented with 10% heat inactivated fetal bovine serum (FBS), 2 mM glutamine, 50 IU/ml penicillin and 50 μg/ml streptomycin (Thermo Electron, USA).

RNA extraction and reverse transcription-PCR

Total RNA was extracted from melanoma cells using Trizol (Invitrogen Australia Pty, Australia) and reverse transcribed to cDNA (SuperScript III First-Strand Synthesis System for RT-PCR, Invitrogen) following manufacturer's instructions. PCR analyses were performed to identify MUC18 transcripts under the following conditions: 35 cycles of denaturation at 94°C for 35 s, annealing at 60°C for 35 s, and elongation at 72°C for 60 s, followed by a 5 min extension at 72°C. Primers used for MUC18 (accession no. M28882.1) amplification were:

- Forward: 5′-ACCCTGAATGTCCTCGTGAC-3′
- Reverse: 5′-TCTCTGTGGAGGTGCTGTTG-3′ (amplicon 209 bp)

PCR products together with a 100 bp DNA ladder (Invitrogen) were run on 1.5% agarose gels and visualised with ethidium bromide using a BioRad GelDoc system and WinFast Software. Products were subsequently extracted and purified from agarose gels, and verified by dye-determination DNA sequencing of both strands.

Immunoblotting

Melanoma cells were seeded in 35 mm dishes (Nunc A/S, Denmark) and cultured to semi-confluence in RPMI or DMEM growth medium for 24 h at 37°C. Growth medium was removed and cells were quickly rinsed in sterile PBS, then lysed in CellLytic™-M Mammalian Cell Lysis/Extraction Reagent (Sigma-Aldrich Pty, Australia) supplemented with protease inhibitor cocktail (Complete Mini Tablets, Roche Products Pty, Australia) for 20 min on a shaking table on ice. Lysed cells were collected and centrifuged for 15 min at 15,000×*g*, after which supernatants were collected and stored at −80°C until used. Protein concentration of cell lysates was determined using the Bio-Rad DC Protein Assay (BioRad Laboratories, Sydney Australia). Equal amounts of protein (15 μg) were diluted in 5× loading buffer [Tris–HCl 300 mM pH 6.8, glycerol 50% (*v*/*v*), bromophenol blue 0.04%, sodium dodecyl sulfate (SDS) 10%, β-mercaptoethanol 5%], denatured at 95°C for 10 min, then loaded onto 10% SDS-acrylamide gels and separated by SDS-PAGE under reducing conditions. Resolved proteins were electrophoretically transferred to 0.45 μm Invitrolon PVDF membranes (Invitrogen) in Tris–glycine/methanol buffer at 250 mA for 3 h.

The membranes were blocked with 5% skim milk powder in 0.05% Tween 20/TBS [20 mM Tris (pH 7.5), 0.5 M NaCl] (TTBS) at 4°C overnight. After rinsing in TTBS, membranes were incubated overnight at 4°C overnight in goat polyclonal anti-MCAM (C-20, MW ~130 kDa; sc18942, Santa Cruz Laboratories, CA, USA), followed by HRP-conjugated anti-goat IgG antibody (Bethyl Laboratories, TX, USA; 1:12,500 dilution in 1% BSA/TTBS) for 2 h. After washing in TTBS and finally in TBS, immunolabelled proteins were visualized with ECL (SuperSignal West Dura Extended Duration Substrate, Pierce Biotechnology, IL, USA) followed by exposure to X-OMAT film (Kodak) and photographic development. To

confirm equal loading of protein lysates, immunoblots were stripped (Restore Western Blot Stripping Buffer, Pierce) and re-probed using mouse GAPDH mAb (0.5 μg/ml, Ambion, Applied Biosystems, VIC, Australia) and then processed as above. Immunoblots (n=3) were visualized using a Syngene G:Box, Chemi XT 16 Camera and GeneSnap (Syngene) software. The average sum of intensities of MUC18 bands and GAPDH bands for each cell line were analysed using GeneTools [Syngene; Ver. 3.07(g)] Software and the ratio of MUC18/GAPDH band intensity calculated.

Immunocytochemistry

We also immunolabelled cultured cells as described previously [26], to assess the cellular distribution of MUC18 protein. Briefly, all melanoma cell lines were grown on glass coverslips, fixed in 2% paraformaldehyde in 0.1 M phosphate buffer with 0.9% sodium chloride (PBS, pH 7.4), and immunolabelled with either polyclonal goat anti-human antibody to the extracellular domain of MUC18 (MCAM, R&D Systems, MN, USA; 2 μg/ml), or goat immunoglobulins (Ig; 2 μg/ml) as a non-specific control, diluted in 2% normal rabbit serum (NRS)/PBS. Antibody binding was visualized using rabbit anti-goat Alexa 488 (1:1000; Invitrogen), nuclei were stained with Hoechst 33342, and cells were examined using a Zeiss LSM 5 Pascal confocal microscope.

Primary uveal melanomas

Specimens

This study was performed under the tenets of the Declaration of Helsinki and with approval from the Human Ethics Committee, University of Sydney. Eyes enucleated for uveal melanoma (n=23 age range: 41 to 85 years) were routinely fixed in 10% neutral buffered formalin (NBF) and paraffin embedded. The specimens included 18 untreated melanomas and 5 I^{125}-treated melanomas, and the majority of tumors were located in the choroid posterior to the equator. Normal human eyes (n=3; aged 39, 50 and 78 years), provided by the Lions NSW Eye Bank with consent and ethical approval, were fixed in 10% NBF, paraffin embedded, and used as controls. Paraffin sections were cut at 8 μm and collected on Super-Frost Plus (Menzel-Glaser) slides.

Tumor histopathology: H&E and periodic acid Schiff staining

Sections were dewaxed and rehydrated through alcohols to water, before staining with hematoxylin and eosin (H&E) according to standard protocols. A separate series of slides was prepared similarly but stained with PAS reagent according to standard protocols. The stained sections were subsequently dehydrated through alcohols and xylenes, then coverslipped in DePeX (BDH Pty), and examined using a Leica light microscope.

From H&E stained sections, tumors ranged from 5 to 24 mm maximum diameter and 2 to 14 mm maximum height, and tumor cell type was classified as spindle, mixed or epithelioid. Extracellular matrix (ECM) patterns in PAS-stained sections were classified as described previously [13, 14], with the following patterns observed: vessel lumens only, straight channels, incomplete loops and complete loops and networks. Complete loops and networks appeared only in epithelioid tumors, while spindle melanomas showed vessel lumens, straight channels or occasionally incomplete loops. From these observations, tumors were graded as follows: 1=spindle; 2=mixed; 3=epithelioid.

Immunohistochemistry

Sections were dewaxed, rehydrated and rinsed in 0.1 M PBS (pH 7.4). For antigen retrieval, sections were incubated in 0.01 M citrate buffer (pH 6.0) at 80°C for 15 min, cooled to 40°C, before rinsing in PBS for 10 min. This was followed by immersion in 5% H_2O_2 in water for 10 min to inhibit endogenous peroxidase activity, then rinsing in PBS. Sections were incubated at room temperature (RT) in 10% NRS for 30 min followed by incubation for 48 h at 4°C in a polyclonal goat anti-human antibody to the extracellular domain of MUC18 (MCAM) at 2 μg/ml in 2%NRS/PBS. After rinsing in PBS, sections were incubated for 1 h at RT in biotinylated rabbit anti-goat antibody (Zymed, San Francisco, USA) diluted 1:200 in PBS, rinsed, then further incubated for 1 h at RT in ExtrAvidin peroxidase (1:200, Sigma Aust. Pty). Antibody binding was visualized using Vector NovaRED substrate (Vector Labs Pty), and a constant time for color development was used for all specimens. After counterstaining in hematoxylin for 10–15 s, sections were dehydrated through alcohols and xylenes and coverslipped in DePeX. Non-specific controls were incubated in 2 μg/ml goat Ig instead of the primary antibody, and processed identically as for the MUC18 antibody.

Immunostaining was graded in masked fashion by 2 independent observers using a semi-quantitative scale where 0=none, 1=weak (low intensity staining of tumor cells regardless of percent, and medium intensity staining of ≤20% cells); 2=moderate (medium intensity staining of >20% tumor cells, high intensity staining of ≤20% tumor cells), and 3=strong (high intensity staining of >20% tumor cells). Differences in grading were resolved by joint review and if necessary, consultation with a third independent

observer. The grading of the two observers was significantly correlated ($P<0.001$; Kendall's rank correlation). Nonparametric linear regression analysis was used to test for a relationship between tumor cell type, maximum tumor height (in mm) and MUC18 immunostaining.

We also assessed the distribution and intensity of MUC18 in retina overlying tumors, and in regions of choroid and retina without melanoma, as well as in normal choroid and retina.

Results

MUC18 in uveal and metastasis-derived cell lines

RT-PCR showed MUC18 mRNA expression in seven uveal and three metastasis-derived melanoma cell lines (Fig. 1). Immunoblotting showed a positive band at ~130 kDa for all cell lines, with variable expression of MUC18 protein between cell lines (Fig. 2a). Membranes re-probed with GAPDH antibody showed a consistent band at ~35 kDa in all samples, confirming that equal amounts of protein were loaded (Fig. 2a). The mean ratio of intensities of MUC18 and GAPDH bands (from three separate immunoblots) showed high levels of MUC18 protein in OCM-3, OCM-8, 92-1, Mel202 and OCM-1A cell lines, relatively less MUC18 protein in OCM-1, OMM-1 and MUM2C cell lines and much reduced MUC18 protein in C918 and MUM2B cell lines (Fig. 2b). All cell lines showed punctuate cell membrane MUC18 immunolabelling, with no immunostaining for goat Ig control (Fig. 2c).

MUC18 in primary uveal melanoma

Immunohistochemical analysis for MUC18 expression was performed on primary uveal melanoma specimens. Overall ≥grade 2 immunolabelling was observed in 60% (3/5) of I^{125}-treated and 78% (14/18) of untreated melanomas. For untreated melanomas, we found a significant relationship between tumor cell type and average MUC18 immunostaining grade ($P=0.031$, nonparametric regression; Fig. 3), with more MUC18 immunostaining in mixed and epithelioid tumors compared to spindle cell tumors. No relationship between maximum tumor height (mm) and MUC18 immunostaining was found for untreated tumors ($P>0.05$). As tumor specimens were collected over a recent period, the relationship between MUC18 expression and survival could not be analysed.

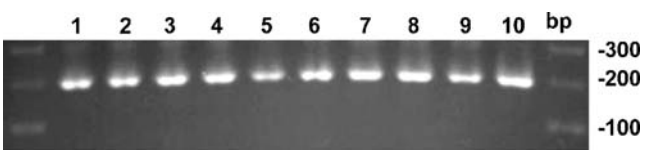

Fig. 1 Diagnostic RT-PCR showing MUC18 mRNA expression in uveal (*lanes 1–7*) and metastasis-derived (*lanes 8–10*) cell lines (accession number M28882.1: Amplicon 209 bp; *Lane 1*=OCM-1, *2*=OCM-3, *3*=OCM-8, *4*=92-1, *5*=Mel202, *6*=OCM-1A, *7*=C918, *8*=OMM-1, *9*=MUM2B and *10*=MUM2C)

In approximately 50% (9/18) of untreated melanomas, cells at the tumor edges showed moderate to strong cell membrane and cytoplasmic MUC18 immunostaining (Fig. 4a; inset). Within tumors, heterogeneous regions of MUC18 membrane and cytoplasmic immunolabelling of tumor cells were observed (Fig. 4b–e). Blood vessels within all tumors showed intense MUC18 immunostaining of vascular endothelium, and often lumen contents (including erythrocytes; Fig. 4a,e; Fig. 5a). We did observe some areas with patterns of MUC18 immunolabelling (Fig. 5b) similar to PAS+ loops and networks; these were seen in 28% (5/18) of untreated specimens (predominantly epithelioid tumors; Fig 5d). PAS+ vessel lumens were also seen in spindle cell and mixed tumors (Fig. 5c).

Blood vessels in the retina and choriocapillaris showed intense MUC18 immunostaining in all specimens (melanoma-affected and normal eyes; Fig. 6a,b). Large retinal vessels and the inner and outer retinal vascular plexus were intensely immunolabelled for MUC18 in normal and melanoma-containing eyes (Fig. 6a,b). No obvious immunostaining of tumor cells or blood vessels, including luminal erythrocytes, was observed in sections when goat IgG (Fig. 6c) was used in place of MUC18 antibody (Fig. 6d).

Discussion

In the present study we found that all melanoma cell lines (primary and metastasis-derived) expressed MUC18 mRNA and protein (western blot and immunocytochemistry). Compared to the other melanoma cell lines, C918 and MUM2B showed reduced levels of MUC18 protein in western blots; C918 uveal melanoma cells and MUM2B metastasis-derived cells are both considered to be highly aggressive [46]. Recent in vitro studies also described variable levels of MUC18 protein in uveal melanoma cell lines [38, 39]. In these studies, two uveal melanoma cell lines with low and moderate levels of MUC18 protein, respectively, displayed similar in vitro invasiveness. However, it was concluded that MUC18 expression was generally associated with an increased potential for in vitro invasiveness [38, 39]. Variable expression of MUC18 protein has also been found in western blots of ovarian carcinoma cell lines and primary cells, with an overall association between tumor progression, and poor prognosis [1]. MUC18 gene expression has also been reported in uveal melanoma cell lines and in primary uveal melanoma

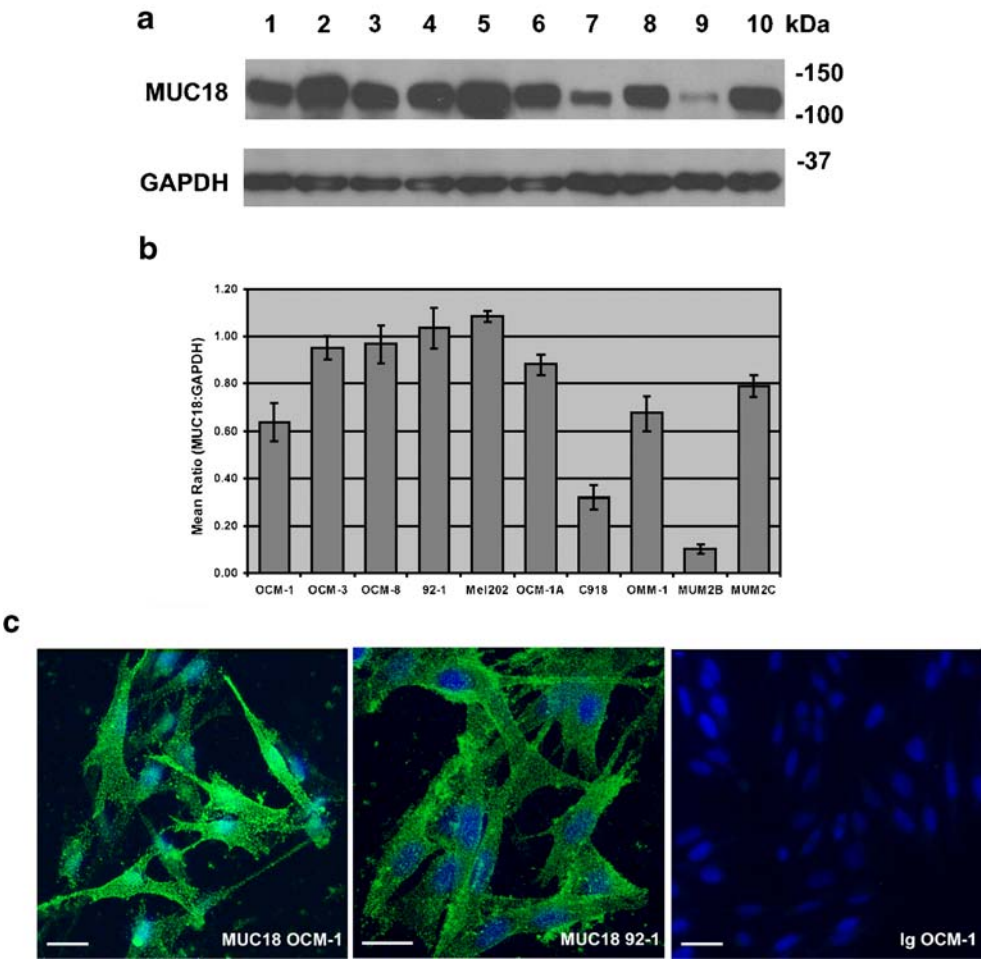

Fig. 2 **a** Representative immunoblot showing a band at ~130 kDa consistent with MUC18 protein expression in uveal (*lanes 1–7*) and metastasis-derived (*lanes 9–10*) cell lines. Reprobing of the membrane with GAPDH antibody showed a consistent band at ~35 kDa for all cell lines. Molecular mass markers (kDa) are shown on the right. (*Lane 1*=OCM-1, *2*=OCM-3, *3*=OCM-8, *4*=92-1, *5*=Mel202, *6*=OCM-1A, *7*=C918, *8*=OMM-1, *9*=MUM2B and *10*=MUM2C). **b** Histogram of the ratio of the average intensities of MUC18 and GAPDH bands calculated from three separate immunoblots. **c** Examples of MUC18 punctate cell membrane immunolabelling of OCM-1 and 92-1 cell lines, and OCM-1 cells with control goat Ig showing no immunolabelling. Nuclei are stained with Hoechst 33342 (*blue*); Bar=15 μm

specimens in gene array studies. *MUC18* was found to be upregulated in a primary uveal melanoma cell line compared to two metastasis-derived melanoma cell lines [56]. In contrast, for primary uveal melanomas, *MUC18* was downregulated in class 2 primary uveal melanomas (associated with epithelioid morphology, monosomy 3 and poor prognosis) compared with class 1 uveal melanomas (spindle morphology with good prognosis) [36]. These findings may reflect the involvement of tumor-stromal cell interactions in regulating gene expression in primary melanomas. The differences in gene and protein expression observed in cell lines and primary tumors most likely suggest that while MUC18 is important for uveal melanoma growth and invasiveness, more than one signalling pathway contribute to these processes as proposed by Pardo et al. (2005, 2006) [38, 39], and discussed below.

Over-expression of MUC18 in primary cutaneous melanoma cells has been reported to enhance adhesion between tumor cells themselves, increased tumor cell invasiveness, as well as greater interactions between tumor cells and endothelial cells, and decreased tumor cell adhesion to laminin; these effects were reversed by anti-MUC18 (MCAM) monoclonal antibody [59]. This study also

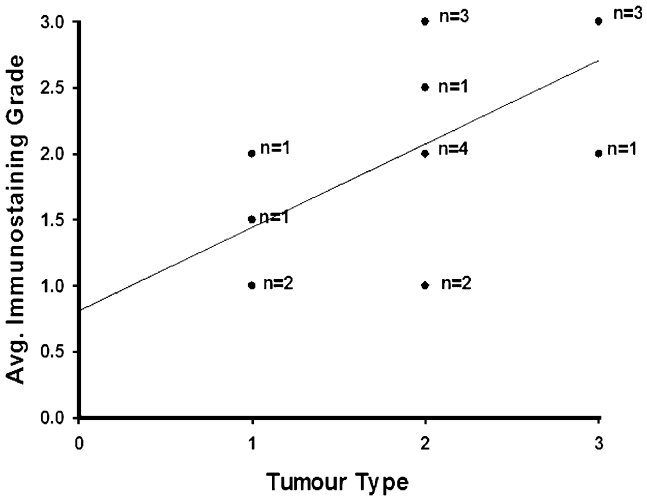

Fig. 3 Tumor type vs Average MUC18 immunostaining for untreated melanomas (n=18; nonparametric regression, P=0.031). Tumor type: (*1*) spindle; (*2*) mixed and (*3*) epithelioid. Average Immunostaining Grade of 2 independent observers: 0=none, 1=weak (low intensity staining of tumor cells regardless of %, and medium intensity staining of ≤20% cells); 2=moderate (medium intensity staining of >20% tumor cells, high intensity staining of ≤20% tumor cells), and 3=strong (high intensity staining of >20% tumor cells)

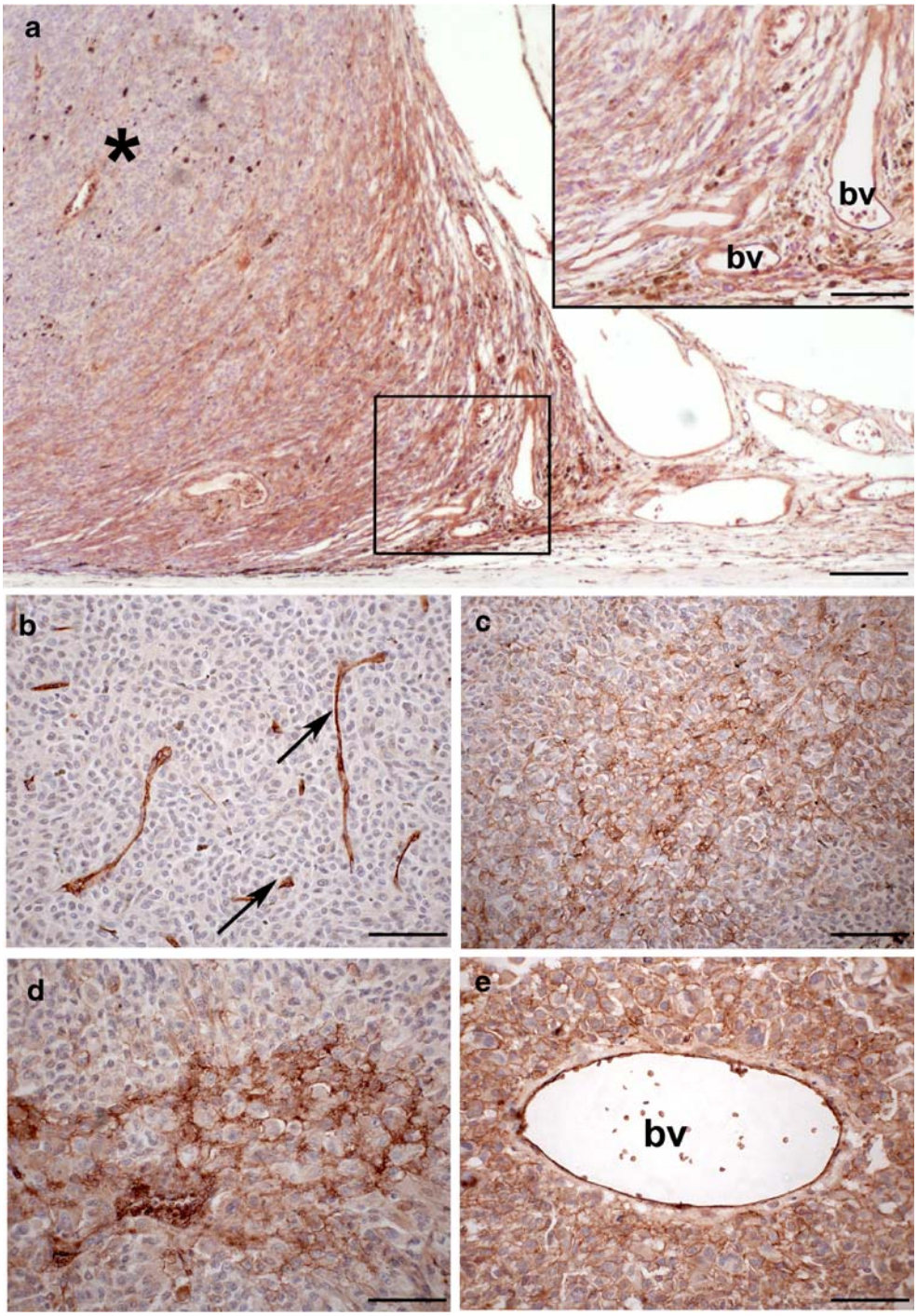

Fig. 4 MUC18 immunostaining in uveal melanoma. **a** Low power micrograph showing MUC18 expression at the tumor edge, where cells display membrane and cytoplasmic MUC18 immunoreactivity (see inset). The more central regions of the melanoma (*asterisk*) show lower levels of MUC18 compared to the tumor edge. Blood vessels (*bv*) in the tumor and adjacent choroid display strong MUC18 immunostaining (bar=100 μm; inset bar=30 μm). **b** Grade 1 (low) MUC18. Blood vessels show intense MUC18 immunolabelling (*arrows*; bar=35 μm). **c**–**e** Areas of moderate to intense (≥Grade 2) cell membrane MUC18. A large blood vessel (*bv*) immunostained with MUC18 is seen in **e**; **c**: Bar=35 μm; **d** and **e**: Bar=30 μm

observed increased collagenase production in MUC18 transfected cells, contributing to increased tumorigenicity [59]. MUC18 over-expressing cutaneous melanoma cells induced tumor growth and metastases in nude mice that could be reversed using anti-MUC18 (MCAM) monoclonal antibody [59]. Further evidence for involvement MUC18 in cutaneous melanoma growth comes from recent in vitro and in vivo studies using antibodies to MUC18 to inhibit melanoma cell tumorogenesis and subsequent metastasis; a therapeutic application for MUC18 antibodies in controlling cutaneous melanoma growth is currently being investigated [30, 32, 34]. Although these studies indicate a major role for MUC18 in cutaneous melanoma growth, other cell adhesion and signaling molecules, for example, N-cadherin, integrins, RAS/ERK and PI3K, are also important for tumor growth [33]. A recent gene and tissue microarray study reported a significant correlation between progression of cutaneous melanoma and expression of N-cadherin and β3 integrin, but not MUC18 [57]. However, MUC18 (and β3 integrin) were implicated in cutaneous

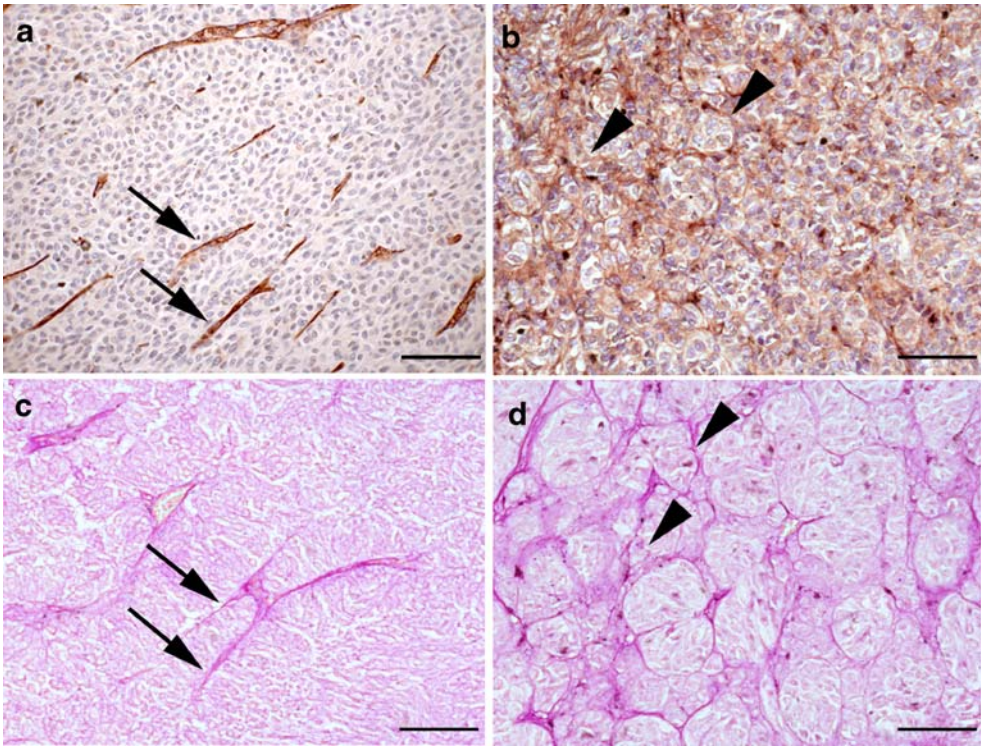

Fig. 5 **a** MUC18 immunostaining (*arrows*) and **c** PAS staining (*arrows*) of tumor blood vessels, with no apparent immunostaining of tumor cells (**a** and **c**: bar= 35 μm). **b** Areas of MUC18+ immunostaining (*arrowheads*) in an epithelioid melanoma appear similar to **d** PAS+ loops and networks (*arrowheads*; **b** and **d**, bar=25 um)

melanoma cell migration and invasion [57]. Overexpression of MUC18 has been associated with tumor progression in prostate and ovarian carcinomas [51, 48, 58]; however, this is not the case for all tumors, as seen for example in breast carcinoma where loss of MUC18 occurs with progression [48]. Furthermore, MCF-7 breast cancer cells transfected with *MUC18* produced smaller tumors when grown in immune-compromised mice, suggesting that in breast cancer, at least, MUC18 increased tumor cell cohesion and reduced the potential for spread into neighbouring tissues [50].

Binding sites for transcription factors including activator protein (AP)-2α, Sp1 and CREB/ATF-1 have been found on the MUC18 promoter [29], and loss of the transcription factor AP-2α has been shown to be involved in progression of several tumors including malignant melanoma [4], breast and prostate cancers [8, 42] and gliomas [18]. Highly metastatic (AP-2α-negative, MUC18-positive) cutaneous

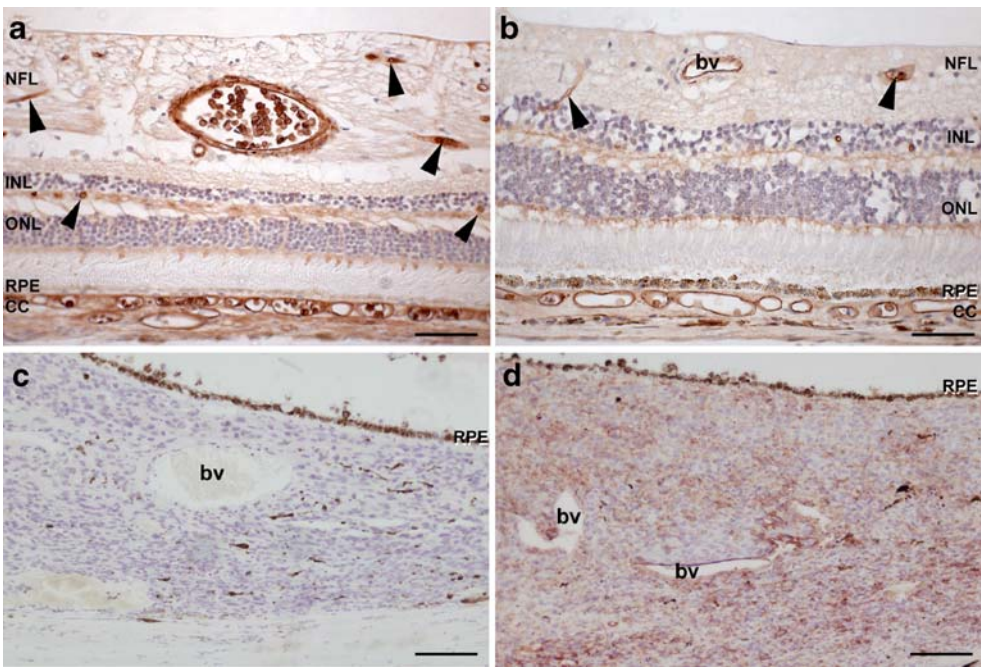

Fig. 6 **a** and **b** MUC18 immunostaining of retina and choriocapillaris (*CC*). Blood vessels (*arrowheads*) display strong MUC18 expression, with both the inner and outer retinal vascular plexus clearly seen in **a** melanoma-containing and **b** control eyes. In **a**, a large vessel also shows MUC18 immunoreactive perivascular cells. **c** No immunostaining of tumor cells or blood vessels (*bv*), including lumen contents, is seen with goat IgG control, compared to **d** MUC18 immunostaining in the same specimen (*NFL*, nerve fibre layer; *INL*, inner nuclear layer; *ONL*, outer nuclear layer; *RPE*, retinal pigmented epithelium; bar=40 μm)

melanoma (A375SM) cells transfected with AP-2α displayed greatly reduced levels of MUC18 mRNA and protein compared to parental cells [20]. These cells also displayed suppressed tumorigenicity and metastatic potential in nude mice that was suggested to be related to AP-2α down-regulation of MUC18 gene expression [20]. However, other transcription factors, including Sp1, have been found to be involved in regulating *MUC18* expression [22, 23, 35]. Other AP-2α-regulated genes important for tumor cell proliferation and apoptosis, invasion and angiogenesis including *c-kit*, *E-cadherin*, *MMP-2* and *PAR-1* have also been found to contribute significantly to cutaneous melanoma growth and metastases [17, 20, 29, 54]. The expression of transcription factors including AP-2, Sp1 and CREB/ATF-1, their role in regulating *MUC18* and other genes, and their relationship to uveal melanoma phenotype and progression remains to be established.

As discussed above, a major role for MUC18 in the growth and metastases of cutaneous melanomas has been proposed, and MUC18 expression has been reported to be strongly up-regulated in malignant melanomas of increasing vertical thickness, especially in metastatic lesions [9, 21, 25, 27, 28]. One study has observed a correlation between MUC18 expression and tumor thickness, associated with the transition of cutaneous melanoma from the radial to the vertical growth phase [59]. We observed heterogeneous areas of moderate to intense MUC18 immunostaining, particularly in mixed and epithelioid tumors; however, no relationship between MUC18 expression and maximum tumor height was established in this series of uveal melanomas.

All vascular endothelium, regardless of location or vessel size, constitutively express MUC18 [5, 7], and consistent with this we observed strong MUC18 immunolabelling of tumor vasculature, and of retinal and choriocapillaris blood vessels in both normal and melanoma eyes. MUC18 has been found localized at endothelial cell junctions, although outside *adherens* junctions, and associated with actin filaments in the endothelial cytoskeleton, suggesting an important role in endothelial cell cohesion [5, 52]. Several studies also show that MUC18 binding in endothelial cells can regulate intercellular permeability, inducing phosphotyrosine-kinase-dependent outside-in signaling associated with proteins including $p59^{FYN}$, $p125^{FAK}$ and paxillin [2, 3]. MUC18-mediated adhesion between melanoma cells and endothelial cells, *via* an unknown heterophilic ligand, may be important for the movement of melanoma cells in and out of the tumor vasculature [34, 47, 50, 59]. Activated peripheral T lymphocytes also express MUC18, with an increased capacity to bind endothelial cells that contributes to T-cell extravasation and/or homing [10, 40, 44]. Overall these observations indicate that interactions between MUC18-positive tumor cells and endothelial cells (and activated T-lymphocytes), may play an important role in (cutaneous and uveal) melanoma hematogenous spread and immunoregulation.

In conclusion, we observed variable expression of MUC18 protein in uveal and metastasis-derived melanoma cell lines. In primary mixed and epithelioid tumors, however, the extent and intensity of MUC18 immunostaining was significantly increased compared to spindle cell tumors. These findings suggest that while MUC18 is important for uveal melanoma growth, its expression may not be essential for invasion and metastasis, consistent with findings in other tumors. The role of transcription factors including AP-2a and the signaling pathways involved in regulating MUC18 expression and activity in uveal melanoma remain to be defined. Finally, interactions between MUC18-positive melanoma cells and vasculature, via an as yet unidentified ligand, may be important for the hematogenous spread of tumor cells during metastases.

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CASE REPORT

Heterotopic respiratory mucosa in the rectum: A first case report

Kunimitsu Kawahara · Hideyuki Mishima · Shin-ichi Nakamura

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Abstract A 50-year-old Japanese woman consulted our hospital complaining of hematochezia. Colonoscopic examination showed Borrmann type 1 rectal carcinoma in the lower rectum, which was later demonstrated to be mucinous adenocarcinoma of Dukes' class A. Along with carcinoma, there was a small sessile polyp with a central depression that mimicked a submucosal tumor. Histologically, the polyp demonstrated tubular structures that coursed from the top of the polypoid surface to the deep submucosa. The tubular structure was 6 mm in length and showed abortive dichotomous branching. It was mainly lined by ciliated pseudostratified columnar to more cuboidal epithelium. Along with the tubular structure, there were bundles of smooth muscle cells and lobules of seromucinous glands. There was no cartilage. The pathological diagnosis was heterotopic respiratory mucosa (HRM) arising in the rectum. To our knowledge, this is the first reported case of HRM in the rectum.

Keywords Heterotopia · Respiratory epithelium · Rectum

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Introduction

The term heterotopia (or choristoma) is applied to microscopically normal cells or tissues that are present in abnormal locations [5]. Previous reports have described heterotopic gastric epithelium (HGE) [6, 7, 9], salivary gland tissue [2, 8], and pancreas [4] in the rectum. However, heterotopic respiratory mucosa (HRM) has not been reported in the literature at any site along the gastrointestinal tract including the rectum. In this report, we describe the first case of HRM arising in the rectum and discuss the possible pathogenesis.

Clinical history

A 50-year-old Japanese woman consulted at our hospital complaining of hematochezia persisting for 5 months. There was no significant past history. Regarding family history, the patient's father died of pulmonary cancer. Colonoscopic examination showed that the patient had a Borrmann type 1 tumor and a coexisting small sessile polyp in the lower rectum. The polyp had a smooth surface covered by normal rectal mucosa with a small depressed area at the top (Fig. 1a). There was no apparent erosion or ulcer on the polyp. There were no evidence of inflammation or polyposis of the large intestine. Endoscopic findings suggested that the polyp was a submucosal tumor. Endoscopic biopsies showed that the Borrmann type 1 tumor was histologically adenocarcinoma, but in the polyp sample, there was only a rectum mucosa without pathological changes. Routine laboratory tests, including serum CEA and CA19–9, were within normal limits. Under a tentative diagnosis of coexisting advanced rectal adenocarcinoma and submucosal benign tumor, low anterior

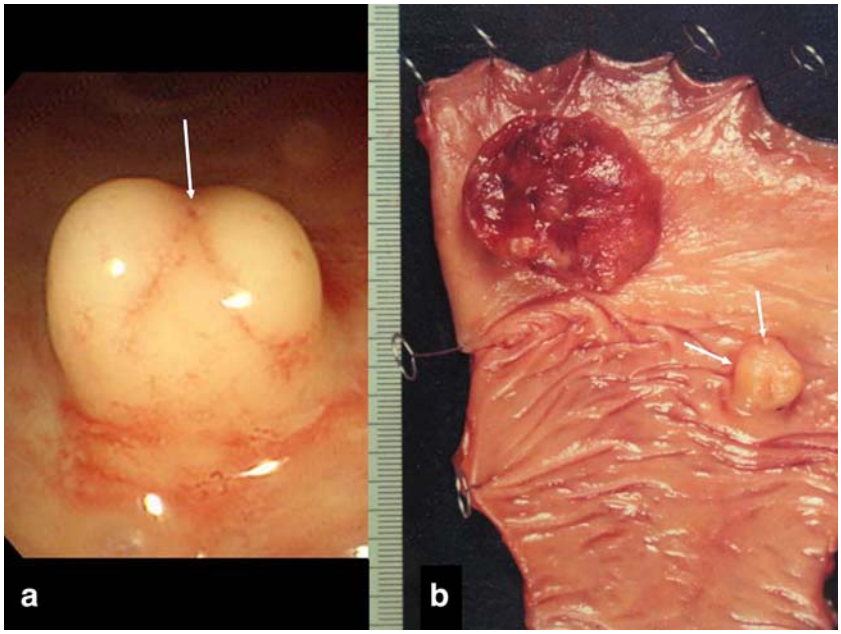

Fig. 1 **a** The endoscopic characteristics suggesting a submucosal origin. The polyp had a smooth surface covered by normal colonic mucosa with a small depressed area (*arrow*) at the top. **b** Surgically resected specimen showing the Borrmann type 1 carcinoma and a small benign-looking polyp (*arrow*)

resection of the rectum and lymph node dissection were performed. The postoperative course was uneventful, and the patient has remained disease free for 7 years after surgery.

Pathologic findings

Macroscopic findings

Macroscopically, a Borrmann type 1 adenocarcinoma, measuring 3.7×2.9 cm, was observed in the surgically resected rectum. A small polyp measuring 1.2×1.3 cm was located 2 cm from the carcinoma (Fig. 1b).

Microscopic findings

From the mucosal surface to the deep submucosa in the polyp (Fig. 2), there were tubular structures with abortive dichotomous branching (Fig. 3a). The proximal segments of the tubular structures, near the rectal mucosa, were lined by pseudostratified columnar epithelium with ciliated cells. There were no goblet cells (Fig. 3b). Deeper in the rectal submucosa, the lining cells demonstrated a low columnar appearance. Around the distal tubular structures, numerous salivary-type glands containing both serous and mucinous cells appeared to mimic respiratory submucosal seromucinous glands (Fig. 4a, b). Smooth muscle bundles were occasionally recognized around the proximal tubular structures. The transition between the colonic epithelium and ciliated epithelium was abrupt. There was no gastric epithelium present in numerous sections examined. Taken together, the pathological diagnosis was HRM arising in the rectum. The Borrmann type 1 carcinoma was diagnosed as mucinous adenocarcinoma of Dukes' class A type. Colonic mucosa in the background demonstrated a slight elongation of the glands surrounded by a proliferation of smooth muscle fibers from the muscularis mucosae, suggesting mucosal prolapse syndrome.

Immunohistochemical studies

Immunohistochemical studies showed that paratubular seromucinous glands were positive for smooth muscle actin (Dako, Glostrup, Denmark), suggesting a myoepithelial cell component (data not shown). CA19-9 (Zymed, South San

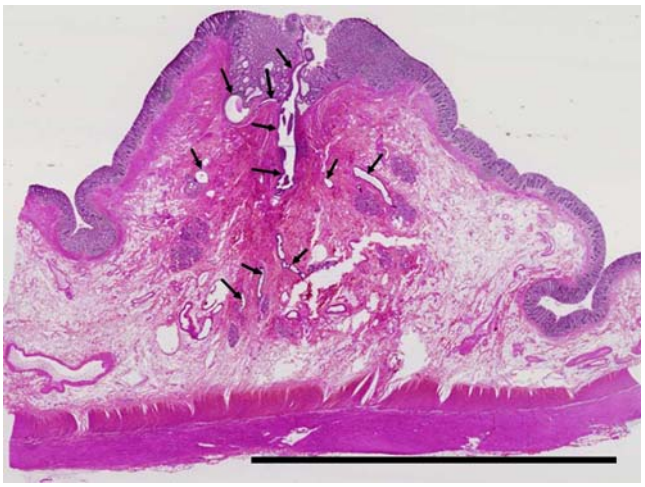

Fig. 2 Tubular structures branching in various directions (*arrows*), ranging from the mucosal surface to the deep submucosa. *Bar* indicates 1 cm

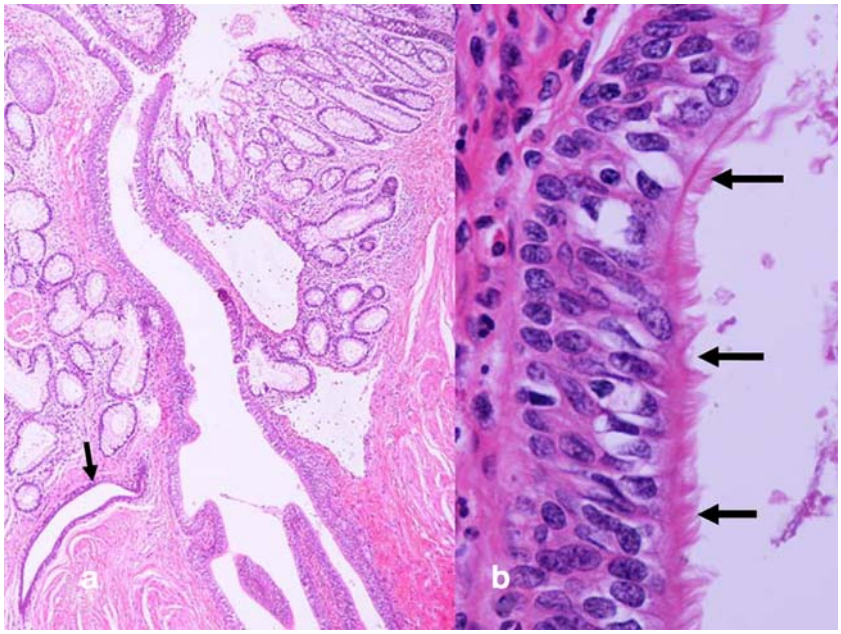

Fig. 3 **a** Proximal tubular structures with abortive dichotomous branching (*arrow*; ×40). **b** Pseudostratified columnar epithelium with ciliated cells (*arrows*) lining the proximal tubular structure (×600)

Francisco, CA, USA) and CEA (Dako) were positive in a small number of cells lining a limited area of the tubular structure. TTF-1 (Dako), Chromogranin A (Dako, CA, USA) and NSE (Dako) were negative.

Discussion

"Heterotopia", a term derived from Greek, implies "other place." It refers to the finding of normal tissues in abnormal locations [5]. Heterotopia in the rectum is relatively rare, but HGE [6, 7, 9] and salivary gland heterotopia (SGH) [2, 8] are well known. HGE is more commonly seen. Wolff [9] reviewed 87 cases of HGE throughout the alimentary tract. Through their clinicopathological analysis, they also divided HGE in the alimentary tract into the following three types: (1) developmental errors of positioning from failure of the stomach to descend between the fourth and seventh weeks of gestational life; (2) dysontogenetic error in the differentiation of the fetal entoderm at that site, rather than error of positioning; and (3) abnormal acquired regenerative process after the destruction of pre-existing mucosa. The

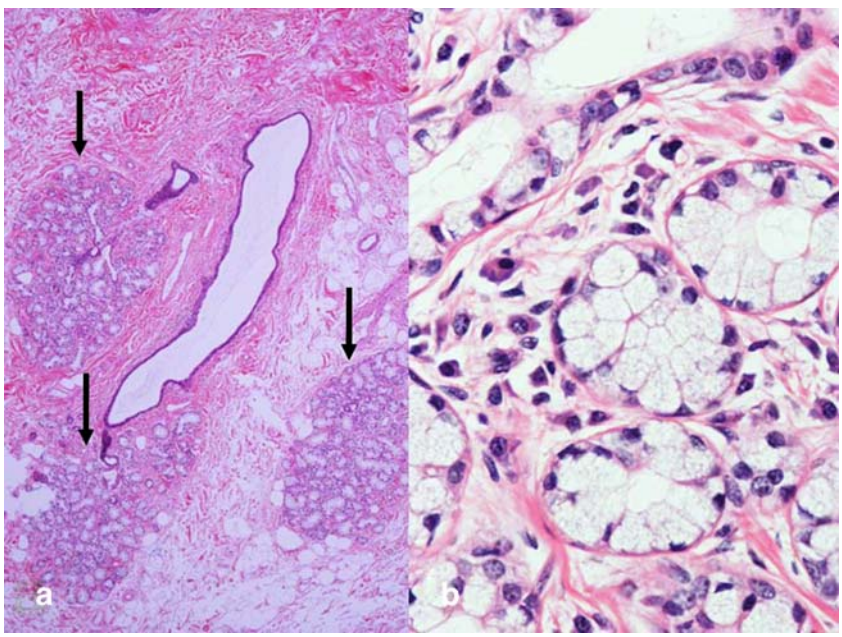

Fig. 4 **a** Lobules of glands (*arrows*) around the distal tubular structure (×40). **b** Lobules composed of seromucinous glands with a duct (×600)

second edition of Gastrointestinal Pathology edited by Cecilia M. Fenoglio-Preiser [3] indicates that: "rectal HGE may coexist with ectopic respiratory epithelium, presumably representing the equivalent of heterotopic foregut" and "gastric heterotopias result from developmental failure of the descent of the foregut, adhesion of ectoderm in the 3- to 4- week-old embryo, a differentiation error of fetal rectal endodermal cells, or proliferation of pleuripotential cells." A unifying concept of foregut heterotopia seems appealing, and the present case could demonstrate one of the expressions in the spectrum of this abnormality. A case of heterotopic pancreas [4] in the rectum was previously described.

Srinivasan et al. [7] reported two HGE cases in the rectum showing a sessile polypoid shape similar to that in our case. In these two cases, there was no endoscopically distinctive feature of HGE as in our case, but endoscopic biopsies of the rectal lesions histologically demonstrated the presence of heterotopic tissue, which differed from our case.

Weitzner [8] first reported a case of SGH in the rectum composed of salivary gland tissue alone. In two previously reported cases of SGH, ectopic gastric mucosa was concomitant [4, 7]. In Weitzner's article, he reported a lesion composed of mixed salivary gland tissue (lobules of serous and mucinous glands and ducts) typically of the submaxillary glands, minor salivary glands, and glands of the respiratory tract. Downs-Kelly et al. [2] reported the fourth case of submucosal SGH in the rectum in a 31-year-old man complaining of rectal bleeding and postulated that SGH may be a form of metaplasia or a developmental abnormality in which undifferentiated stem cells inadvertently migrate to a rectal location and then differentiate into salivary gland tissue. On histology, our case somewhat mimicked SGH arising in the rectum, but the presence of ciliated lining cells was not compatible with SGH [2, 3, 8] because pseudostratified epithelium with ciliated cells is confined to the respiratory tract, which includes nasal mucosa, paranasal sinus, larynx, pharynx, trachea, and bronchus. In the normal salivary gland, the cell surface of the duct system usually contains scattered short microvilli, not cilia.

In the differential pathologic diagnosis, monodermal teratoma (endodermal variant of mature cystic teratoma [1]) was another possibility. Philip et al. first reported a case of endodermal variant of mature cystic teratoma of the ovary [1]. Their ovarian lesion was a multilocular cyst divided by thin fibrous septa without a solid area. The cystic space and occasional smaller gland-like spaces were lined by well-differentiated histologically benign ciliated psuedostratified columnar epithelium with goblet cells, closely resembling normal respiratory epithelium. There was no other teratomatous tissue present. On histology, the lesion in this case was not a teratoma-like cyst or solid mass and lacked goblet cells.

Based on the preoperative endoscopic biopsy specimen in our case, we could not establish a pathological diagnosis of heterotopia because most of the tubular structures were located under the rectal mucosa. Therefore, obtaining an adequate pathologic specimen was very difficult by the standard biopsy sampling method. To establish a final diagnosis, endoscopists should confirm the orifice of the characteristic tubular structure at the depressed area and obtain an adequately deep sample.

In conclusion, to our knowledge, this case of HRM in the rectum arising in a 50-year-old woman is the first such case to be reported. Endoscopists and pathologists should be aware of the rare occurrence of HRM and bear it in mind when considering the differential diagnoses of a submucosal mass in the rectum.

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LETTER TO THE EDITOR

A case of nephroblastoma histologically mimicking rhabdomyosarcoma

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Keywords Rhabdomyosarcoma · Nephroblastoma · Renal tumor

Sir:

We herein report a case of pediatric renal tumor that was difficult to differentiate whether monophasic nephroblastoma or rhabdomyosarcoma (RMS). Although RMS is one of the common malignant tumors in infants and children, RMS of the kidney is extremely rare [2, 3, 5]. There is still controversy as to whether renal RMS is a distinct entity or a variant of nephroblastoma.

A 2-year-old girl presented with a large abdominal mass. Radiological examination showed giant tumor (16×15×11 cm) arising from the left kidney. No distant metastasis was detected. It was clinically diagnosed as nephroblastoma, and left radical nephrectomy was performed. At surgery, the capsule was ruptured and dissemination was suspected. On cut section, the tumor almost occupied the excised kidney and had areas of degeneration, hemorrhage, and necrosis. Histologically, the tumor was composed entirely of sarcomatous elements and contained neither epithelial cells nor structures indicative of blastema (Fig. 1a). Mitotic figures were frequently seen. Anaplastic cells with bizarre nuclei and multipolar mitosis were observed as scattered (Fig. 1b and c). Additionally, atypical rhabdomyoblasts with plump eosinophilic cytoplasm were sporadically found. Cross-striations were not apparent. Many of the tumor cells were immunohistochemically positive for myogenin, MyoD1, desmin, and myoglobin (Fig. 1d). Immunostaining for cytokeratin (AE1/3) was entirely negative. No lymph node metastasis was detected. Ultrastructural, genetic, and molecular examinations were not performed. The patient was treated with chemotherapy using vincristine, actinomycin D and adriamycin, and radiation therapy according to the regimen for nephroblastoma with diffuse anaplasia stage III (according to the National Wilm's Tumor Study). She has no evidence of recurrence 3 years after completion of therapy.

The unique histologic feature of this case was monomorphous proliferation of sarcomatous cells with rhabdomyogenic differentiation. From histological aspects alone, this case was also compatible with embryonal RMS. Although rhabdomyogenesis often occurs in nephroblastoma, predominant proliferation of malignant rhabdomyoblasts is extremely unusual. This case was not compatible with fetal rhabdomyomatous nephroblastoma, as it did not contain the typical triphasic nephroblastoma [4]. Additionally, the histological feature was not reminiscent of fetal rhabdomyomatous tissue. Differential diagnosis includes clear cell sarcoma of the kidney, malignant rhabdoid tumor of the kidney, cellular mesoblastic nephroma, and primary renal synovial sarcoma [1]. They were excluded by the

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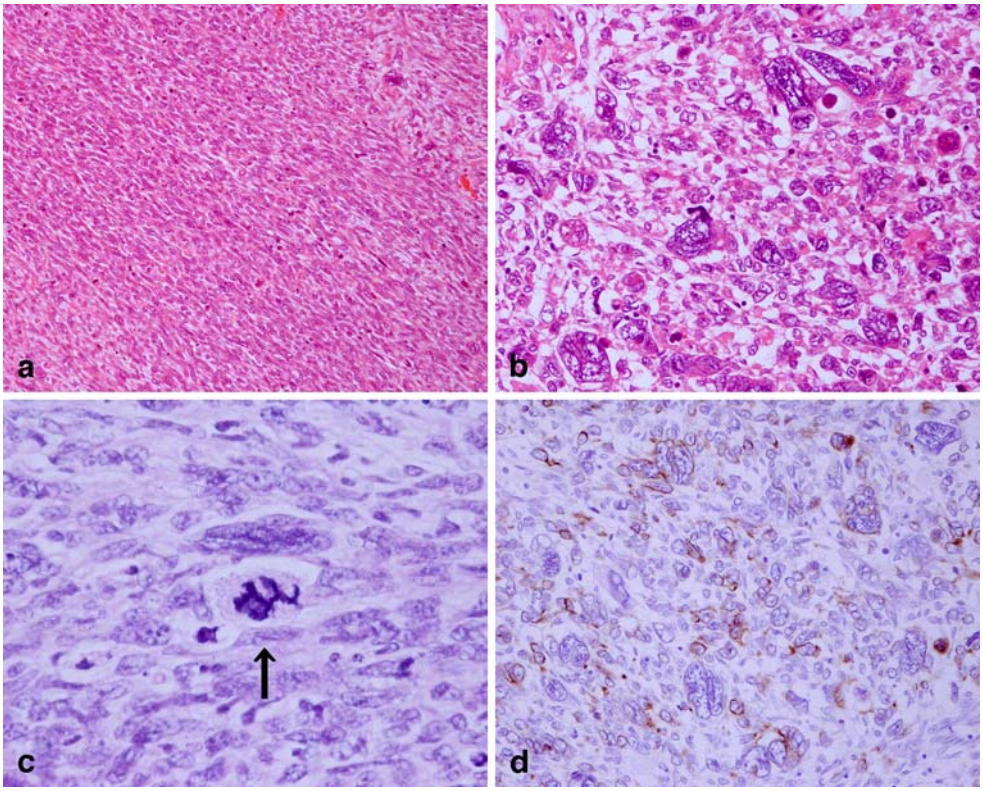

Fig. 1 **a** Low-power view shows monophasic proliferation of mesenchymal components (original magnification×100); **b** Tumor cells with bizarre nuclei are observed (original magnification ×200); **c** Multipolar mitotic figure is seen (*arrow*, original magnification ×400); **d** Many cells demonstrate reactivity for desmin (immunoperoxidase; original magnification × 200)

result of immunohistochemical study showing positive staining for rhabdomyogenic markers. Argani et al. [1] suggest that skeletal muscle differentiation is so sufficiently distinctive as to be a criterion to distinguish nephroblastoma from other primary renal sarcoma.

We diagnosed and treated this case as monophasic nephroblastoma based on the clinical diagnosis and the fact that renal RMS is extremely rare. At present, it remains unclear whether renal RMS in childhood is a distinct entity or in the spectrum of monophasic nephroblastoma. Therefore, there is no criterion for diagnosis and treatment of such cases. The number of reported cases is now limited [2, 3, 5], and further investigations are required to clarify the biological behavior of such renal tumor.

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LETTER TO THE EDITOR

Absence of mismatch repair deficiency in gastric lymphoma: an immunohistochemical study of mlh1 and msh2 protein expression

P. Cuilliere-Dartigues · B. Fabiani · S. Dumont · C. Copie-Bergman · A. Couvelard · T. Molina · A. Duval · J. F. Flejou

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Sir,

A subset of cancers exhibit ubiquitous microsatellite instability (MSI-H) due to mismatch repair deficiency (MMR). These tumors are characterized by improved survival and a better prognosis. Because MLH1 or MSH2 knock-out mice, i.e. the two main MMR genes, often develop lymphoid neoplasms [3], several groups have looked for the presence of MSI in human non-Hodgkin lymphomas (NHL), retrieving contradictory results. Gastric mucosa-associated lymphoid tissue (MALT) lymphomas run an indolent course, but transformation to diffuse large B-cell lymphoma (DLBCL) is responsible for decreasing 10-year survival rate from 90 to 45%. In such a subset of NHL, contradictory results concerning the prevalence of MSI have been reported so far in studies based on molecular testing of these tumors with nonconsensual markers for MSI analysis and biological materials deriving from either frozen or paraffin embedded tissue. International criteria have now been established for MSI testing, saying that it can be detected using a PCR-based molecular assay or by immunohistochemistry (IHC) of MLH1 and MSH2 MMR proteins. To evaluate the contribution of MSI in gastric lymphomagenesis, we thus analyzed MLH1 and MSH2 protein expression by IHC in a retrospective series of 122 MALT NHL and 51 DLBCL. Cases originated from the pathology files of Beaujon, Henri Mondor, Hôtel Dieu, and Saint Antoine hospitals, and from a variety of private laboratories. IHC assay was processed on 48 formalin, 78 Bouin, and 47 Acetic Acid/Formalin/Alcohol (AFA)-fixed specimens, on 162 freshly cut slides from paraffin-embedded tissue and on stored unstained slides that were only available in 11 cases. Immunohistochemical labelling was performed as described [4] with mouse antihuman monoclonal antibodies to MLH1, MSH2, and Ki67. Complete absence of nuclear expression in tumor cells for one MMR protein was considered indicative of an MMR defect. 85 cases were excluded because of absent nuclear signal in tumor and normal cells, related to fixation (100% inconclusive cases with AFA and near 50% with Bouin), and to prolonged slide storage. Using this approach, we failed to find out loss of MMR protein expression in the 88 cases tested (58 MALT NHL and 30 DLBCLs). Of interest, MMR staining was weak and heterogeneous in MALT lymphomas, showing the same intensity in tumor cells compared to internal control cells (Fig. 1), whereas strong nuclear signal with a widespread immunostaining of more than >80% of positive tumor cells was observed in DLBCLs (Fig. 2). Generally, MMR protein expression paralleled the proliferation rate assessed by Ki67 IHC.

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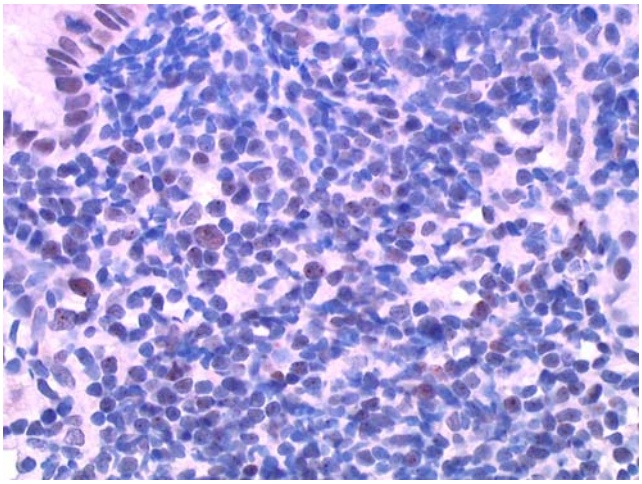

Fig. 1 Expression of MLH1 protein: low and heterogenous nuclear staining in gastric low-grade marginal-zone B-cell lymphoma of MALT-type (×40)

Although IHC has been validated as an accurate technique to identify MMR deficient tumors, MMR protein expression in gastric MALT lymphomas has never been reported. Retained expression of MLH1 and MSH2 has been observed in 31 cases of gastric DLBCLs, which showed a strong nuclear expression of both proteins that parallels the proliferation rate, as reported here [5]. As also reported previously, some technical factors may affect the assessment of IHC, e.g., slide storage (which alters antigenicity), fixative type (which was responsible for 85 inconclusive cases in our study). These technical limits have to be considered when using IHC as a diagnostic test for MSI in unselected series of tumors, as already shown in other tumor subtypes [1].

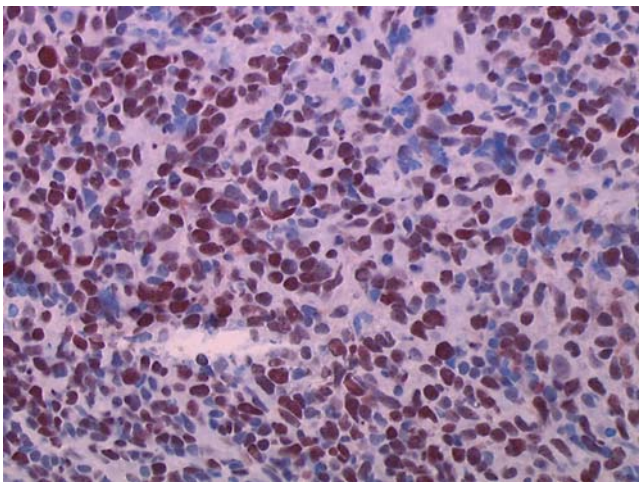

Fig. 2 Expression of MLH1 protein: strong and widespread positivity in a gastric diffuse large B-cell lymphoma (×40)

Consequences of the DNA mismatch repair inactivation pathway is not well established in lymphomagenesis. Our study supports previous reports about the minor role of defects in DNA mismatch repair mechanism in the pathogenesis of gastric MALT lymphoma. It appears, therefore, that MSI only plays a role in NHL occurring in immunodeficient patients, i.e., posttransplant lymphoproliferative disorders and HIV-related NHL, as demonstrated by our group [2].

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LETTER TO THE EDITOR

Early pulmonary manifestations of yellow nail syndrome: a case report

Sarah Liptrot · Geoffrey Hulks · Fiona Roberts

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Dear Editor,

Bronchiectasis with lymphatic obstruction and pleural effusions are well recognised pulmonary manifestations of the yellow nail syndrome. We describe bronchiectasis associated with markedly dilated lymphatic channels in an open lung biopsy from a patient with onycholysis and yellow discoloration of the nails. Although pleural effusions were absent in this patient, we propose that these changes represent the early pulmonary manifestations of yellow nail syndrome.

This 47-year-old man presented with recurrent chest infections and at least a 2-year history of recurrent cough productive of green sputum. His symptoms transiently responded to antibiotics but recurred soon thereafter. He also admitted to mild exertional dyspnoea. Two months before his presentation to the Chest Clinic, a heavy growth of *Pseudomonas aeruginosa* had been isolated from his sputum, and thereafter, his symptoms improved after a course of high dose oral Ciprofloxacin. He admitted to 5-kg weight loss in the previous year, which had since stabilized. The main symptom bothering the patient at the time of presentation was a yellow/green discoloration of his finger and toe nails that were growing very poorly, indeed one thumb and toe nail had come off in whole pieces.

The patient was a lifelong non-smoker with no history of childhood chest disease. He had been investigated for halitosis in 1995 and when he was thought to have a low grade sinusitis. There was no relevant family history or occupational history.

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On examination, all his finger and toe nails were abnormal with a yellowish discoloration, onycholysis and nail thickening (Fig. 1a). There was an increased curvature along their long axis and loss of cuticles. He was slightly built with a body mass index of 19.9. His chest was clear to auscultation, and physical examination was otherwise unremarkable. Spirometry demonstrated a forced expiratory volume in 1 s/forced vital capacity of 2.95:4.15 l (predicted 3.70:4.54). Routine biochemistry, haematology and CRP were normal. Serum immunoglobulins were within normal limits and electrophoresis negative. Aspergillus and micropolyspora precipitins were negative. Nail slicings taken for mycological examination were negative.

His initial computed tomography scan showed some peripheral consolidation in the right middle lobe and lingula and also the left base with some small patchy areas of air space consolidation throughout both lungs (Fig. 1b). Interlobular septal thickening was also noted. The appearances were not considered to be specific but were consistent with infective or inflammatory change.

Video-assisted thoracoscopic biopsy was taken from the right middle lobe and left lower lobes. Histology showed bronchiectasis and organising pneumonia most prominent in the lower lobe biopsy (Fig. 1c). In addition, markedly dilated lymphatics filled with fluid were identified in the interlobular septa and visceral pleura (Fig. 1d). These were considerably more marked than those associated with inflammatory conditions such as bronchopneumonia particularly in the middle lobe where the inflammatory changes were minimal. After discussion, it was considered that these findings in conjunction with the abnormal nails would be consistent with the early pulmonary manifestations of yellow nail syndrome.

The yellow nail syndrome is a relatively rare disorder usually occurring in middle-aged patients and characterized by yellowish, deformed nails, peripheral lymphoedema and recurrent pleural effusions [3, 6]. In addition, a significant number of patients also have sinusitis, bronchiectasis and

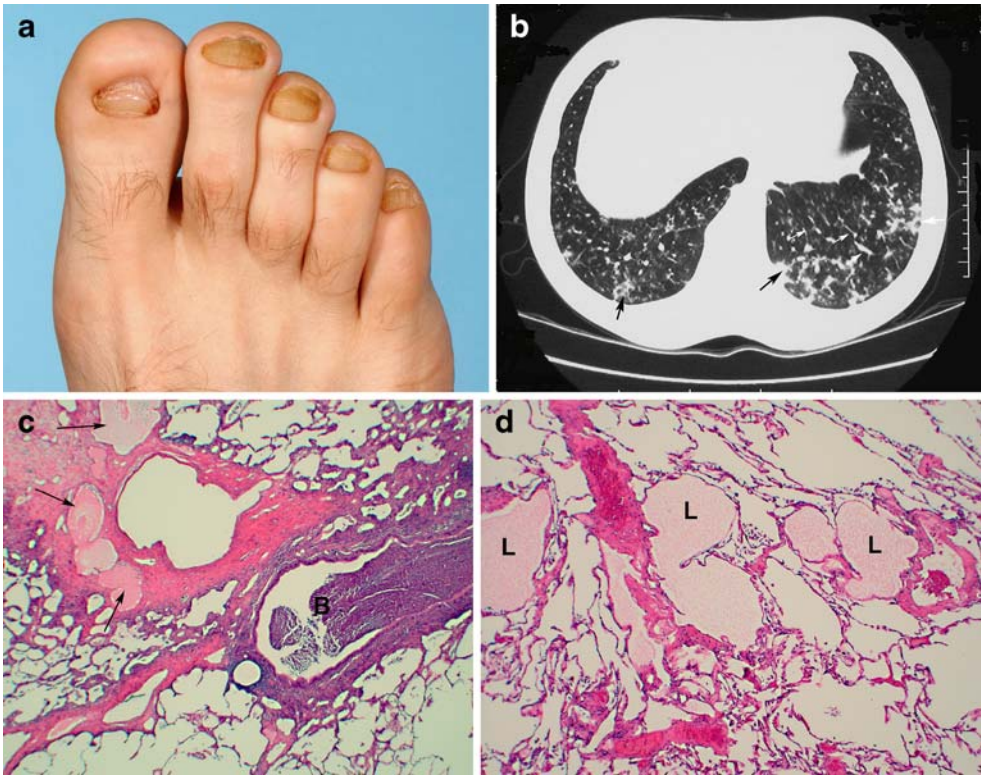

Fig. 1 **a** Discoloration, dystrophy and onycholysis of nails. **b** High resolution computed tomography of chest showing patchy air space consolidation (*black arrows*) and prominent vascular structures (*white arrows*). **c** Histological section of open lung biopsy from the lower lobe showing an ectactic bronchiole (**b**) filled with pus. In the adjacent septa, there are dilated lymphatics (*arrows*) containing eosinophilic fluid. (H&E, ×40 magnification). **d** Histological section of open lung biopsy from the middle lobe showing dilated lymphatics (L) without associated bronchiectasis. (H&E ×40 magnification)

lower respiratory tract infections. The underlying defect in yellow nail syndrome is unclear. Lymphangiography has shown hypoplastic lymphatics in some but not all sufferers [6]. Furthermore, dilated lymphatics have been described in subpleural tissues and small intestines [2, 4, 5]. Lymphoscintigraphy, however, supports a functional rather than an anatomical lymphatic defect [1].

The pulmonary pathology of yellow nail syndrome is not well described with only occasional case reports in the literature. Edwards et al. [2] described one case of yellow nail syndrome where there were characteristic dilated subpleural lymphatics in addition to inflamed bronchioles. Sacco et al. [5] reported the presence of dilated lymphatic channels in the subpleural tissue in association with cystic lung disease with changes of airway obstruction and ongoing inflammation. These latter authors postulated that respiratory infections may damage a previously inadequate lymphatic drainage and result in persistent pleural effusions. In our case, dilated lymphatics were evident both in areas with evidence of bronchiectasis and ongoing infection but also in the middle lobe where inflammation was minimal. The lack of inflammation in the middle lobe biopsy in our case lends support to this theory that there is a pre-existing lymphatic abnormality, which presumably predisposes to recurrent infection and bronchiectasis. Damage from recurrent inflammation may explain the later appearance of pleural effusions in some cases [3, 6].

The treatment of yellow nail syndrome remains largely symptomatic. Currently, our patient's clinical condition is stable, and although he continues to expectorate a little tenacious mucoid phlegm, the overall quantity has decreased and no further pathogens have been isolated from his sputum. The discoloration of his nails appears less, but their morphology remains very abnormal.

Acknowledgements We are grateful to Professor Andrew Nicolson, Brompton Hospital, London for his comments on the pathology of this case.

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REVIEW AND PERSPECTIVE

Pseudoneoplastic lesions of the testis and paratesticular structures

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Abstract Pseudotumors or tumor-like proliferations (non-neoplastic masses) and benign mimickers (non-neoplastic cellular proliferations) are rare in the testis and paratesticular structures. Clinically, these lesions (cysts, ectopic tissues, and vascular, inflammatory, or hyperplastic lesions) are of great interest for the reason that, because of the topography, they may be relevant as differential diagnoses. The purpose of this paper is to present an overview of the pseudoneoplasic entities arising in the testis and paratesticular structures; emphasis is placed on how the practicing pathologist may distinguish benign mimickers and pseudotumors from true neoplasia. These lesions can be classified as macroscopic or microscopic mimickers of neoplasia.

Keywords Testis · Paratesticular structures · Pseudotumor · Tumor-like · Benign mimics

Introduction

There are many lesions that can simulate a neoplasm in the testis or paratesticular structures. Their incidence among tumors arising within the scrotal sac varies according to different series from 6 to 30% [16, 32]. These pseudoneoplastic lesions can be divided into those that only macroscopically imitate neoplasia (Table 1) and those that microscopically imitate neoplasia, regardless of whether they form a macroscopic mass (Table 2). The latter group causes more problems to the practicing pathologist in terms of the correct classification of a giving lesion. Hereby, we summarize main tumor-like lesions and benign mimickers that may be seen in the testis and paratesticular coverages, with emphasis on morphologic criteria for the differential diagnosis from true neoplasia.

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Table 1 Macroscopic mimickers (pseudotumors) of testicular and paratesticular neoplasia

Vascular lesions
Intratesticular hemorrhage
Segmental testicular infarction
Organizad testicular hematocele
Cholesterol granuloma of the tunica vaginalis
Inflammatory lesions
Nonspecific infectious inflammatory lesions
Specific infectious inflammatory lesions
Non-infectious inflammatory lesions
Idiopathic inflammatory lesions
Idiopathic granulomatous orchitis
Testicular malakoplakia
Testicular sarcoidosis
Meconium periorchitis
Sperm granuloma
Cysts
Testicular cysts
Albuginea cysts
Parenchymal cysts (Epidermoid cysts)
Rete-testis cysts- Cystic displasia of the rete testis
Epididymal cysts and Spermatoceles
Spermatic cord cysts
Ectopic tissues
Adrenal cortical rests
Spleno-gonadal fusion
Testicular appendages
Miscellaneous other lesions
Fibrous pseudotumors; (Fibromatous periorchitis- Nodular periorchitis)
Amyloidosis
Polyorchididm
Sclerosing lipogranuloma

Macroscopic mimickers (pseudotumors) of testicular and paratesticular neoplasia

This group of lesions refers to those that macroscopically imitate a neoplasia. Frequently, these lesions affect both compartments and are therefore difficult to establish if one or both are affected. For this reason, we classify the tumor-like lesions according to their etiology.

Vascular lesions

The majority of the vascular disorders of the male gonad are not confused with a tumor because, in addition to causing acute symptoms, they usually affect the entire gonad. However, in the rare situation in which the vascular lesion is segmental, it can simulate neoplasia, as follows:

1. Intratesticular hemorrhage, which occurs spontaneously [24] or in the context of anticoagulation treatment [15], may simulate a tumor at ultrasound examination.
2. Segmental testicular infarction, a lesion clinically characterized by slight local pain unrelated to any acute episode [20], may be related to isolated or systemic vasculitis [43, 103] with morphology of polyarteritis nodosa [21], giant cell vasculitis [94], or Wegener granulomatosis [45]. Up to year 2000, 81 cases of systemic vasculitis with testicular tumor-like lesion have been recorded [45]. Other cases are seen in the context of a hematological disease (sickle cell anemia) [53] or associated with nonspecific perivascular fibrosis. Any of the phases of an infarction can be observed from acute (with hemorrhage) to healing stage (Fig. 1). Currently, the clinical diagnosis can be suspected with Doppler sonography [81], avoiding orchiectomy.
3. Organized testicular hematocele and other hemorrhages in the tunica vaginalis are rarely confused with a neoplasia [89], but this can happen occasionally in long-standing cases because of fibrous thickening with cholesterol granuloma formation in the tunica vaginalis (Fig. 2) [61] Exceptionally, a true testicular neoplasia presents clinically with a hematocele [86].

Inflammatory lesions

Similar to what was stated for vascular tumor-like lesions, inflammation that simulates a neoplasia usually has atypical clinical features; these entities can be grouped as follows:

1. Nonspecific infectious inflammatory lesions with a tumor-like presentation are frequently chronic processes causing

Table 2 Microscopic mimickers of testicular and paratesticular neoplasia

Testicular
Inflammatory-reactive lesions
Lymphocitic orchitis (Testicular pseudolymphoma)
Rosai-Dorfman disease
Sertoli cell hyperplasia
Pick adenoma
Hamartomatous proliferation testicular feminization syndrome
Interstitial cell hyperplasia
Leydig cell hyperplasia
Testicular “tumor” of the adrenogenital syndrome
Hyperplasia of the rete testis
Epididymis
Adenomatoid hyperplasia
Tunicas albuginea-vaginalis
Mesothelial hyperplasia
Spermatic cord
Vasitis nodosa
Inflammatory pseudotumor (Funiculitis proliferans)
Miscellaneous other lesions

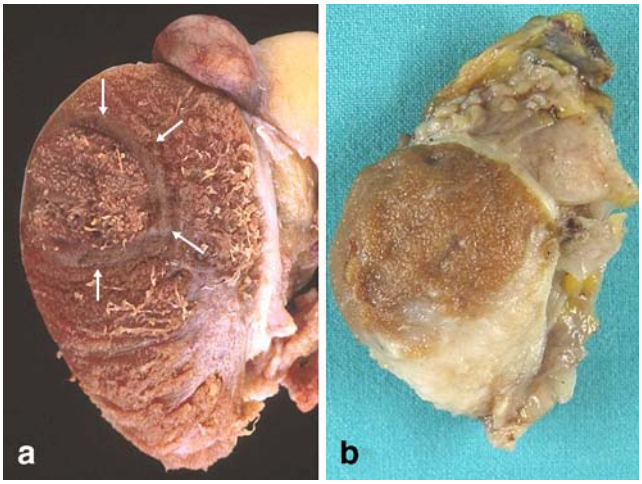

Fig. 1 Segmental testicular infarction. **a** Acute hemorrhagic with reinforcement of the peripheral area (*arrows*). **b** Healing fibrosis

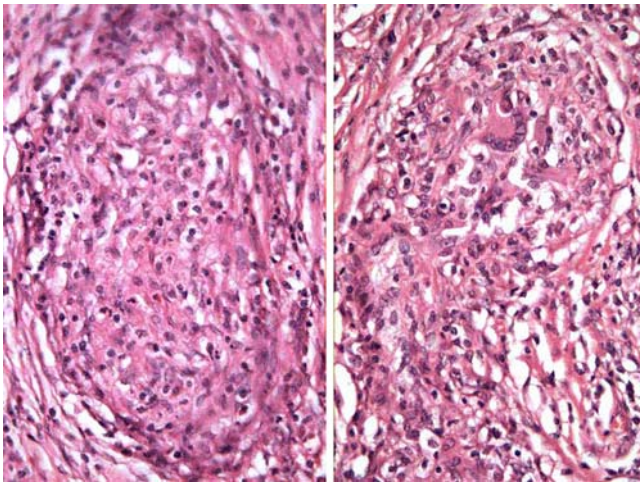

Fig. 3 Idiopathic granulomatous orchitis characterized by tubular granulomas (tubular orchitis)

progressive fibrosis, which may clinically [38] or sonographicaly [23] simulate neoplasia. Rarely a testicular, epididymal, or vas deferens abscess can look like a neoplasia [84]. Very occasionally, testicular neoplasia can clinically imitate an acute inflammatory process [44].

2. Specific infectious inflammatory lesions. The entities of this group that most often have been confused with neoplasias are granulomatous inflammation in tuberculosis [87], brucellosis [49], syphillis [5], fungal infections [42], and parasite diseases [9]. These lesions are usually not problematic for the pathologist.
3. Noninfectious inflammatory lesions. This group of tumor-like lesions include different entities among which can be highlighted:

(a) Idiopathic granulomatous orchitis, probably of autoimmune aetiology, of which around 230 cases have been published [4], is characterized by tubular granulomas (tubular orchitis; Fig. 3) or interstitial granulomas (interstitial orchitis). The presence of intratubular giant cells differentiate this entity from infectious granulomatous orchitis [70]. Diffuse testicular hypoechoic involvement with only peripheral low-resistance flow on color Doppler sonography is a typical but not pathognomonic pattern [72].

(b) Malakoplakia. This lesion is secondary to a mononuclear decrease in cyclin–guanine monophosphate that impairs the killing of bacteria. Fusion of the phagolysosomes with bacterial rests produces the characteristic Michaelis–Gutman bodies in the cytoplasm of the macrophages (von Hansemann cells) [1] (Fig. 4). Giant cells are occasional or absent. Testicular involvement represents only 12% of genital malakoplakia with around 388 cases in the literature [19, 56]; exceptionally, it may affect the epididymis only [31]. It has been related to idiopathic granulomatous orchitis [56, 57] and chronic xantogranulomatous inflamma-

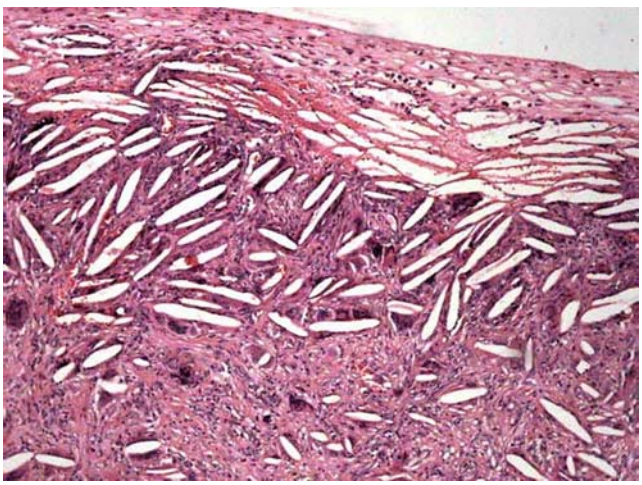

Fig. 2 Cholesterol granuloma in the tunica vaginalis (H&E)

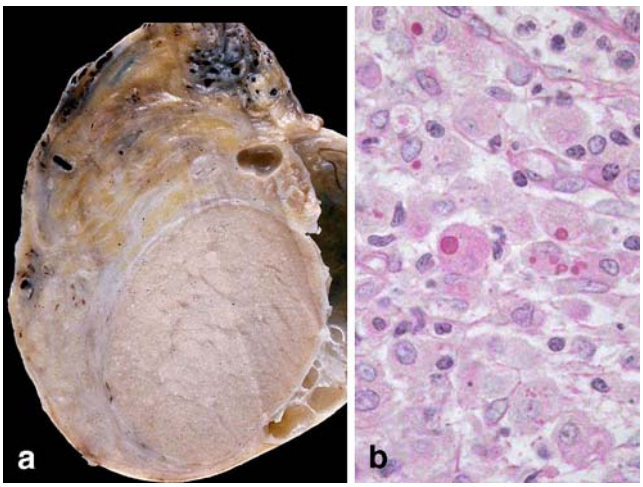

Fig. 4 Malakoplakia. **a** Macroscopic appearance with a homogeneous aspect. **b** The characteristic Michaelis–Gutman bodies in the cytoplasm of the macrophages (von Hansemann cells) (PAS)

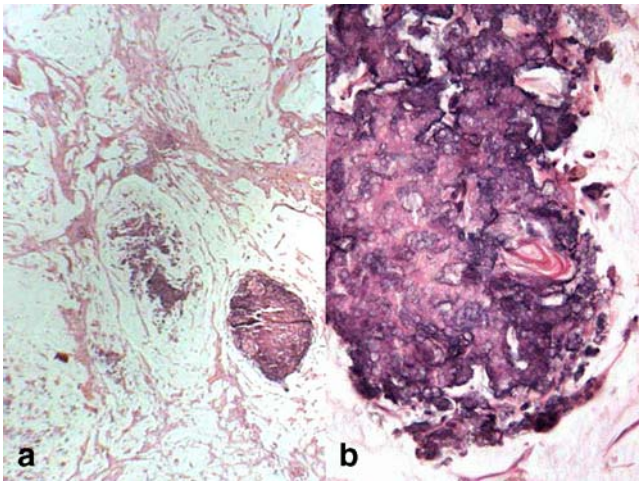

Fig. 5 Meconium periorchitis. **a** calcified pearls resulting from the calcification of the remains of squamous cells or lanugo hairs. **b** Keratin rest in one of the calcifications (H&E)

tion of testis [85, 109, 110]; this last also reported in the spermatic cord [64, 100].

(c) Sarcoidosis. Testicular involvement in a systemic sarcoidosis [39] is exceedingly rare, and its presentation as the primary form is even rarer; in these cases, the epididymis is affected more with the testicle being involved by contiguity [83].

4. Meconium periorchitis. This is an infrequent lesion (around 30 cases being reported) that typically presents in the first months of life; most times there is a clinical history with obstetric problem that has caused the passing of meconium toward the testicular surrounding structures [29]. The macroscopic appearance is a myxoid material with calcified pearls resulting from the calcification of the remains of squamous cells or lanugo hairs [107] (Fig. 5). Suspicion of neoplasia, although very uncommon in this period of life, may be caused by a scrotal mass or sonographically detectable calcifications [6]. Clinically, the peritesticular and spermatic cord enlargement can simulate a paratesticular rhabdomyosarcoma.
5. Sperm granuloma. As its name indicates, this granulomatous lesion with few giant cells is the consequence of extravasation of spermatozoa generally postvasectomy (40% of vasectomized men and 2.5% of general population) [108]. When it produces a tumor-like lesion, it is usually located in the deferent duct or the epididymis [22] with firm nodules of 0.7 to 4 cm with occasional cysts formation.

Cysts

The majority of the cysts with a tumor-like appearance are paratesticular structures, but the testicle may occasionally have some cystic lesions that can be confused with a neoplasia.

1. Testicular cysts occur in approximately 8–10% of patients with a lump in the testis, including those of the tunica albuginea or the parenchyma [33].

(a) Tunica albuginea cysts do not usually cause any diagnostic problem, unless if they are complex cysts [74].

(b) Parenchyma testicular cysts can be more difficult to distinguish from a neoplasia and if there is the slightest suspicion of an intracystic content, one must suspect a malignant neoplasia [107]. Special consideration deserves the testicular Epidermoid cyst, which must only be lined with squamous epithelium (Fig. 6). It is recommended that the specimen is examined "in toto" to avoid underdiagnosis of any area of teratoma (especially among postpuberal patients) or intratubular germ cell neoplasia, as sonographically it is not possible to distinguish between these lesions [54, 106]. Epidermoid cysts represent 1% of the masses of the testes. Recent genetic studies have shown that there is no chromosome 12p abnormality [14], thus supporting its distinction from teratoma.

(c) Tubular ectasia of the rete testis secondary to obstruction and generally located in the mediastinum area of the testes (Fig. 7) [69], is usually bilateral and very different from cystic dysplasia of the rete testis, a congenital lesion with complete testicular parenchyma substitution [68].

2. Epididymal cysts and spermatoceles are relatively frequent and the majority are in relation to the inflammatory processes. The differential diagnosis with other entities are related to its size, and similar to the other cystic formations a true neoplasia must be considered in case of observing any content in its interior [108].

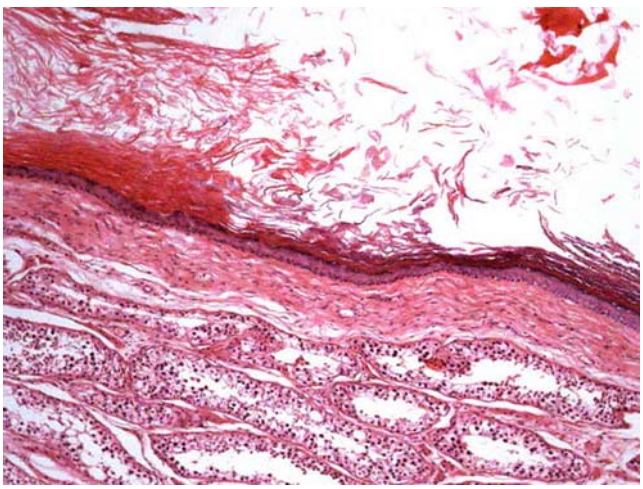

Fig. 6 Epidermoid cyst, which must only be lined with squamous cell epithelium (H&E)

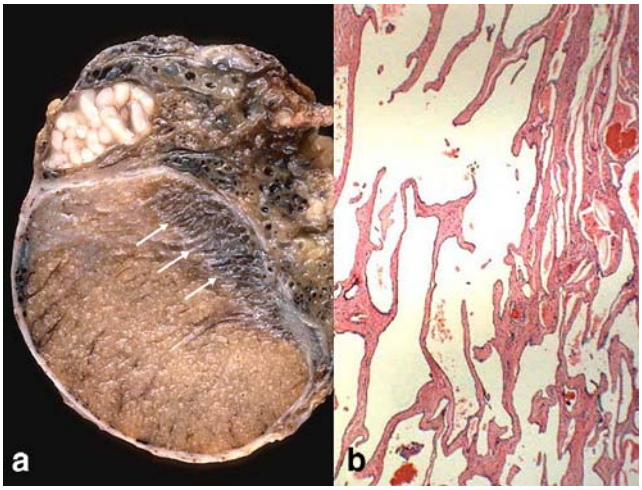

Fig. 7 **a** Tubular ectasia of the rete testis located in the mediastinum area of the testes (*arrows*). **b** Microscopic aspect (H&E)

3. Spermatic cord cysts. The majority of these cysts do not cause any diagnostic doubts, only the occasional Epidermoid cysts [46, 105] can simulate a neoplasia. These cysts can be unilocular or multilocular depending on their origin [65], and the multilocular must be distinguished from the exceptional cystadenomas of probably Müllerian origin [55].

Ectopic tissues

1. Ectopic adrenocortical tissue is relatively frequent in the tunica albuginea, rete testis, epididymis, and spermatic cord and occasionally reaches the size to be symptomatic. Its incidence ranges from 2.5 to 15% [17, 102]. It is made up of adrenal cortical nodes surrounded by a connective tissue band and of about 5 mm in diameter on average (Fig. 8); for which reason, they are not clinically palpable. Only in cases of congenital adrenal hyperplasia or Cushing syndrome, ectopic adrenocortical tissue can be prominent and appear as a tumor-like lesion [90].
2. Splenic–gonadal fusion, as its name indicates, is the fusion of spleen and gonad. It is more frequent on the left side, with about 148 published cases [41]. Morphologically, the ectopic splenic tissue can be in close relation to the head of the epididymis or the upper pole of the testis (Fig. 9) or being separated from it; likewise, there may or may not be a structural continuity between the normal spleen and the ectopic tissue [30]. The same alteration has been described in women, but it is much less frequent that in men, probably because it is much easier to find it clinically in men. In about 30% of the cases, it is associated with complex malformations such as micrognathia, peromelia, or phocomelia (absence of upper portion of a limb) [30, 60, 95]. In three of the reported cases, the fusion was associated with a germ cell tumor of the testis [41].
3. An exceptional hepato-gonadal fusion is reported [26].

Testicular appendages

There are five testicular appendages, but for the surgical pathologist, only three can be of interest (appendix testis or hydatid of Morgagni, appendix epididymis, paradidymis or organ of Giraldes; Fig. 10). These structures are not usually the origin of a tumor-like lesion, but in rare cases of large-sized cysts, it may presents as a paratesticular mass [93]. A case of ectopic epididymal tissue in an appendix testis [101] has recently been reported.

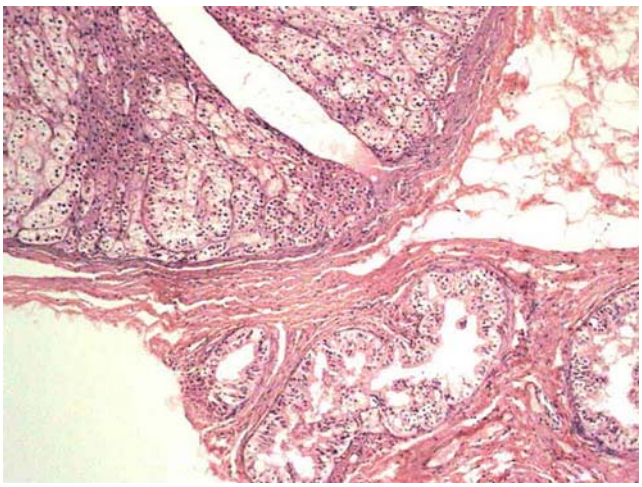

Fig. 8 Ectopic adrenocortical tissue. Adrenal cortical nodes surrounded by a connective tissue band in continuity with epididymis. (H&E)

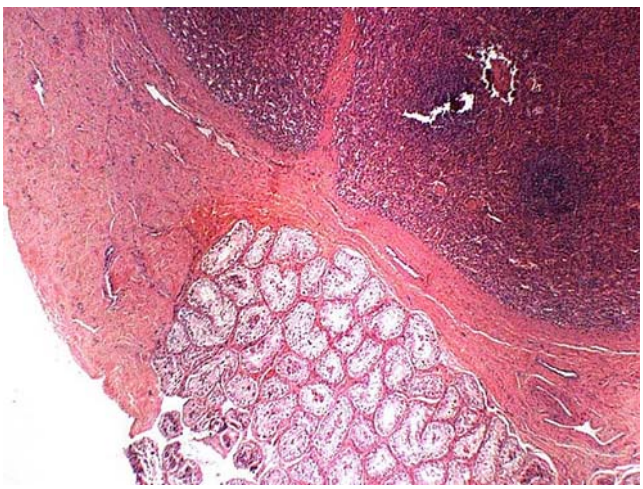

Fig. 9 Splenic–gonadal fusion. The ectopic splenic tissue in close relation with the upper pole of the testis (H&E)

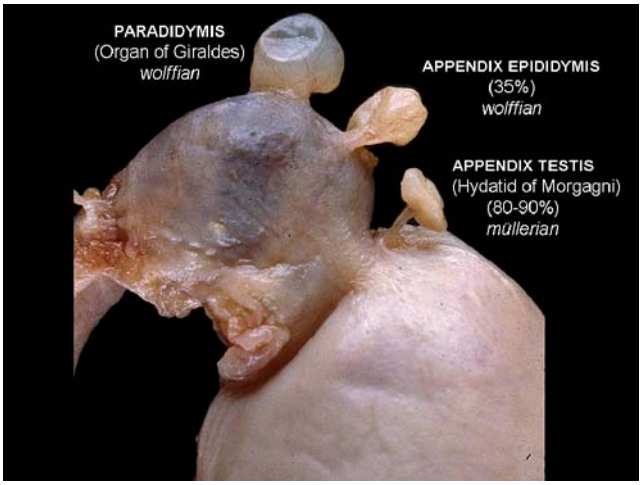

Fig. 10 Normal testicular appendages

Miscellaneous other lesions

1. Fibrous pseudotumors. This name refers to a fibrosis phenomenon with paucicellular hyalinized collagen (Fig. 11) presenting as nodular (single or multiple) or diffuse lesion of the testicular tunics [96, 71]. Sometimes, a node can be free (scrotal mouse) [108]. This broadly considered spectrum of lesions has received diverse names: chronic periorchitis, fibromatous periorchitis, nodular periorchitis, fibrous proliferation of the tunica, nonspecific paratesticular fibrosis, granulomatous periorchitis, nodular fibrous pseudotumor, fibrous pseudotumor, inflammatory pseudotumor, fibroma, reactive periorchitis [108], indicating its controversial pathogenesis. Some cases have been reported preceded by trauma or infection, and on occasion, an inflammatory component can be observed and granulation tissue suggesting the possibility that there might be the healing of an inflammatory pseudotumor, (which will be described later on) [10]. Although radiologically, it is not difficult to recognize, upon occasion, an intraoperative frozen section becomes necessary.
2. Amyloidosis. It is usually bilateral and present in a patient with a prior history of amyloidosis [34]; more rarely, it is a primary form that by being a cryptorquidic patient simulates a testicular tumor [13].
3. Polyorchidism or supernumerary testes is a rare condition, which is easy to recognize. However, the sonography can occasionally be different from that of the normal testis, and the condition may then be tumor suspicious [79].

Microscopic mimickers of testicular and paratesticular neoplasia

Lesions or cellular changes that microscopically imitate a neoplasia are included under this category, whether or not they make a clinical mass. These changes are closely related to the structure of the organ in which they arises, as follows.

Testicular

Inflammatory-reactive lesions Some lesion, already described above such as xantogranulomatous orchitis, idiopathic granulomatous orchitis and malakoplakia could be considered under this category; their microscopic confusion with a neoplasia (seminoma for example) is not currently a usual event; therefore, we preferred to include them in the macroscopic mimickers (pseudotumors).

The situation is different with lymphocitic orchitis or testicular pseudolymphoma [2, 3], which is characterized by a lymphocytic and plasmocellular reaction that may be

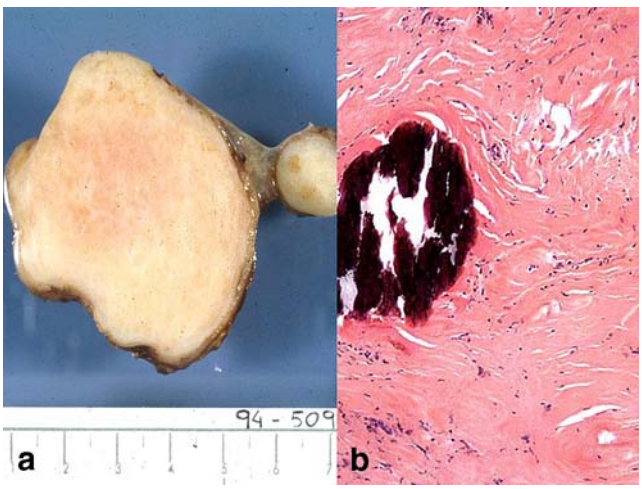

Fig. 11 Fibrous pseudotumor. **a** Well outlined fibrous-like nodule. **b** Paucicellular hyalinized collagen with calcification

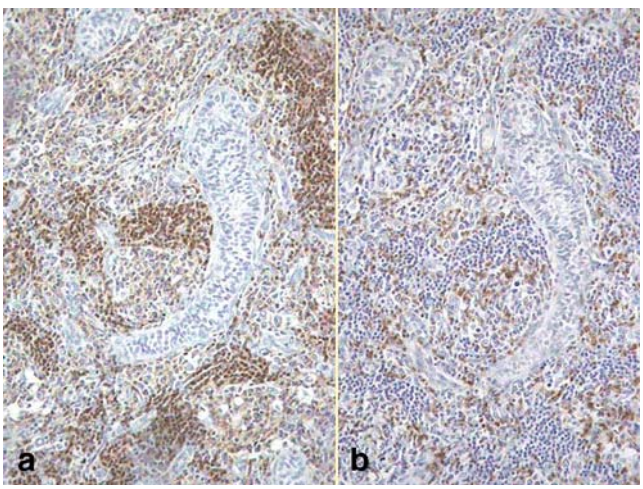

Fig. 12 Lymphocitic orchitis. Lymphocitic cellular infiltration of polyclonal type. **a** CD45RA (lymphocytes B). **b** UCHL-1 (lymphocytes T)

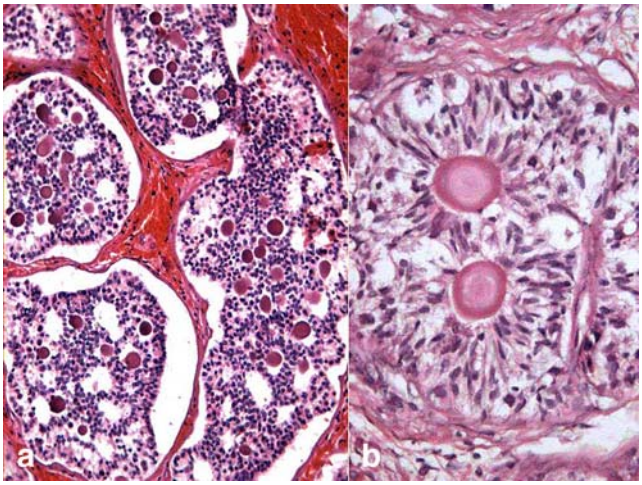

Fig. 13 Sertoli cell hyperplasia. **a** Nonencapsulated nodules of Sertoli cells (Pick's adenoma). **b** Sometimes there are areas that mimic Call–Exner bodies

confused with a lymphoma, but immunohistochemistry will show that the cellular infiltrate is polyclonal (Fig. 12). Among these idiopathic lesions, we can include Rosai–Dorfman disease; histological examination of the testicular mass reveals an inflammatory lesion comprising lymphocytes, plasma cells, and sheets of pale staining histiocytes, some containing lymphocytes within their ample cytoplasm, suggestive of emperipolesis. The histiocytes stained positive for CD68, S100 by immunohistochemistry and negative for CD1a, while ultrastructural examination confirmed emperipolesis [25].

Sertoli cell hyperplasia In a series of situations, nonencapsulated nodules of Sertoli cells can be found, especially known in cryptorchid testes as Pick's adenomas (Fig. 13a) [77]. Because of their appearance, these should be distinguished from the actual Sertoli cell tumors that generally are larger and sometimes there are areas that mimic Call–Exner bodies (Fig. 13b) A differential diagnosis with a yolk-sac tumor is not usually in the scope, but the immunohistochemistry study with AFP, calretinin, α-inhibin, and CD 99 can help [40].

A special consideration merit the androgen insensitivity syndrome or testicular feminization (male pseudohermaphrodism, caused by a failure of androgen receptor binding) that in 63% of cases can have tubular hamartomas (tubules lined by immature Sertoli cells) [82] that must be differentiated from the Sertoli cell adenomas and sex cord tumor with annular tubules [76].

Interstitial cell hyperplasia In testicles with marked tubular atrophy, such as in the Klinefelter's syndrome, it is possible to see Leydig cell nodules that must be distinguished from Leydig cell tumors. An interstitial growth without expansive pattern favors hyperplasia (Fig. 14) [62].

Nodules of eosinophilic cells appearing to be Leydig cells are found in the patients with adrenogenital syndrome [18, 48] and Nelson's syndrome (adrenocorticotropic hormone-secreting pituitary adenoma after bilateral adrenalectomy), some of this last syndrome with excessive testosterone production [91]; proof that these interstitials cells are not only morphologically similar to Leydig cells but also have the functional property of these cells. These nodules are usually bilateral and of a large size with cellular pleomorphism and pigmentation. The clinical history and a complete endocrinological profile avoid an unnecessary orchiectomy [78] because only one case of aggressive behavior is published [18].

Hyperplasia of the rete testis The normal rete testis epithelium is flat, but in some hyperestrogenic situations (treatment or hepatic dysfunction), the epithelium may become columnar and rarely a micropapillary growth of bland cells can be observed. The diagnosis of rete testis hyperplasia is subjective, and adenomatous lesions are rarely seen [35, 63]. In some cases, there are intracytoplasmic hyaline eosinophilic globules resembling a yolk sac tumor, but the negative stains of α-fetoprotein or placental alkaline phosphatase help to rule this differential diagnosis [99].

Pseudohyperplasia of the rete testis and epithelial reaction in case of germ cell invasion and cryptorchidism must be differentiated from real hyperplasia of the rete testis [62].

Epididymis

Benign microscopic mimickers of cancer in the epididymis are very rare. Cysts may occur but do not resemble tumors microscopically. However, some cases of adenomatoid hyperplasia of the rete testis can involve the epididymis, and occasionally, they may become macroscopically apparent [93].

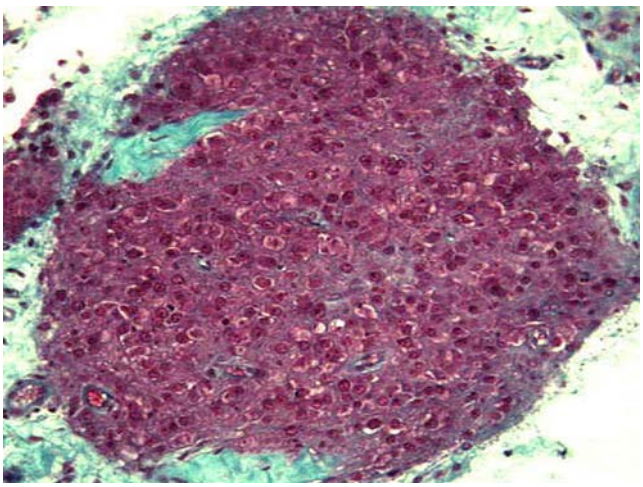

Fig. 14 Leydig cell hyperplasia

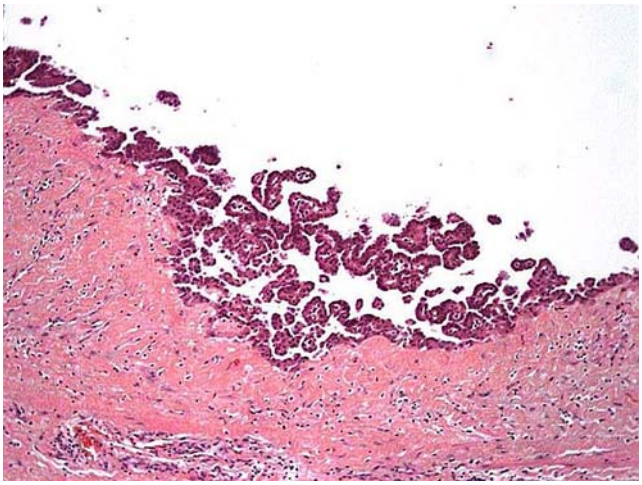

Fig. 15 Mesothelial hyperplasia. The bland nucleus, no true invasion and associated inflammatory elements, can be useful to distinguish from malignant mesothelioma

Tunica albuginea and vaginalis

Non-neoplastic mesothelial lesions involving the paratesticular region include mesothelial cysts and reactive mesothelial hyperplasia [73].

Mesothelial hyperplasia is the most important benign mimicker of the testicular tunics. It is present as a reactive lesion in hydrocele or hernia but may also be found microscopically in older men [80]. The mesothelial proliferation has an epithelial appearance, and rarely, a spindle cell proliferation can be present. In the differential diagnosis with mesothelioma, the bland nucleus, no true invasion, and associated inflammatory elements can be useful (Fig. 15) [11, 98]. Recently, a case was published with "atypical" mesothelial hyperplasia on one side and "well differentiated" mesothelioma on the contalateral [97]. This case is an example of the subjective interpretation of some mesothelial proliferative lesions because some malignant mesotheliomas lack cellular atypia. In these cases, an extensive confluence or prominent infiltration favors a malignant diagnosis. Unfortunately, the immunohistochemical expression of benign and malignant mesothelial proliferations are similar (low- and high-molecular-weight cytokeratins and vimentin) [11, 28], and only the metastatic neoplasias can be differentiated by the CEA, Ber-EP4, and B72.3 expression [27]. Only deoxyribonucleic acid ploidy can distinguish some borderline lesions [28].

Spermatic cord

The vas deferens and the soft tissues of spermatic cord can have benign mimickers.

1. Vasitis nodosa is a ductular proliferation, generally after vasectomy [36], although it can be seen following other trauma on that area, [75]. It has a microglandular morphology (Fig. 16) with mild nuclear atipia and perineural growth [7] or benign vascular invasion [8] that may be mistaken for malignancy [104]. The frequent hyperplasia of nerve fibers in the adventitia can explain the painful symptoms in some patients [36]. The coincidence with microscopic sperm granulomas and inflammatory reaction can help in the correct diagnosis. An analogous epididymal lesion also occur [88].
2. Proliferative funiculitis is the inflammation of the spermatic cord usually the result of an extension of vasitis. The soft tissues of the spermatic cord are also the most common site of an inflammatory pseudotumor in the male genital tract [37, 50, 58]. The lesion is ill defined, myxoid with white-gray color, and a moderate cellular proliferation with loose collagen fibers and irregular infiltration of inflammatory cells. An exceptional case has been reported that was largely infiltrated by mast cells [92]. In some cases, a prominent spindle cell proliferation mimics a sarcoma (pseudosarcomatous myofibroblastic proliferation), but low mitotic index, a capillary pattern, inflammatory cells, and absence of atypical mitoses speak against a diagnosis of sarcoma. Unfortunately, immunohistochemistry is only partly helpful because the cells express actin and vimentin, less strongly desmin and exceptionally cytokeratin. Two cases of this lesion in epididymis [12, 51] and one in the rete testis [47] have been reported.

Embryonic remnants

Although ectopic tissue usually is more problematic for constituting a mass, some of them can cause microscopic diagnostic doubts for which reason they can be included in

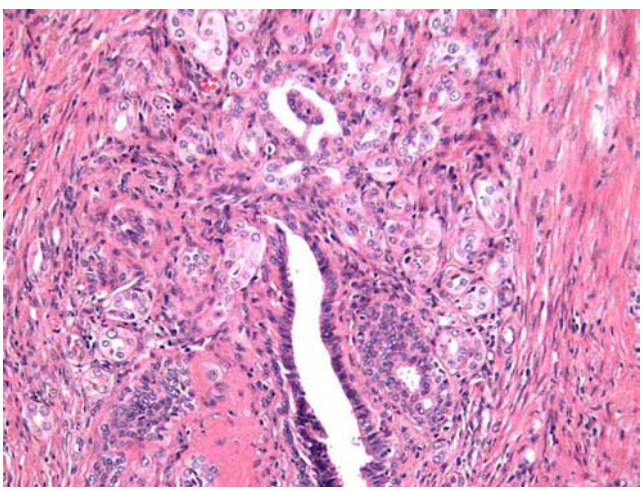

Fig. 16 Vasitis nodosa. Ductular proliferation with a microglandular morphology

this group of benign mimics. The presence of seminiferous tubules within the tunica albuginea [67], Leydig cells in rete testis, albuginea, spermatic cord, or within sclerotic tubules [59, 66], prostate gland in the epididymis [52], and special circumstances with muscular rete testis hypertrophy [27] can mimic a neoplasia.

The lesions described constitute a large heterogeneous group, without etiological or pathogenic relations among them or with true neoplasias. However, in spite of that, the patient with one or more of these tumor-like and/or benign mimickers can have a concomitant or ulterior true neoplasm.

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ORIGINAL ARTICLE

Ezrin and alpha-smooth muscle actin are immunohistochemical prognostic markers in conventional osteosarcomas

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Abstract Ezrin is a cytoskeleton linker protein that is actively involved in the metastatic process of cancer cells. We have searched for a prognostic value of ezrin and some of its partners: α-smooth muscle actin and CD44H in 37 patients with an osteosarcoma. Automate immunohistochemistry (IHC) with anti-ezrin, α-smooth muscle actin and CD44H antibodies was performed in 66 specimens: 37 biopsies before chemotherapy, 16 resected tumours of "poor" responders and 13 metastases. The messenger RNA (mRNA) levels of ezrin of 13 frozen biopsies and 4 metastases were evaluated by real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR). All results were correlated to the following clinical data. Ezrin expression by IHC was found in 62% of 37 biopsies in the different histological subtypes. A good correlation was found between positive or negative samples by IHC and mRNA levels. Ezrin expression was recorded in 84.5% of metastastic samples. The mean expression of ezrin was higher in metastases than biopsies ($p=0.024$). In multivariate analysis, ezrin was an independent prognostic marker for event-free survival and overall survival (OS) with $p<0.001$ and $p=0.003$, respectively, and α-smooth muscle actin for OS only ($p=0.024$). Our findings suggest that ezrin and α-smooth muscle actin are predictive IHC prognostic markers for patients with an osteosarcoma.

Keywords Ezrin · CD44H · α-Smooth muscle actin · Survival · Osteosarcoma

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Introduction

Prognosis of patients with an osteosarcoma has improved during the last two decades with the use of multiagent adjuvant chemotherapy leading to a 5 years disease-free survival of nearly 70% [20, 24]. One of the most powerful prognostic factors in osteosarcoma is the histological response to preoperative chemotherapy evaluated on the resected specimen after surgery [29, 31, 32]. No clinical, pathological or molecular parameter is predictive of the response to chemotherapy. In addition, the use of more intensive preoperative chemotherapy has increased the percentage of patients with good histological response but has not improved outcome, as the event-free survival rate has not changed. At the time of diagnosis, the most important prognostic factor is the presence or absence of metastasis [23]. About 20% of patients present with a metastatic disease and have an extremely poor prognosis with a long-term event-free survival of usually less than 10% [2, 9]. In the group of patients with localised disease, few prognostic factors have been reported such as age of onset of the disease, sex, location and size of tumour [3, 36]. To date, no genetic or molecular prognostic factor can be routinely used to stratify patients into high risk or low risk of metastasis. The finding of novel molecules variably expressed among osteosarcomas cells might provide insight into the mechanisms of tumour progression and could serve to identify tumours with more or less aggressive behavior.

Recently, overexpression of ezrin protein has been correlated to the metastatic potential of several cancers [11, 12, 41]. Ezrin belongs to the ezrin/radixin/moesin (ERM) protein family, which act as membrane organizers and linkers between plasma membrane and cytoskeleton [21]. ERM proteins occur in the cytoplasm in an inactive closed conformation with N- to C-terminal associations within the protein or with the other ERM members. Upon threonine and tyrosine phosphorylation, ezrin assumes an active open conformation, moves to the cell membrane and tethers F-actin directly or indirectly to the cell membrane [37]. Ezrin, also known as cytovillin or villin2, is a component of cell-surface structures, which are involved in cell adhesion to the extracellular matrix and in cell–cell interactions, receptor tyrosine-kinase signalling (cmet/HGF pathway), signal transduction through Rho GTPase and interactions with the Akt-mediated cellular apoptotic machinery. Khanna et al. [13], using sets of cell line variants of osteosarcomas with low or high metastatic properties, found that *vil2* gene mapped to chromosome 6q25–26, was consistently overexpressed in the metastatically capable clones. More recently, they also showed that stable transfection with full length anti-sense ezrin diminished experimental and spontaneous metastases in mice. High expression of ezrin in dog osteosarcomas was also associated with early pulmonary metastases, and a significant association with high ezrin expression and poor outcome was also found in a small cohort of pediatric osteosarcomas [14]. CD44 is a family of cell surface glycoproteins implicated in the invasiveness and metastasis [22]. Ezrin directly interacts with the cytoplasmic tail of the CD44 gene product, the hyaluronic-acid receptor [37]. Increased expression of CD44 potentially leading to increased functional activation of ezrin has also been shown to correlate with increased invasion in osteosarcomas [28]. Shiratori et al. [34] reported that lung metastasis was accelerated by up regulation of CD44 in an osteosarcoma cell line: POS-1. Weber et al. also showed that disruption of CD44 gene led to aborted metastasis formation in osteosarcoma [21]. However, prognostic value for the standard CD44 form (CD44H) or variants in osteosarcomas remains controversial [18, 28, 40].

To further evaluate the prognostic value of the expression of ezrin and CD44H in osteosarcomas, we performed automated immunohistochemistry in 66 specimens of conventional osteosarcomas: a series of 37 biopsies of osteosarcomas before chemotherapy, 13 metastases, 16 resected tumour specimens of "poor responders" and correlated them to patients' outcome. We also investigated messenger RNA (mRNA) level of ezrin in 13 frozen biopsies of osteosarcomas and 4 metastases. As ezrin is a protein that links cell surface glycoprotein such as CD44H to the actin cytoskeleton and as in conventional osteosarcomas, α-smooth muscle actin expression has been variably reported [10, 39, 42]; we also studied α-muscle actin expression by immunohistochemistry (IHC) in the same specimens.

Materials and methods

Patients and tumour specimens

Thirty-seven patients treated for an osteosarcoma at the La Timone hospital between 1993 and 2004 in the pediatric and adult oncological departments were included in this study. All tumours arose de novo. All patients received preoperative and postoperative chemotherapy derived from T10 regimen [33] or SFOP OS94 regimen [19]. All patients received high-dose Methotrexate and had wide conservative surgery (except one case of amputation) with microscopically complete resection (R0). The response to preoperative chemotherapy was assessed on resected specimens according to Rosen's protocol [31]. Good responders were defined by no more than 5% viable tumour. Surgical biopsies of osteosarcomas made before chemotherapy were available for all patients. Histological diagnosis was conventional osteosarcomas type in all cases. They encompassed

osteoblastic subtype in 17 cases (45.9%), pure chondroblastic subtype in 2 cases (5.4%), telangiectatic subtype in 2 cases and mixed subtype (mainly osteoblastic and chondroblastic or fibroblastic and osteoblastic) in the 16 remaining cases (43.2%).Thirteen metastases from 13 patients were also available for immunohistochemistry. For seven patients, biopsy and metastasis were both available for immunohistochemistry study. The location of the metastases was lung in 11 cases and brain in 2 other cases. Total RNA extraction was performed for 13 frozen biopsies (10 was positive and 3 negative for ezrin by IHC) and 4 metastatic specimens (all positive for ezrin by IHC).

Immunohistochemistry

Tumour specimens were fixed in 4% formalin. Fleshy tissue was separated from calcified areas in biopsy specimens to avoid unuseful decalcification and then parafin embedded. Resection specimens were decalcified in a solution of 22% acid formic about 2 or 3 weeks. Sections of 4-μm thickness were prepared from one representative block of the 37 biopsies before chemotherapy, 16 resection specimens after chemotherapy (among the 19 poor responders) and 13 metastases. Automated immunohistochemistry was performed with avidin–biotin–peroxidase complex on a Ventana 320 Device (Tucson, AZ) with Ventana kits (Strasbourg, France) including AEC reagent. Sections were counterstained with hematoxylin and mounted in glycergel (Dakocytomation).The following antibodies were used: ezrin (sigma, clone 3C12, diluted to 1/250) and CD44H (RD system, clone 2C5, diluted to 1/2,000). Heat antigen retrieval was used for both with citrate buffer (pH 6). For α-smooth muscle actin immunohistochemistry, the antibody was purchased from Dako (clone 1A4) and used prediluted without heat pre-treatment. For ezrin immunohistochemistry, paraffin sections from a placenta collected after birth were used as external positive control. Lymphocytes in biopsies or bronchial epithelium in pulmonary metastasis served as internal positive controls. An anaplastic oligodendroglioma served as external positive control for CD44H, and vessels served as internal positive control for α-smooth muscle actin [4]. Slides incubated without antibodies were performed for negative controls. Immunohistochemistry was recorded as positive for ezrin when a membranous and/or cytoplasmic staining was observed. For CD44H, the staining was membranous only, and for α-smooth muscle actin, the staining was cytoplasmic. More than 1% of the cells was the arbitrary cut-off to accept positivity. A mean percentage of stained cells was scored for each positive case in representative cellular areas avoiding necrosis and abundant matrix. One hundred cells were counted in the most stained areas.

RNA preparation

Total RNA extraction was performed using the acid guanidinium isothiocyanate/phenol/chloroform procedure [6] and analysed on both nanodrop spectrophotometer and Agilent 2100 bioanalyzer (Agilent Technologies, France). Only samples with 28S/18S ratio of >1.5 and no evidence of ribosomal degradation were included. Before use, RNA samples were treated with 10 U ribonuclease-free deoxyribonuclease (Promega, France) at 37°C for 15 min.

Real-time quantitative RT-PCR

Real-time quantitative reverse transcriptase polymerase chain reaction (QPCR) was used to accurately detect the changes of Ezrin and ribosomal 18S gene copies. Total RNA (1 μg) DNA-free was reverse transcribed into complementary DNA (cDNA) using 1 μg of hexamers (Pharmacia Biotech, Orsay, France) and M-MLV reverse transcriptase (Invitrogen Life Technologies, France). Human Ezrin and 18SrRNA were amplified, detected and quantified in real-time using the Light Cycler Detector System (Roche Applied Science, Meylan, France) using the following primers: for ezrin, EZS1, 5′CGAAACCAAT CAATGTCCG3’ and EZAS2, 5′GGGCAGACACCTTCT TATC3′ and for 18S rRNA, 18SS, 5′CTACCACATCCAA GGAAGGCA3′ and 18SAS, 5′TTTTTCGTCACTACCT CCCCG3′. The QPCR conditions for 18SrRNA expression level analysis have been previously described [7, 30]. The amplification mixture contained cDNA derived from 50 ng of total RNA, 0.2 μM of primer and mix from Light Cycler Fast Start DNA Master Sybr Green I kit (Roche Applied Science) in a final reaction volume of 20 μl. For ezrin, a three-step PCR was performed for 40 cycles as follows: denaturation at 95°C for 10 s, annealing at 56°C for 5 s and elongation at 72°C for 15 s after one cycle of denaturation/activation at 95°C for 15 min. A melting curve was performed at the end of amplification cycles to verify the specificity of the PCR products, and each amplicon was sequenced to validate the assay. All determinations were performed in duplicate.

Efficiencies (E) were calculated from the given slopes in the Light Cycler software using dilutions of total RNA prepared from renal cDNA samples.

The relative expression ratio (R) of the target gene transcript and reference gene transcript (18SrRNA) was calculated from the QPCR efficiencies (E) and the crossing point (Cp) deviation of a tumour sample vs the renal cDNA used as control:

$$R = \frac{\left(E_{\text{target}}\right)^{\Delta Cp\ (\text{control}-\text{sample})}_{\text{target}}}{\left(E_{\text{ref}}\right)^{\Delta Cp\ (\text{control}-\text{sample})}_{\text{ref}}}$$

Statistical analysis

Correlation between quantitative covariates was analysed by Pearson correlation test or non-parametric Spearman correlation test when Pearson correlation test assumptions was not verified. Relations between quantitative and qualitative covariates was analysed by ANOVA or non-parametric Kruskall–Wallis test when analysis of variance assumptions was not verified.

Overall survival and event-free survival (survival without local relapse or metastasis) were both analysed. Survival analysis was first provided using Kaplan–Meier estimation and Log-rank test for covariate selection, with the usual level of significance of 0.25 [8]. Secondly, multivariate analysis was provided using Cox proportional hazard model with forward stepwise based on likelihood ratio. The IHC parameters were recorded as a continuous variable. As the presence of initial metastasis and preoperative chemotherapy response are known as prognostic factors [2, 9, 29, 31, 32], the model was adjusted (as usual) for these two confounder covariates. Furthermore, because ezrine and α-smooth muscle actin expressions were biologically related, the model was also adjusted for interaction between these two covariates. The level of significance for the multivariate model was set at 0.05. The association of the results of QPCR with IHC parameters was assessed by the no parametrics Spearman test. Data analysis was performed using SPSS version 11.5.1. (SPSS, Chicago, IL) software.

Threshold value of ezrin/18S/rRNA ratios was determined by receiver operator characteristic (ROC) curve using the Analyze-It software (Excel™, Microsoft).

To compare ezrin expression by IHC (mean percentage of positive cells) of biopsies and metastases, we used non parametrics Wilcoxon test.

Results

Patients

Data are summarised in Table 1. Mean age was 15 years. Seventy-five percent of patients were younger than 17 years. Thirty-one patients were treated in the paediatric unit and six in the adult unit. Sex ratio was 62.2% male and 37.8% female. The tumour was located in long bones except for one case where it was located in a rib. Tumour size varied from 3.5 to 25 cm. The duration of preoperative symptoms ranged from 2 weeks to 7 months. Two patients had lung metastases at the time of diagnosis. There were 18 "good" responders to preoperative chemotherapy and 19 "poor" responders. The median of follow-up was 4.5 years (minimum, 10 months; maximum, 150 months). At the end of the follow-up 8 patients had died of disease, and 13 had developed metastases (11 lung metastasis, 1 bone metastasis, and 1 node metastasis). The median of metastasis onset in our cohort was 21 months (minimum, 4 months; maximum, 78 months). One patient developed local recurrence and then metastases. Event-free 3-year survival rate for good responders was better: 72 vs 48% for poor responders.

Table 1 Clinical data

Clinical characteristics	Values
Number of patients	37
Median age at diagnostic (years)	15 (6.7; 54.2)
Male (%);	62.2%
Female (%)	37.8%
Patients of medical pediatric oncology	31 (83.7%)
Patients of medical adult oncology	6 (16.3%)
Histologic response, number of patients	
Good responders	18 (48.6%)
Poor responders	19 (51.4%)
Metastatic disease at diagnosis, number of patients	
Absent	35 (94.6%)
Present	2 (5.4%)
Preoperative chemotherapy, number of patients	
Polychemotherapy or monochemotherapy with Methotrexate	37
Surgical treatment, number of patients	
Wide conservative surgery	37
Amputation	1
Limb salvage	36
Microscopically complete resection (R0)	37
Histological diagnosis and subtype, nb. of patients, %	
High grade osteosarcomas of central "conventional" type	37
Osteoblastic	17 (45.9%)
Chondroblastic	2 (5.4%)
Telangiectatic	2 (5.4%)
Mixed subtype	16 (43.2%)
Tumor localisation, number of patients	
Long bone	36 (97.3%)
Flat bone	1 (2.7%)
Tumor size	
Maximum	25 cm
Minimum	3.5 cm
Mean	11.5 cm
Var.	33.5
Duration of preoperative symptoms	
Maximum	7 months
Minimum	2 weeks
Median	1.25 months
Follow-up	
Median follow up	4.52 years
Minimum	10 months
Maximum	150 months
Death	8 (21.6%)
Local recurrence and metastatic recurrence	1 (2.7%)
Metastatic recurrence	12 (32.4%)
Localisation, number of patients	
Lung	10
Bone	1
Node	1
Median of metastasis onset	21 months
Event-free 3-years survival rate	
Good responders	72%
Poor responders	48%

Ezrin, CD44H and α-smooth muscle actin expression in biopsies of osteosarcomas before chemotherapy

Among the 37 patients, 23 biopsies were positive for ezrin (62%), 23 for CD44H (62%) and 15 for α-smooth muscle actin (40%) (Table 2). No statistically significant correlation was seen between each of the three IHC parameters and histological subtypes. The positivity for ezrin IHC was mainly cytoplasmic with sometimes a membranous signal (Fig. 1a). Atypical multinucleate giant cells and mononuclear cells were both positive for ezrin in telangiectatic osteosarcomas (Fig. 1b). However, positivity with ezrin antibody was present in chondroblastic areas (Fig. 1c) and in osteoblastic or fibroblastic areas.

Quantification of Ezrin mRNA with 18S rRNA reference gene transcript confirmed higher Ezrin mRNA levels in biopsies positive for Ezrin in IHC compared with negative samples (Fig. 2). The mean level of Ezrin mRNA expression was 0.698±0.404 in positive samples vs 0.173±0.116 in negative samples. Statistical correlation was found between ezrin expression by IHC scored as a continue variable and the QPCR (p=0.003). The correlation coefficient was 0.75.

Ezrin, CD44H and α-smooth muscle actin expression in residual tumour of "poor responder" osteosarcomas

Of the 19 cases of osteogenic sarcomas that were "poor responders" to chemotherapy, 16 resection specimens were available for IHC analysis. For 13 cases that were immunoreactive for ezrin before chemotherapy, only 2 maintained their immunopositivity after chemotherapy. For CD44H, 11 cases were positive: 9 both on biopsy and resection and 2 on resection only. For α-smooth muscle actin, four cases were positive both on biopsy and resection.

Ezrin, CD44H and α-smooth muscle actin expression in metastases

Among the 13 metastatic specimens, 11 were positive for ezrin (Fig. 1d,e), 11 for CD44H and 5 for α-smooth muscle actin (Table 2). Mean ezrin expression (percentage of cells positive by IHC) was 38.1% (95% CI, 21.4–54.7) in metastases vs 17.3% (95% CI, 7.6–27.1) in biopsies (p=0.024). Mean CD44H expression was also statistically higher in metastases than in biopsies 60.8% (95% CI, 42.9–78.6) vs 27.1% (95% CI, 15.2–39; p=0.004). However, for α-smooth muscle actin expression, no significant difference was observed (p=0.88).

Table 2 Immunohistochemistry data

	Biopsy: positive samples, number (%)	Metastasis: positive samples, number (%)	Tumour on resected specimen: positive samples, number (%)
Ezrin	23/37 (62%)	11/13 (84.5%)	2/16 (12.5%)
α-smooth actin	15/37 (40%)	5/13 (38.5%)	4/16 (25%)
CD44H	23/37 (62%)	11/13 (84.5%)	11/16 (68.7%)

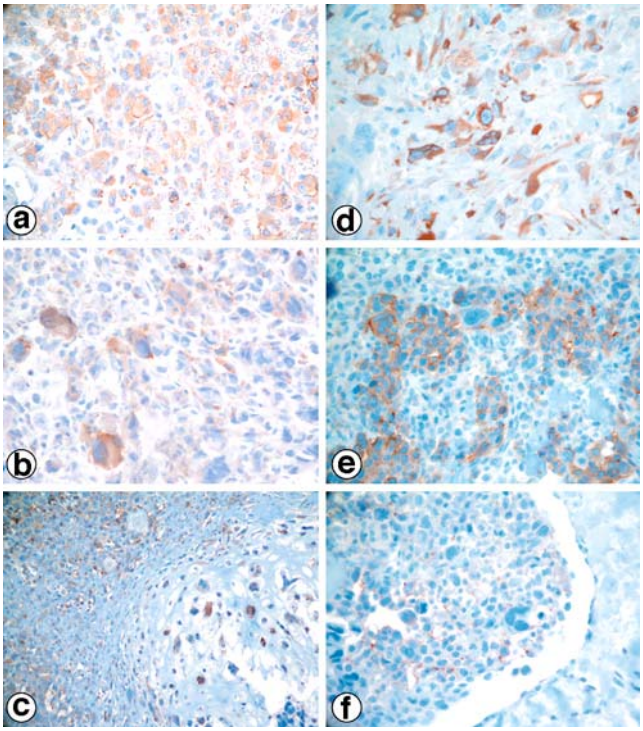


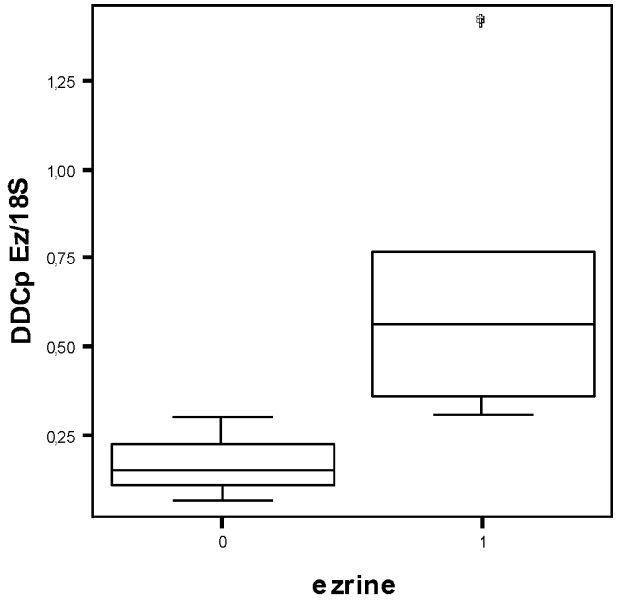
Fig. 1 **a** Ezrin immunostaining in an osteoblastic osteosarcoma: membranous and cytoplasmic staining of 90% of cells, ×40. **b** Ezrin cytoplasmic immunostaining in atypical multinucleated and mononucleated cells of a telangiectatic osteosarcoma, ×40. **c** Ezrin immunostaining in both osteoblastic (*left*) and chondroblastic (*right*) areas, ×40. **d** Ezrin immunostaining in a lung metastasis, ×40. **e** Ezrin immunostaining in a cerebral metastasis, ×40. **f** Membranous ezrin expression in tumour cells in a vessel, ×40

Among patients for whom biopsy and metastasis were available (seven patients), four had ezrin expression in both biopsy and metastasis. In one case, only metastasis was positive. In two cases, biopsy was positive but metastasis was not. CD44H was expressed in both biopsy and metastasis in all cases. For α-smooth muscle actin, only one case showed positivity in biopsy and metastasis.

By QPCR, we found in the four samples of metastases tested a mean level of Ezrin mRNA of 5.95±0.2. All samples were positive by IHC.

Ezrin, CD44H and α-smooth muscle actin expression and patient outcome

The two patients who had metastases at the time of diagnostic showed positivity for both ezrin and CD44H on prechemotherapy incisional biopsies. In one patient, α-



Real-time quantitative RT-PCR (QPCR)

Fig. 2 *Box plot figure* mRNA level of ezrin in negative (*0*) and positive (*1*) biopsies by immunohistochemistry

smooth muscle actin was also positive. Among the 11 patients who presented with metastases during the follow up, nine showed positivity for ezrin in the biopsy and ten for CD44H. For α-smooth muscle actin, the biopsy was positive in 6 of 11 patients. Interestingly, in two biopsies, tumour vascular invasion was present, and positivity for ezrin (Fig. 1f), CD44H and α-smooth muscle actin in tumour cells was seen.

No significant statistical correlation was found between IHC parameters scored as positive/negative or as a continuous variable and the onset of metastasis (for ezrin, p=0.183; for α-smooth muscle actin p=0.851; and for CD44H p=0.658).

No statistical correlation was found between the response to chemotherapy and IHC parameters (for ezrin, p=0.57; for α-smooth muscle actin, p=0.12; for CD44H p=0.79).

In Cox analysis, ezrin (Fig. 3a) was the only prognostic marker for event-free survival (p<0.001, relative risk RR= 1.070; 95 CI [1.033–1.108]), adjusted for the presence of initial metastasis (p=0.06), the preoperative chemotherapy response (p=0.28), the α-smooth muscle actin expression (p=0.77) and the interaction between ezrin and α-smooth muscle actin expressions (p=0.08). Ezrin (Fig. 3b) and α-smooth muscle actin were prognostic markers for overall survival in Cox analysis with p values being, respectively: 0.003 and 0.024 with RR=1.071 (95% CI: 1.024–1.120) and RR=2.547 (95% CI: 1.131–5.735), adjusted for the presence of initial metastasis (p=0.2), the preoperative chemotherapy response (p=0.16) and the interaction between ezrin and α-smooth muscle actin expression (p=0.02).

Discussion

The cytoskeleton linker protein ezrin was initially found in osteosarcoma cell lines and more recently in osteosarcoma specimens [13, 14]. In our series, 62% of paraffin-embedded samples of conventional osteosarcomas were positive for ezrin, which is higher than what Park et al.[27] and Kim et al. [16] recently reported (respectively, 43 and 51,6%). Ezrin staining was mainly cytoplasmic with sometimes a membranous signal as previously reported

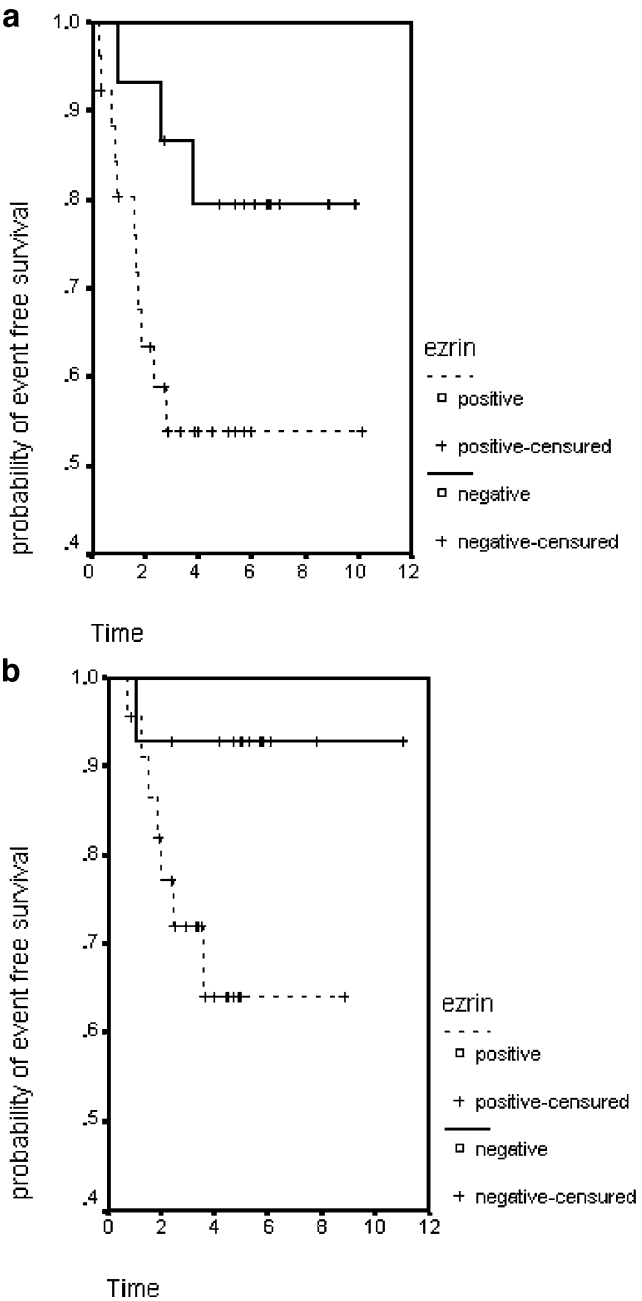


Fig. 3 Survival curves according to ezrin expression by IHC in 37 biopsies of osteosarcoma. **a** Event-free survival, time in years; **b** overall survival, time in years

[41]. Positivity was seen in all histological subtypes: osteoblastic, chondroblastic, fibroblastic and telangiectatic. In contrast to Kim et al., we did not use tissue microarray for IHC. This allowed us to observe ezrin expression in the different histologic components: osteoblastic, chondroblastic or fibroblastic, which often coexist in a same specimen of osteosarcoma.

In the series of Park et al., no ezrin expression was found in central low-grade osteosarcomas, underlining the main difference in prognosis and behaviour between conventional and central low-grade osteosarcomas [27]. In the latter category, metastasis rarely occurs except if multiple recurrence and/or de-differentiation appear. On the other hand, pulmonary metastasis is the most common systemic relapse and the main cause of death in patients with conventional osteosarcomas. Recently, overexpression of ezrin has been correlated with the metastatic potential of several cancers such as melanomas, prostate carcinoma or malignant gastrointestinal stromal tumour (GIST) [12, 17, 26]. Among sarcomas, forced ezrin expression can induce a highly metastatic state in rhabdomyosarcoma cell lines [43]. Ezrin overexpression was also correlated to clinical stage in children with embryonal rhabdomyosarcoma [43]. In adults' high-grade soft tissue sarcomas, ezrin positivity was a predictive factor for metastatic relapse and also an independent prognostic factor for overall survival in multivariate analysis [41]. In a small series of paediatric osteosarcomas (19 cases), the risk of metastatic relapse was 80% greater for patients with high ezrin expression compared to those with low ezrin [14]. In our series, ezrin expression increases during tumour progression, as it was more frequently observed in metastases than in biopsies. However, it was not possible to predict the onset of metastasis on the basis of ezrin expression by IHC on biopsy at the time of diagnosis. This might be explained in part not only because of the size of the cohort and the time of follow up but also because metastasis is a complex progress involving a cascade of linked sequential steps regulated by the activation and/or downregulation of many specific genes. The ezrin gene may be involved in the initiation and/or a transformation of osteosarcoma cells. However, it is unlikely that the ezrin gene alone controls the metastatic behaviour.

In line with a recent study, we found that ezrin expression by IHC was an independent prognostic marker for DFS and overall survival [16]. In particular, no link was found to preoperative chemotherapy, the main prognostic factor in osteosarcoma. This means that molecules and pathways implicated in chemoresistance and metastasis are different. Compared with the histological response to preoperative chemotherapy, ezrin expression is available at the time of diagnostic.

A statistically significant correlation was found between ezrin expression by IHC and mRNA levels assessed by QPCR. Mechanisms of ezrin overexpression are to date unknown. Little or no alteration of the *VIL2* gene copy number detected by comparative genomic hybridisation (CGH) array in malignant high-grade soft tissue sarcomas and by fluorescent in situ hybridization (FISH) in high-grade prostatic intraepithelial neoplasia have been reported [26, 41]. In osteosarcomas, chromosome 6 is not frequently altered, and usually deletion of chromosome 6q is reported [5, 25, 35]. Mechanisms explaining increased ezrin expression may be regulatory effect, epigenetic alteration or activating chromosomal mutation.

For the first time, we report ezrin expression by IHC on resected tumour after chemotherapy in "poor responders". Only two patients had positive cells for ezrin. Although no definite conclusion can be drawn, as prolonged decalcification of the bone can alter antigenicity, a real effect of chemotherapy cannot be excluded. The two patients had a poor prognosis. One patient had a metastasis at the time of diagnosis and died within 10 months. The other patient developed lung metastases 20 months after diagnosis. Positivity for ezrin for both biopsy and residual tumour after chemotherapy seems highly indicative of metastasis.

Concerning CD44H, we found that 62% of the biopsies were positive, which is in line with the literature data (range of positivity between 50 to 85% [18, 41]). However, no correlation was found with metastasis or survival rate in our cohort. Prognostic value of CD44 remains controversial in the literature. Peng et al. [28] found a higher rate of positivity for CD44H in poorly differentiated osteosarcoma variants and those with lung metastasis. Other studies have found a prognostic value only for V5 [15] or V6 variants [18].

Previous studies have shown that nearly 50% of osteosarcomas of various histological subtypes were positive for α-smooth muscle actin [10, 39]. Yamamura et al. [42] have reported the expression by RT-PCR analysis of several smooth muscle differentiation-specific genes, calponin, SM22α, caldesmon and α-actin, in clonal osteosarcoma cell lines and osteosarcomas tissues suggesting a lineage relationship between osteogenic and smooth muscle cells. Moreover, survival rate was found to increase in a group of patients whose tumour cells had basic calponin expression while no prognostic value was found for the other proteins. The mechanism proposed was related to the inhibitory action of calponin on actin-myosin interaction that promotes migration and cytokinesis of tumour cells. In contrast, we have found an unfavourable prognostic value for α-smooth muscle actin for overall survival in multivariate analysis. Maybe osteosarcomas expressing α-smooth muscle actin are a subgroup with a different cell of origin and a different prognostic value. Interestingly, α-smooth muscle actin expression by IHC was not modified by preoperative chemotherapy. Poor prognosis is usually observed in primitive bone sarcomas with more advanced

smooth muscle differentiation such as leiomyosarcomas, which are resistant to chemotherapy [1]. Further studies are required to confirm that expression of smooth muscle proteins could generate different prognostic subgroups of patients with an osteosarcoma.

In conclusion, we have shown that ezrin is expressed in the different histological subtypes of conventional osteosarcomas. Ezrin was an independent prognostic factor for event-free and overall survival rate in multivariate analysis. These data confirm the role of ezrin signalling pathway for tumour dissemination in osteosarcomas in vivo. This also might be of interest for therapeutic strategy to select patient for dose intensification or to use new anticancer agents such as rapamycin, which reduces experimental lung metastases through an ezrin-related pathway [38]. Further prospective studies are required to confirm the prognostic value of ezrin in a large cohort of osteosarcomas to confirm its usefulness in clinical practice. The pattern of ezrin expression in other bone and cartilage forming tumours would also be interesting to investigate searching for a putative diagnostic value for this marker (manuscript in preparation).

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ORIGINAL ARTICLE

Actinomycosis of the jaws—histopathological study of 45 patients shows significant involvement in bisphosphonate-associated osteonecrosis and infected osteoradionecrosis

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Abstract Actinomycosis of the jaws is a rare disease, which has been recently described in patients with infected osteoradionecrosis (IORN) and bisphosphonate-associated osteonecrosis (BON). We investigated our archive material for Actinomycosis of the jaws with special regard to underlying disease. Out of a total number of 45 patients with Actinomycosis, 43 (93.5%) suffered from BON (58.7%) or IORN (35.6%), while there were only 3 patients (6.7%) without anti-tumor treatment. In all cases, we found direct association of *Actinomyces* colonies with bone; in the surrounding medullary space, mixed inflammatory infiltrates with variable amounts of osteoclasts were a typical finding. Pseudoepitheliomatous hyperplasia occurred in 60.9% of patients. Cell-rich vessel obliteration was seen in less than 25.9% of BON patients, while hyalinized vessel obliteration was obtained in 37.5% of IORN patients. Additionally performed polymerase chain reaction (PCR) on paraffin-embedded and ethylene diamine tetracetic acid (EDTA)-decalcified tissue specimens confirmed the presence of *Actinomyces israelii* in seven of seven cases analyzed. We conclude that Actinomycosis of the jaws is a particular complication in patients with BON and/or IORN. Patients with Actinomycosis of the jaws during or after these forms of anti-cancer therapy are suggested to represent a distinct patient cohort with a relevant impairment of their general condition.

Keywords Actinomycosis · Osteomyelitis · Bisphosphonate-associated osteonecrosis · Infected osteoradionecrosis · Polymerase chain reaction

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Introduction

Actinomycosis is a chronic disease caused by non-spore-forming, anaerobic, or microaerophilic bacterial species of the genus *Actinomyces*. *Actinomyces* species were once believed to be fungi due to their branching filaments, but they are now classified as higher prokaryotic bacteria. *Actinomyces* spp. are Gram-positive, pleomorphic, and commonly delicately filamentous microbes [33]. Characteristically, Actinomycosis is a polymicrobial infection, but the most frequent species is *Actinomyces israelii* [32]. By means of histology, *Actinomyces* spp. form typical colonies with Splendore–Hoeppli phenomenon [30, 33]. The latter is described by eosinophilic, pseudomycotic structures composed of necrotic debris and immunoglobulin [28]. The most common form of Actinomycosis is oral-cervicofacial disease. Generally, the disorder presents as a soft tissue induration with frequent abscess formation and multiple fistulae, while genuine actinomycotic osteomyelitis of the

jaws is rarely seen [6, 30]. However, there is increasing evidence from several recent reports that *Actinomyces* osteomyelitis comprises a crucial complication in patients treated for different malignant tumors [3, 15, 17, 20, 23, 25, 36].

The first group with this observation consisted of patients who were treated by radiotherapy due to cancer of the head and neck region. A variable percentage of these patients present with a serious complication of radiotherapy, which is called infected osteoradionecrosis (IORN). Several case reports emphasize a close relationship between Actinomycosis osteomyelitis and IORN [3, 15, 19, 34]. Støre et al. [36] demonstrated DNA of several *Actinomyces* spp., especially *A. israelii* in 10 of 11 tissue probes of patients suffering from IORN. In their investigation, the biopsy material was harvested from the depth of the bone tissue specimens to rule out a superficial contamination effect from the oral microflora. Furthermore, in a recent histological study with 31 IORN patients, we showed that about two thirds of IORN patients (20/31) carried *Actinomyces* in the affected bone tissue, rendering it likely that infection by this microbe was a more frequent problem than previously believed [11].

The second group with a striking occurrence of *Actinomyces* osteomyelitis during anti-cancer therapy comprised the patients suffering from bisphosphonate-associated osteonecrosis (BON). Bisphosphonates are synthetic analogs of the naturally occurring pyrophosphate, and they principally lead to a decrease of bone resorption. Therefore, they are nowadays an important part of the therapy for patients with osseous tumors such as multiple myeloma or bone metastases [7, 16, 27, 29]. Besides these malignant disorders, bisphosphonates are also used in the therapy of osteoporosis and M. Paget. In addition, BON is reported in these patients group. However, following the published data, the frequency of BON is lower as compared with patients suffering from malignant tumors [1, 21]. In contrast to numerous detailed clinical studies, there are only a few histological analyses addressing BON. However, *Actinomyces* was consistently detected in a significant percentage of BON [2, 12, 20–23]. Based on our hitherto examined histological archive material, *Actinomyces* occurs in more than 90% of BON patients (data not published). Besides these two patient groups, there are only a very few reports on patients treated with other forms of anti-cancer treatment (such as chemotherapy) giving an association with *Actinomyces* [2, 17].

In the present study, we investigated the clinicopathological features of patients with Actinomycosis of the jaws with special regard to underlying anti-cancer therapy. Additionally, we complete previous molecular pathological studies [11] performing polymerase chain reaction (PCR) for *A. israelii* on formalin-fixed and paraffin-embedded bone tissues with emphasis on the decalcification procedure.

Materials and methods

Patient data

The records from the archives of the Institute of Pathology (Mainz) from January 2002 to January 2007 were searched for cases encoded as "osteomyelitis of the jaws" and "*Actinomyces*". For definition of Actinomycosis of the jaws, only these cases were accepted, which met the following criteria:

–Actinomyces colonies were confirmed by classical histological morphology, i.e., they exhibited the so-called Drusen with the classical Splendore–Hoeppli phenomenon; alternatively, they were confirmed by at least one of the known special staining techniques for *Actinomyces* (i.e., periodic acid Schiff (PAS), Gram, or Grocott reaction).
–They revealed at least in one focus (means high power field (HPF)=0.306 mm^2) a direct contact with the bone tissue.
–They contained at least one focus with inflammatory tissue.

Using these criteria, we found a total number of 45 cases. They could be divided into three groups: I, bisphosphonate-associated osteonecrosis (BON group); II, infected osteoradionecrosis (IORN group); and III, no specific underlying disease (NOS group). Patient data are summarized in Table 1. The subgroups are briefly described in the following paragraphs. It should be mentioned that several of the patients belonging to group I or II were already described in previous studies [11–14].

The BON group consisted of 26 patients (58.7%; 13 men and 13 women, median age: 67 years). They were treated with bisphosphonates due to bone metastases or osseous involvement of the malignant tumor. The primary neoplasia was multiple myeloma in ten cases, breast carcinoma in nine patients, prostate carcinoma in six cases, and non-Hodgkin lymphoma in one patient. Most patients received zoledronate (Zometa, Novartis, Nürnberg, Germany), followed by treatment with pamidronate (Aredia, Novartis). Patients presented with necrotic bone exposed to the oral cavity. The extent of bone destruction was assessed by standard radiographs or computed tomography. The osteonecrosis occurred in the mandible in 13 patients (50%) and in the maxilla in 7 patients (27%), while 6 patients (23%) presented with osseous lesions in both parts of the jaws.

The IORN group included 16 patients (35.6%, 15 men and 1 woman, median age, 60.7 years). These patients were treated by external radiotherapy due to cancer of the head and neck region. Underlying malignancy was squamous cell carcinoma except of one patient (no. 34), who suffered from an adenoid cystic carcinoma. IORN was defined as

Table 1 Summary of the clinical and morphological findings

Patient no.	Age	Sex	Cause	Tumor	Site	Tooth extraction	PH	Bone	Vessels
1	59	F	BON	BC	Md	Yes	No	Patchy	–
2	65	M	BON	MM	Md and Mx	Yes	Yes	Patchy	Obl
3	65	F	BON	NHL	Mx	ND	No	Patchy	–
4	74	M	BON	MM	Md	Yes	Yes	Patchy	Obl
5	69	M	BON	PC	Md	Yes	Yes	Patchy	–
6	66	M	BON	PC	Mx	No	No	Patchy	–
7	72	F	BON	BC	Md and Mx	Yes	Yes	Patchy	-
8	60	M	BON	MM	Md	No	Yes	Complete	-
9	62	M	BON	PC	Mx	Yes	Yes	Patchy	Obl
10	62	F	BON	BC	Mx	Yes	No	Complete	–
11	74	M	BON	MM	Mx	Yes	No	Patchy	–
12	66	F	BON	BC	Md	Yes	Yes	Patchy	–
13	54	M	BON	MM	Md	Yes	No	Patchy	Obl
			BON-R (6)	MM	Md and Mx	No	No	Complete	–
14	69	F	BON	BC	Mx	Yes	No	Patchy	–
			BON-R (4)	BC	Md and Mx	No	Yes	Patchy	-
15	67	M	BON	PC	Mx	Yes	No	Patchy	–
16	74	M	BON	PC	Md	Yes	Yes	Patchy	Obl
17	55	F	BON	BC	Mx	ND	Yes	Patchy	–
	56		BON-R (3)	BC	Md	No	Yes	Patchy	–
			BON-R (1)	BC	Mx	Yes	Yes	Patchy	–
18	82	F	BON	BC	Md	ND	Yes	Patchy	Obl
			BON-R (6)	BC	Md	ND	Yes	Complete	–
19	75	F	BON	BC	Md	Yes	Yes	Patchy	–
			BON-R (4)	BC	Md	No	No	Patchy	–
20	58	M	BON	MM	Md	ND	Yes	Patchy	–
21	84	F	BON	MM	Md	ND	No	Complete	–
22	44	M	BON	MM	Md	Yes	No	Complete	–
23	71	F	BON	MM	Mx	Yes	Yes	Patchy	Obl
	73	F	BON-R (22)	MM	Mx	No	Yes	Patchy	–
24	80	M	BON	PC	Md	Yes	Yes	Complete	–
25	72	F	BON	MM	Md	ND	Yes	Complete	–
			BON-R (2)	MM	Md and Mx	ND	Yes	Complete	–
26	62	F	BON	BC	Md	Yes	No	Patchy	–
27	37	M	NOS	No	Md	ND	No	Complete	–
28	69	F	NOS	No	Md	ND	Yes	Patchy	–
29	50	M	NOS	No	Md	Yes	Yes	Patchy	–
30	77	F	IORN	ND	Md	No	No	Patchy	Obl
31	72	M	IORN	PECA	Md	No	Yes	Patchy	–
32	48	M	IORN	PECA	Md	No	Yes	Patchy	Obl
33	57	M	IORN	ACC	Md	Yes	No	Complete	-
34	65	M	IORN	PECA	Md	No	Yes	Complete	-
35	46	M	IORN	PECA	ND	Yes	No	Complete	Obl
36	63	M	IORN	PECA	Md	No	Yes	Patchy	–
37	57	M	IORN	PECA	Md	No	Yes	Complete	Obl
38	49	M	IORN	PECA	Md	No	Yes	Patchy	–
39	59	M	IORN	PECA	Md	No	No	Complete	–
40	47	M	IORN	PECA	Md	Yes	No	Complete	Obl
41	55	M	IORN	PECA	Md	No	No	Complete	–
42	77	M	IORN	PECA	ND	No	Yes	Complete	–
43	66	M	IORN	PECA	ND	Yes	No	Complete	Obl
44	68	M	IORN	PECA	ND	Yes	No	Complete	–
45	65	M	IORN	ND	Md	No	Yes	Patchy	–

Abbreviations: *ACC* adenoid cystic carcinoma; *BC* breast cancer; *BON* bisphosphonate-associated osteonecrosis; *BON-R* recurrence of BON (duration time between primary and next event given in months); *F* female; *IORN* infected osteoradionecrosis; *M* male; *MM* multiple myeloma; *ND* no data; *obl* obliterated vessel; *PC* prostate cancer; *PH* pseudoepitheliomatous hyperplasia; *SCC* squamous cell carcinoma

bone necrosis after irradiation of the jaws with clinical signs of infection either as exposure of necrotic bone, inflammatory infiltration, and fistula formation or gross bone sequestration. Bone necrosis was again confirmed by radiography.

NOS group consisted of three patients (6.6%, two men and one woman, median age: 52 years). Underlying systemic disease could not be determined in these cases. Especially, there was no malignant tumor or immunosuppressive therapy. Patients suffered from exposed or sequestrated bone. Osteonecrosis was also demonstrated by radiography.

Histological preparation

In most cases, biopsies or resection specimens were taken both from the mucosal site and the affected bone tissue. Tissue specimens were fixed in 4% phosphate-buffered saline (PBS)-buffered formalin and processed according to standard protocols. In the case of molecular studies, decalcification was performed by ethylenediaminetetraacetic acid (EDTA; 48–96 h). In the other cases, bone tissue was decalcified by trichloric acetic acid for 24–72 h. The specimens were embedded in paraffin, and 4-μm slides were cut. Besides standard histology by hematoxylin and eosin, slides were stained for the Gram, PAS, and Grocott reaction to detect *Actinomyces* colonies. Bone tissue structure was further analyzed by either Goldner or Elastica-van Gieson staining. All histochemical special stains were performed according to previous protocols [8].

Slides were studied and photographed with a Zeiss microscope (type Axiophot, Germany; digital camera type Camedia, Olympus, Hamburg, Germany).

Furthermore, we performed scanning electron microscopy (SEM) of the paraffin-embedded tissue specimens of a total number of five patients (nos. 2, 11, 16, 36, and 45). In brief, dewaxed sections were incubated in 100% ethanol for about 12 h at room temperature (RT). After treatment with hexamethyldisilazane (Polysciences, Warrington, PA, USA), slides were air-dried for about 1 h. Sections were then mounted on metal slide (Plano, Wetzlar, Germany). Slides were sputtered with gold (Sputter Coater S150B; Edwards, Crawley, West Sussex, UK). Finally, slides were microscoped with a scanning electron microscope type DSM 962 (Zeiss).

Molecular testing

For PCR testing, DNA was extracted from formalin-fixed and EDTA-decalcified paraffin-embedded tissues of seven patients according to standard protocols [31]. *Actinomyces*-colonized tonsillar tissue served as positive control, while specimens of acutely inflamed appendicitis were used as negative control. For detection of *A. israelii*, a semi-nested PCR approach was designed targeting the 16S ribosomal RNA gene (GenBank accession no. X82450.1). The procedure was performed as previously described [11].

Results

Bone tissue specimens showed variable amounts of *Actinomyces*. In the case of major resection of the mandibular ramus, large areas of bone marrow spaces and surrounding osseous tissue were colonized by these microbes (Fig. 1). In these regions, bacteria tended to occupy the medullar spaces and showed a close proximity to the bone matrix (Fig. 2). *Actinomyces* was mainly attached to the bone in the absence of inflammatory cells (Fig. 2). In 3 of 45 cases, we additionally found fungi forming non-septated hyphae and spores, which therefore most likely belong to the *Candida* family (Fig. 3). In contrast to *Actinomyces*, mycotic filaments were always broader and showed a double-linear lining (Fig. 3). Furthermore, they were negative in the Gram stain.

Pseudoepitheliomatous hyperplasia was a found in more than the half of the cohort (28/45 patients, 62.2%). It was encountered in a similar percentage in BON patients (59.3%) and in IORN patients (50%). In the NOS group, it was found in two out of three patients. Including the recurrences and excluding the patients without respective data available, pseudoepitheliomatous hyperplasia was found in 12 cases out of a total number of 24 tooth extractions (50%), respectively, while we found the same percentage revealing pseudoepitheliomatous hyperplasia without tooth extraction in the patients' history. In addition,

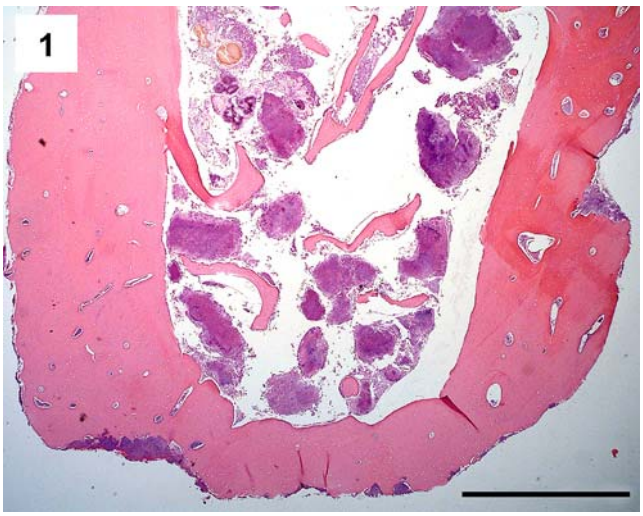

Fig. 1 Histological overview shows the ramus of the mandible occupied with numerous *Actinomyces* colonies (patient 36; HE, *bar*=1 mm)

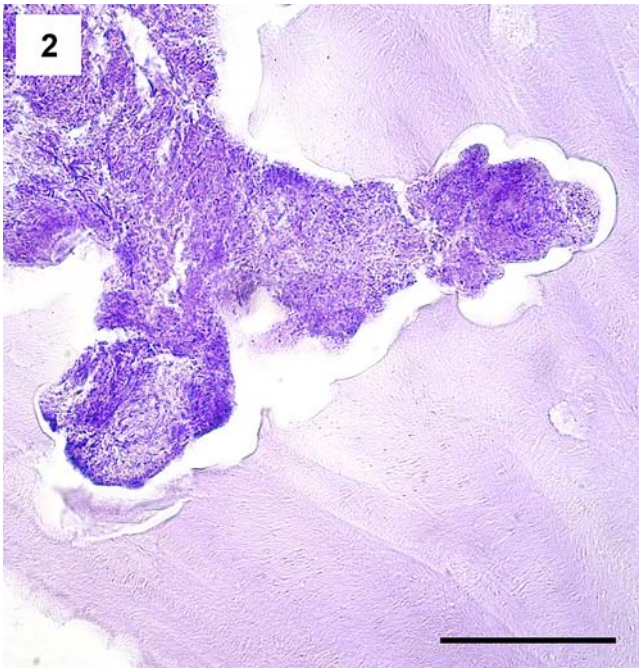

Fig. 2 Gram staining reveals *Actinomyces* colonies that are directly adjacent to the bone tissue (patient 12; Gram, *bar*=50 μm)

we found 17 of 41 cases (41.5%) without tooth extraction in the patients' history, and the large majority of this cohort (12/17; 70.6%) also revealed pseudoepitheliomatous hyperplasia. Sometimes, *Actinomyces* colonies were found between the epithelial lining and the bone tissue (Fig. 4). In one patient (no. 14) we also found a fistula reaching from the epithelial lining of the oral mucosa to the bone. It seems remarkable that, at the bottom of the fistula, *Actinomyces* could be observed in direct contact with bone tissue (Fig. 5), while the fistula itself and the surrounding soft tissues were not directly affected by this microbe. This was consistent with the SEM findings. Using this technique, we found colonies of long and short rods in direct

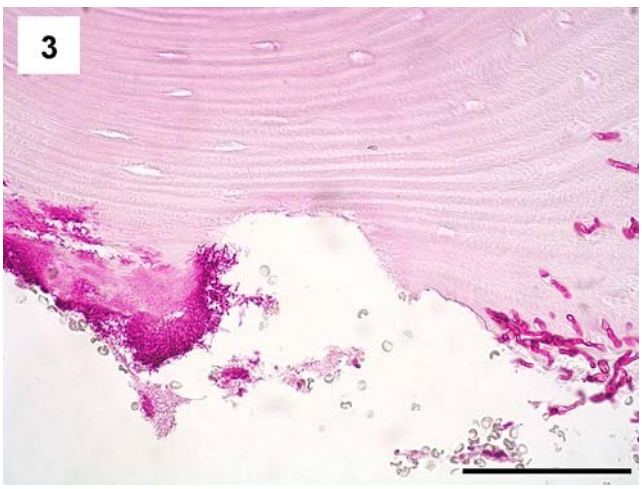

Fig. 3 PAS staining shows *Actinomyces* colonies on the *left side* and fungal spores and hyphae on the *right side* (patient 34; *bar*=50 μm)

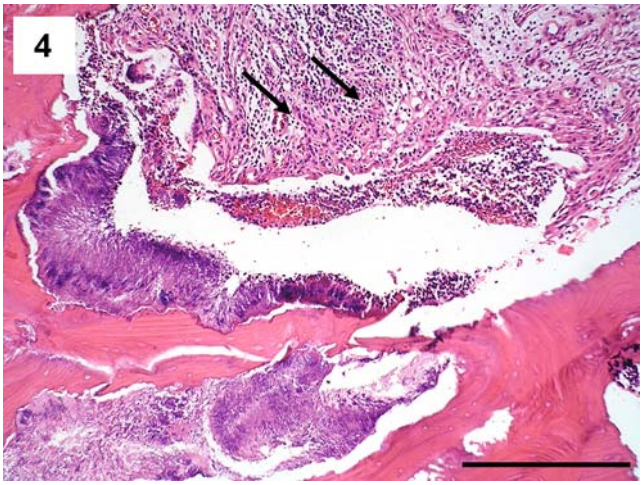

Fig. 4 *Actinomyces* colonies are closely attached to bone. Note that, in this area, there is no inflammatory infiltrate. In the neighborhood, pseudoepitheliomatous hyperplasia is found (*arrows*) surrounded by mixed inflammatory infiltrates (patient 2; HE, *bar*=200 μm)

contact with bone tissue in both BON and IORN cases (Figs. 6 and 7).

Inflammatory infiltrates mainly consisted of variable amounts of predominantly two cell types, namely, neutrophilic granulocytes and plasma cells. The latter revealed sometimes binucleated forms and little variability of the nuclear size; however, signs of atypia were never found. Furthermore, in the case of MM patients, light chain restriction was excluded by additional immunohistochemistry (data not shown). Recurrence of the underlying malignancy was only found in one patient (no. 34) with an adenoid cystic carcinoma. While in BON inflammatory infiltrates were more cellular, there were typically areas of hypocellular fibrosis in the IORN group.

Apart from completely devitalized bone within the genuine sequestration area, BON and IORN showed different patterns of bone necrosis. In BON, necrotic areas characterized by empty osteocyte lacunae were interspersed with bone containing viable osteocytes leading to a non-homogeneous and patchy appearance of bone necrosis. By contrast, uniform osteonecrosis with large areas of empty osteocyte lacunae was typical in the case of IORN. Obliterated vessels were infrequently seen in both BON

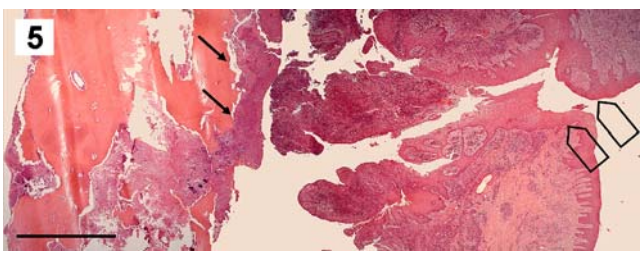

Fig. 5 Histological overview shows a fistula that reaches from the epithelial lining (*arrowheads*) to the bone tissue (*arrows*), here with numerous *Actinomyces* colonies (patient 14; HE, *bar*=1 mm)

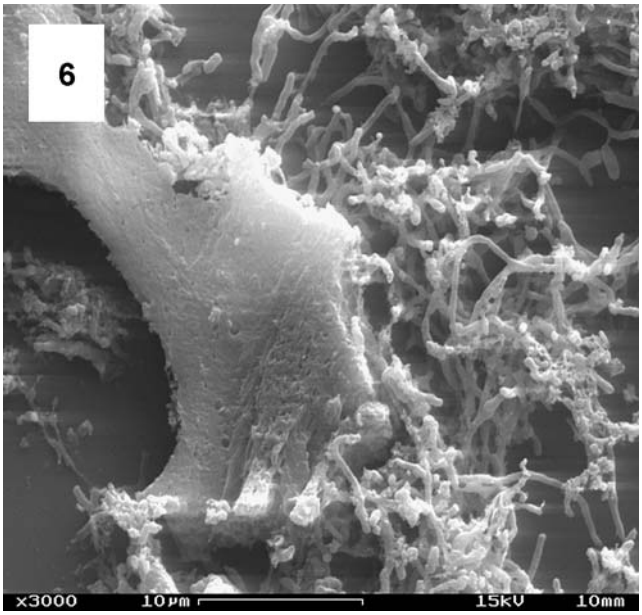

Fig. 6 SEM reveals numerous rods forming microbial colonies, which are directly attached to the bone (*arrow*; patient 45, *bar*=10 µm)

and IORN group. They occurred in 7 of 27 BON patients (25.9%) and in 6 of 16 IORN patients (37.5%). While vessel obliteration was predominantly cell-rich in the case of BON (Fig. 8), it was typically cell-poor in the case of IORN. Furthermore, hyalinization of the vessel wall and the surrounding tissue was a typical feature in the latter group (Fig. 9).

Furthermore, tissue probes of seven patients were subjected to PCR method for the presence of *A. israelii* specific DNA sequences as previously described. A single PCR product could be obtained from six of seven cases after first PCR testing resulting in a 237-bp product. In particular, all patients revealed positive results by nested PCR testing exhibiting a 170-bp product (Fig. 10).

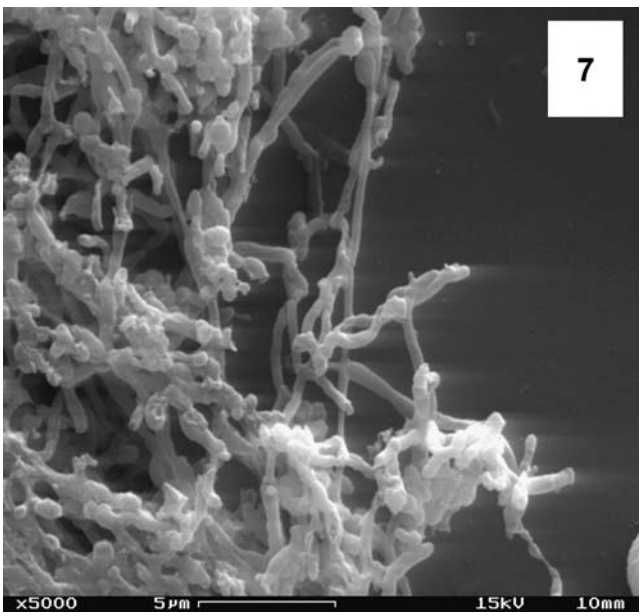

Fig. 7 Detailed view of the bacterial colony (patient 45, *bar*=5 µm)

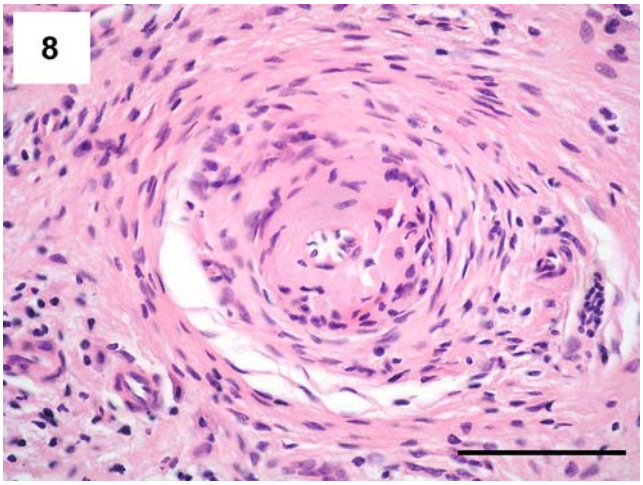

Fig. 8 Obliterated vessel in a BON patient demonstrates cell-rich infiltrate of spindle-shaped cells in the media and intima (patient 18; HE, *bar*=50 µm)

Discussion

In the present study, we analyzed patients suffering from Actinomycosis of the jaws. The large majority (93.4%) of these patients was either treated by radiotherapy or by bisphosphonates because of malignant tumors, while only three patients revealed Actinomycosis without primary neoplasia (6.6%). This remarkable relationship could be explained by the fact that tumor patients generally are closely observed during the clinical follow-up control of the anti-tumor therapy, while this might not be the case in the sporadic Actinomycoses. Nevertheless, these findings em-

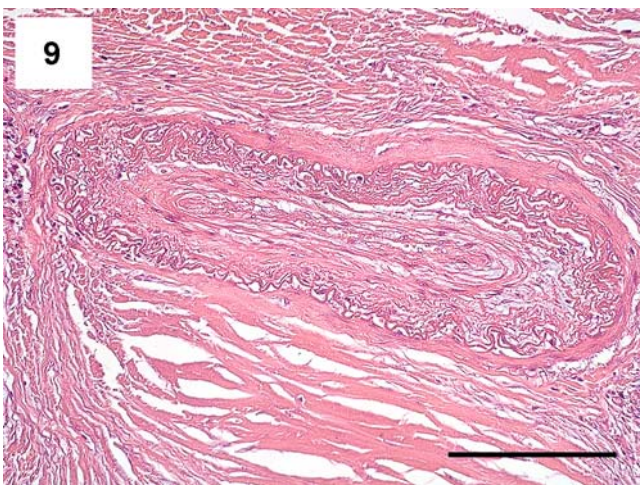

Fig. 9 Obliterated vessel in an IORN patient shows hyalinization and a hypocellular wall. Note that the surrounding stroma shows fibrosis (patient 38; HE, *bar*=100 µm)

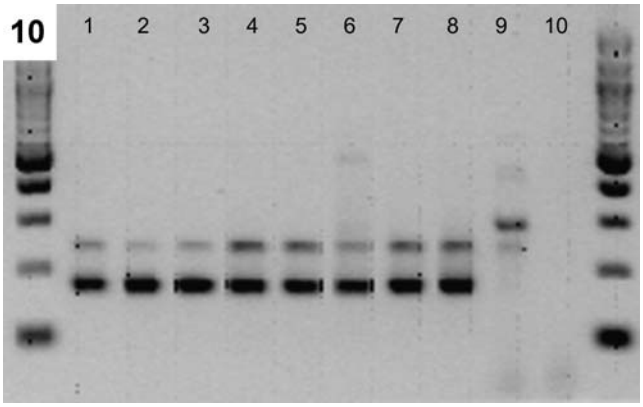

Fig. 10 Results of the semi-nested PCR testing using the first ACM primer and the second ACM primer revealing a 170-bp product. All patients are positive; no cross-amplification is seen. The numbers encode for the following tissues: *1* positive control (tonsillitis); *2–5* BON patients (nos. 10, 16, 3, and 15); *6* IORN case (no. 46); *7* BON case (no. 11); *8* IORN case (no. 38); *9* negative control (appendicitis); *10* water control

phasize the role of Actinomycosis of the jaws as a specific complication in patients with anti-tumor treatment. It is worthy to note that particularly this microbe type, but no other forms of bacteria or fungi (such as *Candida*), is histologically detected in such a large proportion. Concerning the morphological presentation of this lesion, all patients show similar histological features regardless of the primary disorder: First, they show direct association of *Actinomyces* colonies with bone; second, in the surrounding medullary space, mixed inflammatory infiltrates occur with variable amounts of osteoclasts; and third, pseudoepitheliomatous hyperplasia is present in up to 60% of the patient cohort.

Although each of these features has been described in *Actinomyces* of the jaws before, the increased frequency and their combination has not been addressed in such a *large proportion* of patients. Thus, pseudoepitheliomatous hyperplasia of the mandible has been investigated by Warter et al. [38] in three patients suffering from mandibular osteomyelitis, which also was associated with the occurrence of *Actinomyces*. Besides the mandible, we also observed pseudoepitheliomatous hyperplasia in Actinomycosis involving the maxillary bone, which has not been described so far. Furthermore, it should be emphasized that pseudoepitheliomatous hyperplasia is uncommon in the other forms of chronic osteomyelitis [38] and is strongly associated with the occurrence of fistula.

Actinomyces can be commonly found in gingivodental crevices [33]. Moreover, it is well known that the initial step in the pathogenesis of Actinomycosis is disruption of the mucosal barrier, which leads to the implantation into damaged tissue [30, 33]. One cause of this mucosal defect may be tooth extraction, which is frequently seen in both BON and IORN [5, 22, 24, 39] and which was found in about 50% of our patients. We found pseudoepitheliomatous hyperplasia in half of the cases with tooth extractions. On the other hand, in BON and in IORN, osteomyelitis has been shown in several cases without dental extractions [23, 35, 36] suggesting that superficial trauma is not the only portal of entrance for infectious agents. In fact, we found a number of cases without tooth extraction in the patients' history. Interestingly, the large majority of this cohort (70.6%) also revealed pseudoepitheliomatous hyperplasia. Thus, other causes of mucosal defects, like endodontal and periodontal infection [35, 36], have to be considered. Taken together, we suggest that pseudoepitheliomatous hyperplasia is a relatively common feature that reflects the mucosal disruption in Actinomycosis. Regarding the clinical context, we confirm previous data that dental extraction may be an important but not the only cause of mucosal disruption.

The close contact between the bacterial colonies and the bone tissue could be additionally confirmed by SEM. Using this technique, *Actinomyces* colonies show numerous rods, which are in close apposition. In the case of IORN, SEM was previously applied by Støre and Olsen [37]. Microorganisms could be detected in 64% of the patients, again strongly suggesting an association between microorganisms and osteoradionecrosis [37]. In general, tissue probes for SEM are not embedded in paraffin, as presented in our study. However, a few authors have previously performed this modified SEM method as well [18]. The advantage of this method is reflected by the fact that both light microscopical studies with immunohistochemistry and electron microscopical analysis can be performed on the same tissue specimen. In particular, areas of interest can be evaluated by light microscopy first, and afterwards, they can be studied by SEM, even in the case of osseous tissue. The sections are thick enough to perform three-dimensional analysis of the tissue specimens, and thus, information can be obtained concerning the structural relationship between the bacteria and the surrounding tissue.

Actinomycosis is generally considered to present as polymicrobial infection in most cases [30]. For evaluation of the relevant microbes affecting osteomyelitis of the jaws, molecular testing is a suitable method. In a previous study, we evaluated PCR for *A. israelii* in bone tissue specimens, which were decalcified in trichloric acetic acid and therefore revealed a remarkable reduction of sensitivity. Out of a number of 14 probes, which were histologically positive for Actinomyces, we found only three cases that showed positive signals by testing for the housekeeping gene β-actin and for *A. israelii* [11]. In the present study, we confirmed that PCR analysis of relevant infectious agents (*A. israelii* in our case) results in a higher sensitivity if milder decalcification, such as EDTA, is applied. Thus, even bone biopsies primarily used for histology can be used for this molecular approach.

Since the first histological descriptions of BON, the question arose as to whether it is possible to differentiate between BON and IORN by means of histomorphology. Vessel obliteration has been described to occur in both IORN and BON. As this vessel lesion is considered to play a crucial role for the development of avascular bone necrosis, it is suggested that it comprises a major feature of both disorders [4, 10, 22]. Moreover, the association of anti-cancer therapy and osteonecrosis due to vessel obliteration might explain why the overwhelming majority of osteomyelitis of the jaws occurs in BON and IORN but not during other forms of anti-tumor treatment. In a previous histological study, we demonstrated that in IORN, the wall of an obliterated vessel was hyalinized and obtained only few viable cells, while numerous spindle cells narrowing the vessel lumen was a feature encountered in BON [12]. However, on analyzing a larger patient group in the present study, we found obliteration of vessels in less than 30% of BON patients and in less than 40% of IORN patients. Therefore, one has to accept that in the majority of tissue specimens tested, the typical vascular features of BON and/or IORN are absent. Another feature that might help in the differential diagnosis is the pattern of bone necrosis. While in BON osteonecrosis tended to reveal a patchy pattern, bone tissue was rather completely necrotic in the case of IORN. However, the morphological differentiation can be difficult if only small bone biopsies or bone *sequestrae* are studied.

If one bears in mind that the underlying malignancy and the subsequent treatment, particularly in the case of radiotherapy, lead to suppression of the immune response, accordingly to other groups of tumor patients [1], Actinomycosis of the jaws in both BON and IORN patients can be interpreted as an opportunistic infection [26]. Moreover, the general condition of these patients is markedly impaired, as this infectious disease needs an additional long-term antibiotic regime and almost always substantial and even repeated surgical treatment [2, 3, 9, 22]. With regard to the patients' clinical course and outcome, it remains to be investigated whether the histological differentiation between IORN and BON is significant or whether a classification according to the *Actinomyces* status regardless of the origin could be of greater benefit.

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ORIGINAL ARTICLE

Comparison of RNA amplification techniques meeting the demands for the expression profiling of clinical cancer samples

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Abstract Available ribonucleic acid (RNA) amplification methods are extensively tested for reproducibility, but only a few studies additionally deal with potential amplification bias. On targeted arrays, we evaluated three amplification protocols, which are less time consuming than the commonly used T7-RNA polymerase based in vitro transcription protocols and therefore may be more suitable for clinical use: Template-switching polymerase chain reaction (PCR), Ribo-single primer isothermal amplification and a random primer-based PCR. Additionally, a more sensitive labelling method, Dendrimer labelling, was evaluated. All methods were compared to unamplified RNA labelled at reverse transcription. From our results, we conclude that RNA amplification with template-switching PCR is highly reproducible and results in a reliable representation of the starting RNA population. We then assessed whether RNA amplification of clinical breast and thyroid cancer samples with template-switching PCR showed robust performance when altered cycle numbers or partially degraded RNA were used. Template-switching PCR proved to be a very reliable method for global RNA amplification, even when starting from partially degraded RNA down to a RNA Integrity Number of 4.3. In conclusion, template-switching PCR amplification promises to help micro-array expression profiling of limited amounts of human samples on its way to a clinical routine.

Keywords RNA amplification techniques · RNA stability · Gene expression chips

Introduction

It is not the expression of a single gene but the statistically secure profile of a set of genes that allows ribonucleic acid (RNA) expression to enter clinical practice. Using micro-array expression profiling, genes enabling tumour detection [19], tumour classification [26] and derivation of prognostic information [30] can be identified. Van't Veer et al. showed a 70-marker signature to be a better single prognostic indicator in breast cancer than currently used histological parameters.

A single cell typically contains roughly 10 pg of total RNA. Yet, micro-array experiments require microgram amounts of complementary deoxyribonucleic acid (cDNA) or complementary RNA. Replicate experiments are mandatory for statistical validation, therefore requiring even higher amounts of starting material. One strategy is to enhance the signal intensity by increasing the signal output per molecule using dendrimer technology [27], radioactive labelling, tyramide signal amplification [8] or amino-allyl labelling [14]. However, for minute amounts of samples, those techniques are not sufficient. Therefore, for micro-arrays

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analysis of samples obtained by needle biopsies, laser capture microscopy, microdissection and flow-sorted methods, the RNA of the starting material has to be amplified. Several exponential and linear RNA amplification methods are reported: (a) template-switching (TS) polymerase chain reaction (PCR) [15, 17, 18, 22], (b) random-primed amplification methods [7, 9, 10], (c) single primer isothermal amplification (Ribo-SPIA) [1, 2, 23], (d) in vitro transcription (IVT) [29] and its extended form with two rounds of IVT [3] and (e) modifications or combinations of the various amplification techniques [5, 24, 31, 34]. Currently, T7 promoter-based IVT with several slight modifications to the original method, first described by van Gelder and Eberwine [29], are used for most micro-arrays, including Affymetrix GeneChips (Affymetrix, Santa Clara, USA). However, IVT is reported to be time consuming and technically demanding. To introduce micro-array-based gene expression profiling of small samples into clinical practice, the amplification method of choice should be quick and easy to perform, potentially automatable and generally robust.

Two decisive features of a RNA amplification method are its reproducibility and its ability to maintain the relative abundance of the starting RNA population, the so called fidelity of amplification. The fidelity indicates whether a bias towards a RNA sub-population was introduced. Most studies focused mainly on reproducibility, whereas fidelity was scarcely investigated. In general, methods are highly reproducible with correlation coefficients of gene expression ratios above 0.95 between replicate experiments. However, correlation coefficients between methods are usually lower than 0.9. To date, a few studies compared more than one technique to unamplified RNA [5, 7, 18, 20, 28, 33], all of them chiefly involving time-consuming IVT protocols.

In this study, we meet the urgent need to comprehensively revalidate overhastily adopted amplification techniques to ensure quality of micro-array results in the future, especially for the clinical routine. We compare fidelity and reproducibility of amplification techniques, which could potentially be used in a routine clinical setting. The best performing techniques, TS-PCR and Ribo-SPIA, retrospectively, were further tested for their use with clinical breast and thyroid cancer samples.

Materials and methods

Isolation of total RNA from clinical samples and cell lines

Total RNA from MCF7 and Hs578T human breast cancer cell lines was extracted using Trizol (Invitrogen, Paisley, UK) followed by a RNeasy mini column clean up (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Primary breast cancer samples from patients 180/03 and 254/03 with oestrogen receptor (ER)-negative and ER-positive invasive ductal mammacarcinoma were chosen for the study. Primary thyroid cancer samples presenting papillary thyroid cancer entities, as well as additional benign nodular goitre were obtained from the University Hospital Vienna. Clinical samples were collected with approval of the local ethics committee. Primary samples were dissected into smaller pieces if necessary and shock frozen in liquid nitrogen. RNA later ice (Ambion, CA, USA) was applied to the samples at least 1 day before RNA isolation and then kept at −80°C. Samples were disrupted in liquid nitrogen with a mortar and pestle-like device (developed in our laboratory). Samples were homogenized using Qiashredder Columns (Qiagen), and total RNA was isolated by an RNeasy mini-column (Qiagen). Total RNA was concentrated by ethanol precipitation and dissolved in appropriate amount of diethylpyrocarbonate-treated distilled water (dH_2O). All total RNA samples were assessed for integrity using the Agilent 2100 BioAnalyzer and quantified using a NanoDrop 1000 spectrophotometer.

Labelling and amplification procedures

Standard protocol—unamplified RNA

Twenty micrograms of total RNA containing 1:90 diluted Spike-RNA from Lucidea Scorecard (Amersham, Vienna, Austria) was mixed with 4 μl 0.5 μg/μl oligo$(dT)_{12–18}$ primer (Invitrogen). dH_2O was added to 15 μl. The mixture was incubated at 70°C for 10 min and chilled on ice. Five microlitres 5× first strand buffer (Invitrogen), 3 μl 0.1M dithiothreitol (DTT), 0.6 μl of a nucleotide mix composed of 25 mM dA/dT/dGTP and 15 mM dCTP (Invitrogen), 3 μl Cy3 or Cy5 dCTP (Amersham, PA53021, PA55021) and 400 U SuperScript II (Invitrogen) were added. Reaction mixture was incubated at 42°C for 2 h and stopped by addition of 15 μl 0.1 N NaOH followed by incubation at 70°C for 15 min and addition of 15 μl 0.1 N HCl. Labelled first-strand cDNA was purified using a DNA-Clean&Concentrator 5-μg column (Zymo Research) according to the manufacturer's instruction. Purified labelled cDNA was dried down with a vacuum centrifuge.

Dendrimer—labelling

In brief, this method starts with a reverse transcription step where a special RT dT primer is used. Then, the cDNA is hybridized to the micro-array. Last, the fluorescent 3DNA reagent hybridizes to the cDNA because it includes a 'capture sequence' that is complementary to a sequence on the 5′ end of the RT primer. The entire procedure using 2 μg of total RNA with 1:90 diluted Spike-RNA from

Lucidea Scorecard (Amersham) was performed using a 3DNA Array 350-kit (Genisphere, Hatfield, USA) as described in Genisphere's Array 350 protocol: http://www.genisphere.com/pdf/array350_10_19_04.pdf. cDNA was concentrated using Millipore Microcon YM-30 centrifugal filter devices. cDNA hybridization was carried out at 60°C using vial 6 hybridization buffer.

Linear amplification by ovation Ribo-SPIA

Ribo-SPIA amplifications used Ovation Aminoallyl RNA Amplification and Labeling Systems kit (Nugen Technologies, San Carlos, USA). Ribo-SPIA is an alternative isothermal linear amplification method. Excess chimeric RNA/DNA primer is added to reverse-transcribed cDNA. As an anti-sense strand is elongated by a DNA polymerase, its RNA part of the primer is digested by RNase H, which allows a new copy of the primer to anneal. A new DNA polymerase displaces the original strand. Numerous repeats of this procedure allow the same cDNA to be processed by many DNA polymerases, resulting in high amplification. All steps were performed according to the manufactures protocol: http://www.nugentechnologies.com/pdfs/Ovation_RNASys_UserGuide.pdf. Amplified cDNA was purified using a DNA-Clean&Concentrator 25-μg column (Zymo research) washing with 80% EtOH instead of wash buffer. Amionallyl cDNA was labelled with Cy Dyes according to Nugen's recommendations using CyDye Post Labeling Reactive Dye Packs (Amersham). Labelled cDNA was purified using a DNA-Clean&Concentrator 5-μg column (Zymo research) washing with 80% EtOH instead of wash buffer. Purified labelled cDNA was dried down with a vacuum centrifuge.

Exponential amplification by random-primed PCR

Random primed PCR was accomplished by the primer extension pre-amplification (PEP) method, initially applied for whole-genome amplification [35]. Total RNA of 100 ng containing 1:90 diluted Spike-RNA from Lucidea Scorecard (Amersham) were transcribed into first-strand cDNA as described for "Standard protocol—unamplified RNA." Reagents were then added as follows: 2 μl $(N)_{10}$ primer (optical density [OD]=125), 6 μl 2 mM deoxyribonucleoside triphosphate (dNTP; Invitrogen), 6 μl F511 10× buffer, 5 U *Taq* polymerase (Invitrogen) and H_2O in a total volume of 60 μl. The following programme was executed on an ABI GeneAmp 2700 thermocycler: 95°C for 5 min followed by 25 cycles of 95°C for 30 sec, 25°C for 5 min, 45°C for 5 min and 72°C for 3 min and ended by incubation at 72°C for 7 min. PCR product was purified, labelled and precipitated exactly the same way as described for the TS-PCR product in "Exponential amplification by TS-PCR."

Exponential amplification by TS-PCR

TS-PCR amplification was performed as previously described by Petalidis et al. [17] using PowerScript RT kit and Advantage 2 PCR Kit (BD Biosiences, CA, USA). Briefly, 50 ng total RNA with 1:90 diluted Spike-RNA from Lucidea Scorecard (Amersham) was mixed with 10 pMol 3′SMART CDS primer IIA, 10 pMol SMART IIA oligonucleotide and H_2O to a total volume of 5 μl. The mixture was denatured at 70°C for 2 min and chilled on ice. For first strand synthesis 2 μl 5× first strand buffer, 1 μl 20 mM DTT, 1 μl 10 mM dNTP and 1 μl PowerScript RT were added and then incubated at 42°C for 1h and chilled on ice to stop the reaction. The following reagents were added to the total first strand reaction mixture: 72 μl H_2O, 10 μl 10× Advantage 2 PCR buffer, 2 μl 10 mM dNTP, 4 μl PCR Primer IIA and 2 μl 50× Advantage 2 PCR polymerase mix. PCR was performed with a ABI GeneAmp 2700 thermocycler with the following settings: 95°C for 1 min and 17 to 26 cycles of 95°C for 10 s, 65°C for 10 s, 68°C for 6 min. PCR product was purified using Qiaquick PCR Purification Kit (Hilden, Qiagen) according to manufacturer's instructions and labelled with Klenow polymerase. Two micrograms of PCR product were mixed with 2 μl $(N)_{10}$ primer (OD=125), 8 μl 10× Klenow buffer (Epicentre, Madison, USA) and dH_2O. The mixture was incubated at 98°C for 5 min and chilled on ice. Next, the following reagents were added: 6.9 μl of a dNTP mix that contained 0.23 mM dA/dT/dGTP and 0.07 mM dCTP, 1.1 μl Cy3- or Cy5 dCTP (Amersham, PA53021, PA55021) and 20U Exo Minus Klenow DNA polymerase (Epicentre) to a total volume of 80 μl. The labelling reaction mixture was incubated at 37°C for 2 h and heated to 95°C for 3 min to stop the reaction. The labelled product was purified and collected by ethanol precipitation.

Array construction and hybridization

Oligonucleotide probes representing 131 sequences (genes of interest with several isoforms, positive and negative controls) and 116 sequences were designed for breast cancer and thyroid cancer micro-array, respectively. All probes are represented in four replicate spots on the array.

Precipitated or dried-down labelled target was resuspended in 20 μl DIGeasy hybridization solution (Roche, Basel, Switzerland). Dissolved labelled target was heated to 99°C for 2 min, followed by prehybridization at 37°C for 10 min. The entire labelled target was applied to the prewarmed micro-arrays, under M-Series Lifter Slips (Erie Scientific, Portsmouth, USA) and incubated at 42°C for 16 h. Hybridized arrays were washed with 1× sodium chloride–sodium citrate (SSC)/0.2% sodium dodecyl sul-

phate (SDS) for 5 min and 0.1× SSC/0.2% SDS for 5 min and then plunged repeatedly in 0.1× SSC. Slides were dried by centrifugation and scanned using an GenePix 4000A (Axon Instruments, Jersey City, USA).

Data analysis

All data analysis was performed in *R* (www.r-project.org) using bioconductor packages *limma* and *geneplotter* [4, 25]. Every chip was scanned twice at different pmt gains, and images were analysed using either GenePix 5.0 or Spot 3.0. Output files from images analysis were then uploaded into a Microarray database (BASE). Using the BASE-plugin *pmt-merging*, data from the two scans were merged to increase the dynamic range of our assays [13]. Raw data was extracted from BASE using SQL and RODBC at the R-prompt.

First, data was background substracted, and the same offset was added to all arrays to remove negative signal. We fitted the loess curve on the calibration controls (spike controls with a known ratio of 1 [$M=\log2(R/G)=0$]) and applied it to the whole array. Correlation between the methods was assessed in a heatmap based on differentially expressed genes (B value>0 from limma topTable). We also introduced concordance-at-the-top (CAT) plots according to Irizarry et al. [6]. We used the repeated dye-swap direct labelling experiments as the gold standard method to which we compared all other methods on the basis of their most regulated genes (topTable). The lines in the plots show the percent overlap in the topTable of an experiment compared to the topTable of the gold standard. Raw data as well as normalized log2 ratios are available at the Gene Expression Omnibus, series entry GSE5588.

Results

Experimental design

To compare the methods under test, we generated two total RNA pools from breast cancer cell lines MCF7 and Hs578T, respectively, which were used for all techniques under test (Fig. 1). Lucidea Scorecard Spike RNA was diluted 1:90 into those total RNA pools for normalization purposes. RNA quality control using Agilent Bioanalyzer showed a RNA integrity number (RIN) of 9.5 for the MCF7 pool and 8.7 for the Hs578T pool. For each method, a replication including a dye-swap hybridization was performed because of its additional ability to detect dye effects. Overall, non-amplifying methods were replicated two times and amplifying methods four times.

Mean yield for Ribo-SPIA amplification from 5 ng total RNA is 4.4 μg, for 19 cycles of TS-PCR from 50 ng, total RNA is 9.0 μg, and for 25 cycles of PEP-PCR from 100 ng, total RNA is 1.9 μg. One to 2.5 μg of the amplification product was labelled, depending on the various labelling efficiencies. For the standard protocol and dendrimer labelling 20 or 2 μg of total RNA were used, respectively. The labelled target was hybridized onto our breast cancer micro-array, which contains 1,040 spots of 131 unique 65-mer oligonucleotides plus Lucidea Scorecard probes (Amersham). Signal intensity data were preprocessed, normalized, and log2 ratios of intensity values between MCF7 and Hs578T were calculated (M-values).

Reproducibility and fidelity

Pearson correlation coefficients between and within methods are listed in Table 1 and summarized in Table 2.

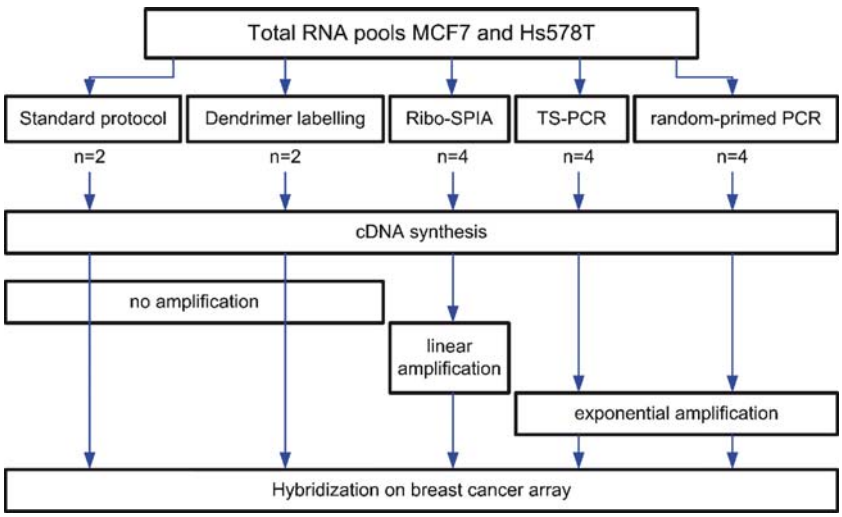

Fig. 1 Experimental design. Flow diagram showing strategy to compare techniques using common total RNA pools from two breast cancer cell lines

Table 1 Detailed correlation matrix showing Pearson correlation between all experiments done with total RNA from breast cancer cell lines

	BC54 direct	BC77 direct	BC79 Dendrimer	BC13 Dendrimer	BC24 RiboSPIA	BC29 RiboSPIA	BC91 RiboSPIA	BC16 RiboSPIA	BC92 tsPCR	BC52 tsPCR	BC90 tsPCR	BC89 tsPCR	BC74 PEP	BC40 PEP	BC62 PEP	BC51 PEP
BC54 direct	1															
BC77 direct	0.930	1														
BC79 Dendrimer	0.910	0.925	1													
BC13 Dendrimer	0.885	0.931	0.940	1												
BC24 RiboSPIA	0.792	0.819	0.798	0.788	1											
BC29 RiboSPIA	0.799	0.836	0.806	0.785	0.938	1										
BC91 RiboSPIA	0.782	0.810	0.788	0.743	0.907	0.895	1									
BC16 RiboSPIA	0.730	0.739	0.724	0.694	0.888	0.890	0.915	1								
BC92 tsPCR	0.918	0.895	0.851	0.832	0.873	0.885	0.864	0.831	1							
BC52 tsPCR	0.914	0.891	0.848	0.804	0.851	0.854	0.853	0.816	0.967	1						
BC90 tsPCR	0.893	0.879	0.842	0.801	0.869	0.883	0.874	0.843	0.971	0.972	1					
BC89 tsPCR	0.879	0.873	0.814	0.781	0.846	0.862	0.843	0.799	0.973	0.959	0.959	1				
BC74 PEP	0.604	0.675	0.593	0.720	0.484	0.498	0.400	0.342	0.576	0.546	0.529	0.535	1			
BC40 PEP	0.851	0.896	0.939	0.900	0.752	0.799	0.749	0.655	0.791	0.788	0.782	0.747	0.644	1		
BC62 PEP	0.655	0.756	0.666	0.769	0.542	0.597	0.499	0.438	0.633	0.615	0.592	0.599	0.874	0.725	1	
BC51 PEP	0.747	0.750	0.793	0.767	0.550	0.595	0.509	0.384	0.604	0.615	0.576	0.568	0.594	0.851	0.639	1

Correlation values between 0.85 and 0.9 are highlighted by grey areas, values above 0.9 by dark grey areas. Prefix BC indicates the breast cancer micro-array used

tsPCR Template-switching PCR

Reproducibility was defined as the mean Pearson correlation coefficient of spot log2 ratios between all replicates of the same method. Fidelity was defined as the mean Pearson correlation coefficient of spot log2 ratios between all replicates of the method under test and all replicates of the standard method.

Dendrimer labelling, a method without RNA amplification, was most comparable to the standard method (r=0.91). TS-PCR proved to be the most reproducible method (r=0.97) and was the RNA amplification method that correlated most to the standard method (direct labelling, r=0.89). Correlation of Ribo-SPIA-amplified RNA with the standard method is somewhat lower (r=0.79). Random-primed PCR amplification by PEP-PCR performed worst in both categories. Correlation within and between methods is shown in more detail by a heatmap (Fig. 2a). Methods generally cluster together because the techniques are more reproducible than comparable to other methods. Differences in expression ratios could not be attributed to either GC content, distance from the 3′ end of the target, longer stretches of GC nor transcript length (Supplementary Fig. 1).

A CAT plot visualizes concordance of differential expression of Ribo-SPIA, TS-PCR and Dendrimer labelling with the standard method [6] (Fig. 2b). Briefly, this CAT plot shows agreement between differential expression calls, defined by ranking genes by their log odds ratio of differential expression. Among the amplifying methods, TS-PCR shows slightly higher concordance in differentially expressed genes with the standard method.

Validation with clinical samples

Next, we investigated whether Ribo-SPIA and TS-PCR are suitable for amplification of rather heterogenous clinical breast cancer samples (e.g., containing connective tissue, invading leukocytes, endothelial cells, fat cells and other cells). The resulting impurities in the RNA may cause problems with subsequent RNA amplification. Total RNA from ER− (patient no. 180/03) and ER+ (patient no. 254/03) breast cancer samples were chosen as targets for hybridization, based on the observation that the expression of roughly a tenth of all genes is associated with ER status [30], therefore resulting in many differentially expressed genes. RNA quality control showed a RIN of 7.8 for 180/03 and 8.5 for 254/03. As tumor material was limited, only one hybridization of unamplified RNA could be performed. For Ribo-SPIA and TS-PCR a replicate including a dye-swap hybridization was carried out. Again, even with heteroge-

Table 2 Summary of the performance of the amplification techniques under test

	Fidelity	Reproducibilty	Starting Material	n	Time required
Direct Labeling		0.930	20 000 ng	2	1.5 days
Dendrimer	0.913	0.940	2 000 ng	2	2 days
Ribo-SPIA	0.788	0.906	5 ng	4	1.5 days
TS-PCR	0.893	0.967	50 ng	4	1.5 days
PEP-PCR	0.742	0.721	100 ng	4	2.5 days
Ribo-SPIA	0.708	0.767	50 ng	2	1.5 days
17 cycles of TS-PCR	0.916	0.926	50 ng	2	1.5 days
19 cycles of TS-PCR	0.892	0.912	50 ng	2	1.5 days
26 cycles of TS-PCR	0.921	0.934	50 ng	2	1.5 days
All TS-PCR	0.909	0.924	50 ng	6	1.5 days

Results obtained by breast cancer cell lines are highlighted by blue color. Result obtained from clinical breast cancer samples are highlighted by grey shade. 'Reproducibility' was defined as the mean Pearson correlation coefficient of spot log2 ratios (*M* values) between all replicates of the same method. 'Fidelity' was defined as the mean Pearson correlation coefficient of spot log2 ratios between all replicates of the method under test and all replicates of the standard method. 'Starting material' indicates total RNA used. *n* is number of replicates performed and 'time required' corresponds to the time required, to accomplish a total micro-array experiment including labelling, hybridization and scanning.

nous clinical samples, TS-PCR proved to be highly reproducible and was comparable to the standard method (r=0.91, Table 2).

Robustness of TS-PCR over cycles and varying RNA integrity

For breast cancer samples, TS-PCR was repeated with 17 and 26 cycles instead of the initially tested 19 cycles. Mean yield for 17, 19 and 26 cycles is 4.8, 11.1 and 14.5 μg, respectively. In summary, TS-PCR amplifies total RNA of two distinct cancer types reliably in a range of 17 to 26 PCR cycles (Table 2).

RNA integrity can vary a lot among clinical samples, depending on the handling and time required until cryopreservation. The RNA amplification of choice should therefore be able to equally amplify total RNA of different quality. Total RNA from both breast cancer samples was subjected to artificial degradation by heating to 50°C for 0, 25 and 50 min (Fig. 3), and RNA integrity was assessed on an Agilent Bioanalyzer. Incubation of total RNA for 50 min resulted in a RIN decrease of 1.4 and 1.3 for samples 180/03 and 254/03, respectively. TS-PCR with 19 cycles was performed on total RNA from both samples, and the amplification product was labelled with Cy3. Universal Reference RNA (Stratagene) was used as a common reference and labelled with Cy5. Correlation of *M* values between 25 min incubated RNA and original RNA ranges from 0.93 to 0.99 (Fig. 3). Correlation of *M* values between 50-min-incubated RNA and original RNA ranges from 0.94 to 0.97.

To further pay attention on the clinical setting, we asked whether this was also true for degraded thyroid cancer samples. Thyroid nodules from three different patients (604/05, 607/05 and 618/05) were disrupted into several pieces (suffices Gx in Fig. 4 and Table 3), each of which RNA was extracted from using different tissue lysis approaches (suffice Ex in Fig. 4 and Table 3). This resulted in different RNAs of different quality from the same piece of tumor (Fig. 4). Gene expression profiles are largely maintained with TS-PCR amplification with partially degraded samples. Even after RNA degradation to RIN 4.8, correlation to the original RNA of RIN 7.3 is as high as 0.95 (Table 3).

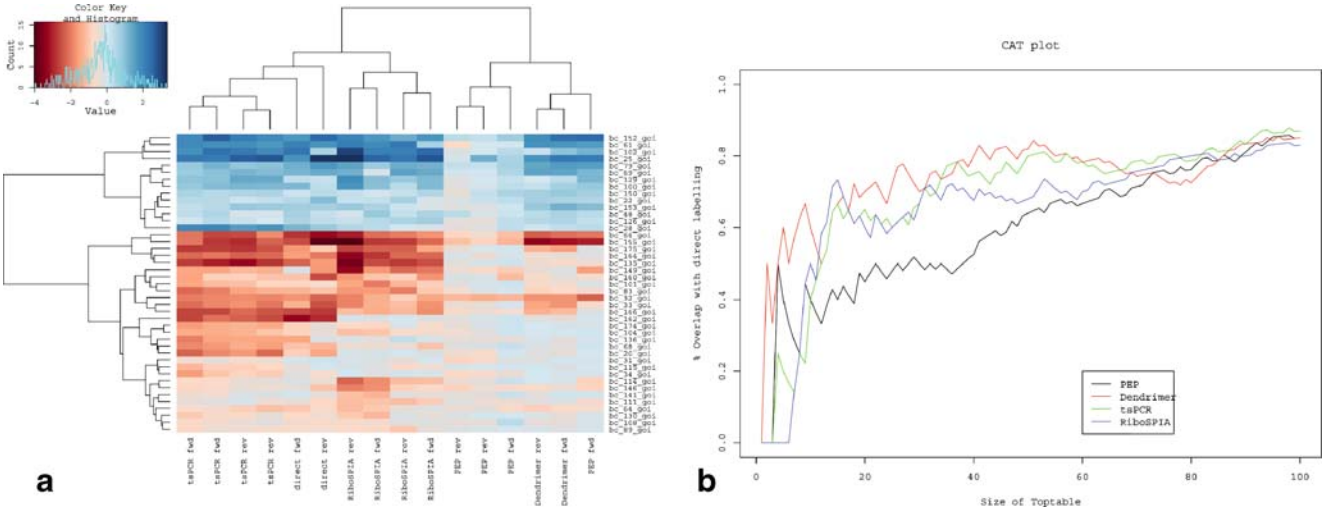

Fig. 2 Heatmap and Concordance-at-the-top (CAT) plot of experiments with total RNA from breast cancer cell lines. **a** Heatmap: Unsupervised hierarchical clustering using manhattan distance measure and complete linkage clustering. Techniques used are clustered horizontally, and probes are clustered vertically. *Color key* indicates the differential expression (*M* values, $M=\log2(R/G)$) between MCF7 and Hs578T breast cancer cell lines. **b** CAT plot: For explanation, see "Materials and methods"/"Data analysis"

In summary, partial degradation of RNA of two breast cancer samples and three thyroid cancer samples cannot corrupt the fidelity of TS-PCR amplification, as correlation to original RNA proves to be high.

Discussion

In the present study, we used a two-colour oligonucleotide micro-array platform to compare one linear and two

	180/03	*254/03*
0 min	7.8	8.5
25 min	7.3	8
50 min	6.4	7.2

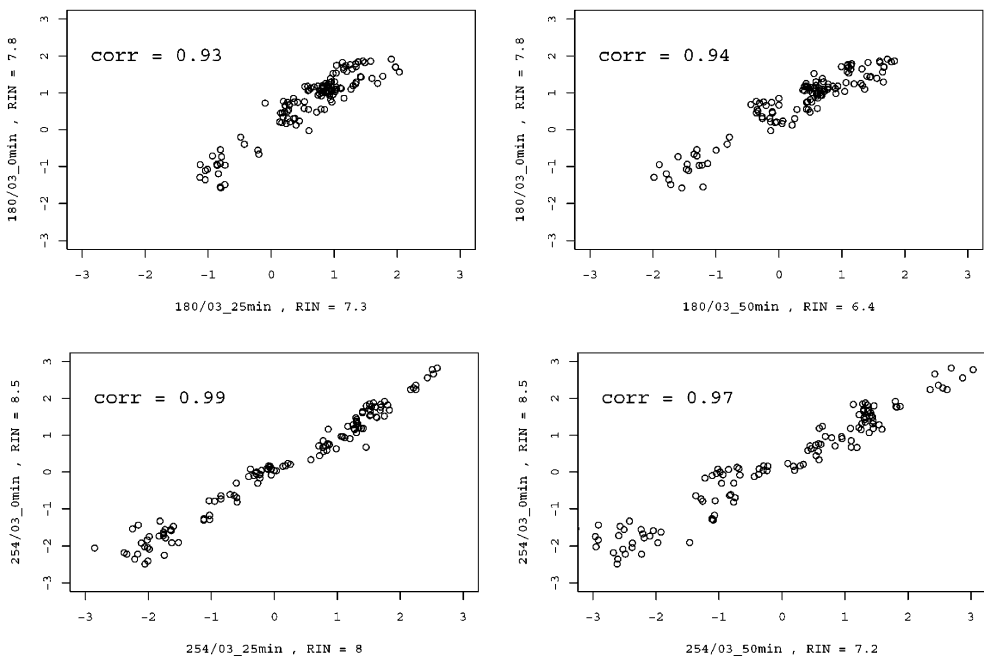

Fig. 3 TS-PCR amplified total RNA of differing integrity from two primary breast cancer samples. RNA integrity numbers (*RIN*) of primary mammacarcinomas from patients 180/03 and 254/03 were assessed at different time points of artificial digestion (see table). Correlation plots with Pearson correlation of degraded RNA to corresponding originally undegraded (0 min) RNA after TS-PCR amplification are shown

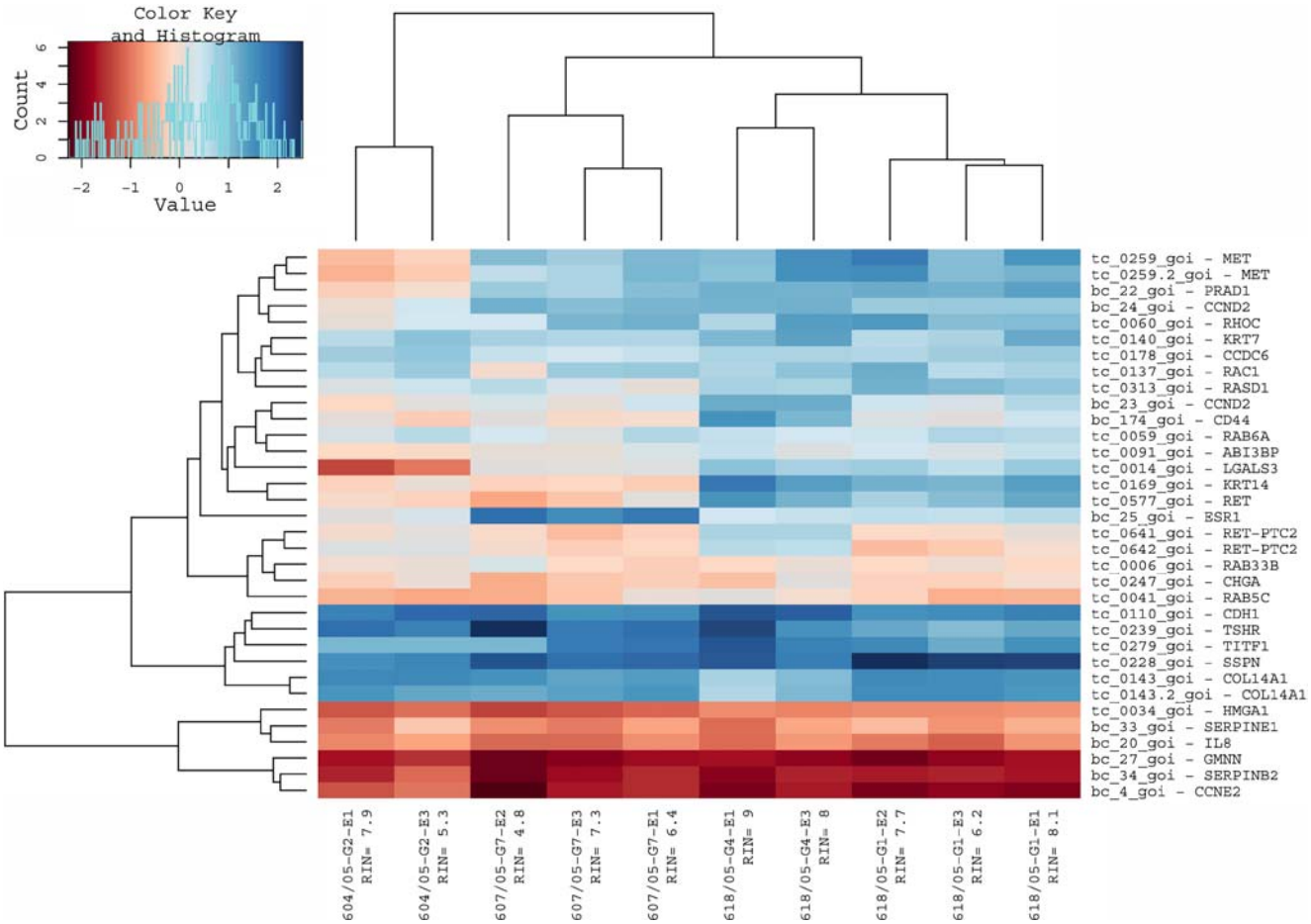

Fig. 4 TS-PCR amplified total RNA of differing integrity from three primary thyroid cancer samples. Unsupervised hierarchical clustering using Manhattan distance measure and complete linkage clustering. Samples with RNA at given integrity (*RIN*) are clustered horizontally, and probes are clustered vertically. *Color key* indicates the differential expression (*M* values, $M=\log2(R/G)$) relative to a common reference (Stratagene's universal human reference)

exponential RNA amplification methods and a signal amplification method to the standard method of labelling RNA directly at reverse transcription. We chose the micro-array as the tool to detect gene expression profiles because qualitative PCR cannot measure as many targets simultaneously.

We aimed to find a RNA amplification technique suitable for clinical routine, considering only methods that can be accomplished within 1 day including labelling. Therefore, we did not evaluate IVT approaches in this study because IVT is time consuming and requires a great amount of manual work. To date, with one round of IVT, the lowest amount of starting total RNA is 50 ng RNA (MessageAmp II kit, Ambion) suggesting 14 h of IVT to provide sufficient amplification product for micro-array experiments. To obtain detectable signals from less total RNA, a second round of IVT is needed. This usually takes around 3–4 days to be completed and introduces a new source of bias and results in 5′-truncated transcripts. Degradation of RNA progresses with time and additional rounds of IVT, resulting in 5′-truncated transcripts, as reported in several studies [10, 12, 16, 21, 33]. Consequently, GeneChip arrays preferentially contain oligonucleotides derived within 600 bp to the 3′ end of the transcript. Median size distributions of roughly 2 kb for TS-PCR and 1 kb for Ribo-SPIA amplification products are described elsewhere [1, 17, 23]. Not fully in agreement, slightly smaller median sizes of amplification product for both techniques have been observed for the clinical breast cancer samples (Supplementary Fig. 2). Yet, in contrast to the IVT product, TS-PCR amplification product leaves more space in oligo design for carefully considering cross-hybridization conditions. Oligonucleotides designed for splice variants are also more likely to be located further on the 5′ end.

Notably, there are great discrepancies in measured gene expression when either mRNA or total RNA is used [11, 20]. Important for comparability, all methods in this study use total RNA that is first reverse transcribed relying

Table 3 Matrix of Pearson correlation coefficients between thyroid cancer samples with differing RIN (RNA integrity numbers)

Patient				618/05			607/05			604/05	
Tissue				G1		G4	G7			G2	
Extract	E1 Rin 8.1	E2 RIN7.7	E3 RIN6.2	E1 Rin 9.0	E3 Rin 8.0	E3 Rin 7.3	E1 Rin 6.4	E2 Rin 4.8	E3 Rin 5.3	E1 Rin 7.9
618/05-G1E1; RIN 8.1	**1**	**0,97**	**0,98**	0,92	0,95	0,89	0,88	0,87	0,76	0,75
618/05-G1E2; RIN 7.7	**0,97**	**1**	**0,98**	0,87	0,93	0,91	0,9	0,86	0,74	0,73
618/05-G1E3; RIN 6.2	**0,98**	**0,98**	**1**	0,88	0,92	0,9	0,89	0,87	0,79	0,78
618/05-G4E1; RIN 9	0,92	0,87	0,88	**1**	**0,95**	0,82	0,81	0,82	0,7	0,74
618/05-G4E3; RIN 8	0,95	0,93	0,92	**0,95**	**1**	0,87	0,86	0,84	0,73	0,74
607/05-G7E3; RIN 7.3	0,89	0,91	0,9	0,82	0,87	**1**	**0,99**	**0,95**	0,84	0,84
607/05-G7E1; RIN 6.4	0,88	0,9	0,89	0,81	0,86	**0,99**	**1**	**0,94**	0,81	0,81
607/05-G7E2; RIN 4.8	0,87	0,86	0,87	0,82	0,84	**0,95**	**0,94**	**1**	0,84	0,84
604/05-G2E3; RIN 5.3	0,76	0,74	0,79	0,7	0,73	0,84	0,81	0,84	**1**	**0,98**
604/05-G2E1; RIN 7.9	0,75	0,73	0,78	0,74	0,74	0,84	0,81	0,84	**0,98**	**1**

All RNA was amplified by TS-PCR. Correlation values between extracts derived from the same piece of tumour are highlighted by bold numbers and are above 0.94.

somehow on oligo $d(T)_n$ primers. Dendrimer labelling showed good reproducibility, and results correlated well to the standard method, but with our micro-array, as much as 2 μg of total RNA were necessary to obtain an evaluable signal. Of the RNA amplification methods under test, TS-PCR was most reproducible (r=0.97) and showed the highest correlation of log2 expression ratios to the standard method (r=0.89 for cell culture and r=0.91 for primary mammacarcioma). Results for reproducibility [17, 20, 21, 22] and fidelity [22, 32] of TS-PCR are in good agreement to published data, although they are derived using slightly modified methods or qPCR platforms. In contrast, Puskas et al. observed lower values for reproducibility and fidelity than the other studies.

Recently, Subkhankulova et al. [28] performed a comparison of RNA amplification at the single-cell level. In this study, TS-PCR clearly outperformed two rounds of random-primed PCR and three rounds of IVT in terms of fidelity. In our study, TS-PCR also showed the highest concordance of differentially expressed genes with unamplified RNA among the amplification techniques. Reproducibility of Ribo-SPIA is consistently higher in literature [1, 2, 23]. Surprisingly, inter-method correlation of Ribo-SPIA to unamplified RNA [1, 5] or to one round IVT [23] is lower than fidelity reported by us. Random-primed PCR amplification is reported to be biased [7, 10]. A new approach of random-primed PCR amplification, PEP-PCR, tested in this study even performed slightly worse in terms of fidelity and reproducibility. Taken together, we could confirm results from the literature in an unprecedented comprehensive manner.

Starting amounts of RNA differ between amplification methods because they either amplify linearly or exponentially and additionally vary in amplification efficiency. However, the amount of starting material was not arbitrarily chosen but derived from the manufacturer's recommendation or from the literature.

Clinical samples are usually very heterogenous, which potentially poses a problem for the amplification method of choice. However, TS-PCR (and Ribo-SPIA) showed very similar reproducibility and fidelity when total RNA was amplified from clinical samples. Yields only differ slightly when either breast cancer or thyroid cancer RNA is used. From product yields, we conclude that amplification is still in its exponential phase at 17 cycles and has completely reached its plateau at 26 cycles. However, reproducibility and fidelity remain nearly the same in a range of 17 to 26 cycles. Therefore, laborious optimization of cycle numbers for each tissue under investigation is not necessary. Still, as indicated by Petalidis et al., we also recommend working with the amplified product obtained in an exponential phase of PCR.

RNA integrity is considered decisive for subsequent gene expression analysis, and RNA extracted from clinical samples usually is of varying quality. However, to our knowledge, the influence of RNA integrity on the performance of RNA amplification has not yet been investigated thoroughly. Dependent on the handling after surgery and on

the disruption protocol, clinical samples are often more or less degraded. In our experiments with breast cancer samples, TS-PCR proved to reliably amplify total RNA that diverges in 1.4 and up to 2.6 RIN points. To further prove the good reproducibility of TS-PCR in the light of differing RNA integrity, we compared RNAs from the same thyroid nodule. This serves us as a model for a clinical scenario, where some samples will be more degraded than others. In the presented experiments, we still observed a Pearson correlations of 0.95 when comparing the same RNA with RIN 7.2 and RIN 4.3 and a correlation of 0.98 when comparing RIN 7.9 with RIN 5.3.

In a research environment, RNA amplification methods have long been used for micro-array experimentation. Therefore, much effort has been put into proving the reproducibility and fidelity of the various methods available today. We believe, however, that for use in a routine clinical scenario, a RNA amplification method also needs to be robust against differing RNA integrity and be time effective. We further believe that by these standards, TS-PCR is a promising method on the move of micro-arrays from the research laboratory into clinical practice.

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Competing Interests Statement The authors declare that no conflicts of interest exist.

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ORIGINAL ARTICLE

Upregulation of cIAP2 in regenerating colonocytes in ulcerative colitis

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Abstract It has been reported that colonocytes in ulcerative colitis (UC) upregulate anti-apoptotic cytoprotective pathways. An expression-profiling study of apoptosis-related genes suggested that the cellular inhibitor of apoptosis protein-2 (cIAP2) could be upregulated in epithelial cells in UC. The role of cIAP2 in active UC was therefore investigated. Fourteen patients with active UC and 12 control subjects who underwent routine colonoscopy for control of their disease or as part of their examination program for irritable bowel syndrome were included. cIAP1 and cIAP2 expression was investigated by polymerase chain reaction, Western blotting, and immunohistochemistry. The regulation and role of cIAP2 for apoptosis was further investigated in cell cultures. cIAP2, but not cIAP1, was upregulated during active UC in regenerative epithelial cells. A similar upregulation was found in cell lines stimulated with proinflammatory cytokines and was dependent on nuclear factor κB activation. Inhibition of cIAP2 increases the susceptibility of epithelial cells to Fas ligation. Inflammation during active UC thus causes an upregulation of cIAP2 in regenerating epithelium, which renders the cells less susceptible to Fas ligation. This might play a role in regeneration of the epithelium but might additionally be implicated in carcinogenesis of UC.

Keywords Apoptosis · cIAP2 · Fas ligand · Fas receptor · Ulcerative colitis

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Introduction

Ulcerative colitis (UC) is commonly characterized by continuous inflammation or cyclic episodes of relapse and remission [17]. On the histological level, the cycling or ongoing disease activity is accompanied both by increased numbers of apoptotic cells throughout the crypt axis and by corresponding areas of regeneration of the epithelial lining. The inherent ability of the epithelium to regenerate during inflammation indicates that the epithelial cells respond to inflammation by activating cytoprotective programs. Previously, the effect of UC inflammation on cell destiny has focused on the study of apoptosis mechanisms [11, 15, 35]. The interest in apoptosis was caused by the possible pathophysiological role of increased apoptosis and the apoptosis-derived loss of epithelial integrity, which is thought to contribute to the ongoing immunological drive from luminal pathogens allowed to pass the epithelium and diarrhea caused by intestinal leakiness [2, 10, 11, 14, 24]. Accordingly, it has been shown in neoplastic cell lines that exposure to interferon-γ (IFN-γ) and tumor necrosis factor-

α (TNF-α) increases epithelial leakiness through apoptosis and that the cells are more susceptible to cell-death inducers, including Fas ligand [10, 27]. The increased susceptibility to Fas ligand has been shown to be mediated through the IFN-γ-controlled signal transducers and activators of transcription 1 transcription factor [16, 26, 27, 39]. In line with the cell-line studies, it was shown that primary human colonocytes from normal colon undergo apoptosis when stimulated by Fas ligand [35]. The apoptosis response to Fas ligand has, however, never been investigated directly in patients with UC.

We recently performed an mRNA-expression profiling of apoptosis-related genes in an attempt to characterize the pathways of importance for apoptosis in isolated colonocytes, which showed that the primary difference between inflammation and no inflammation in UC was the upregulation of anti-apoptotic cIAP2 (inhibitor of apoptosis protein 2) [33]. This finding is, in particular, interesting, as cIAP2 is well known to be upregulated by cellular stimulation by TNF-α in a nuclear-factor-κB (NF-κB)-dependent manner [5, 38], and as cIAP2 has been implicated as a main regulator of Fas susceptibility in human colonocytes [30].

The current investigation is therefore aimed to investigate regulation of expression and function of cIAP2 and its possible role in UC.

Materials and methods

Patients

Fourteen patients with active UC (patient characteristics, see Table 1) [18], who underwent colonoscopy as part of evaluation of their disease, and 12 control subjects (7 men, median age 57, range 26–86 years), who underwent routine colonoscopy and where the diagnosis of irritable bowel syndrome was later established, were included in the study. Irritable bowel syndrome was defined as abdominal pain or discomfort with altered bowel habits in the absence of organic disease, including a normal endoscopy. No patients with postinfectious colitis were included. No significant differences were found in gender or age between the groups. All UC patients were medicated with mesalazine in the dose range 1.6–3.2 g/day, and 3 of 14 patients were taking glucocorticoids (prednisone in the dose range of 20–40 mg/day). Biopsies in active UC were taken from areas with moderate macroscopic inflammation to avoid biopsies with denuded mucosa due to severe inflammation. Biopsies were taken from inflamed areas in the sigmoideum. Inflammation and diagnosis was confirmed by histology. In three patients, biopsies were obtained from both macroscopically inflamed and uninflamed areas. Biopsies were evaluated by conventional histological criteria, including crypt distorsion, goblet-cell loss, inflammation in the mucosal lamina propria, subcryptal leukocyte infiltration, and absence of changes specific for other diseases such as granulomas. Evaluation of inflammation focused on the presence and degree of activity, which in chronic inflammation bowel diseases is defined as the presence of infiltration of neutrophil granulocytes in the crypts (i.e., cryptitis), formation of crypt abscesses, and ulcerations [9].

Table 1 Patient characteristics

Characteristics	
Gender	
Male	11
Female	3
Age	35 (18–76) years
Duration of disease (from diagnosis)	2.7 (0.3–35.4) years
Extent of disease	
Left-sided colitis	11
Pancolitis	3

Medians and ranges are shown.

Isolation and culture of human colonic epithelial cells

Colonocytes were isolated as earlier described [32]. In brief, seven colonoscopically obtained biopsies were kept on ice in phosphate-buffered saline (PBS) without Mg^{2+}or Ca^{2+}, containing 50 IU/ml penicillin, 50 μg/ml streptomycin, and 0.5 mg/ml gentamycin and washed three times within 15 min in ice-cold PBS. The biopsies were then incubated in 1 mM ethylene-diamine-tetraacetic acid and 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid in PBS without Mg^{2+}or Ca^{2+}at 21°C for 75 min. The chelating buffer was replaced by ice-cold PBS, and crypts and surface epithelium were liberated from the biopsies by shaking. The crypts and surface epithelium were transferred to a new tube and centrifuged in PBS at 20×*g* for 2 min. The loose pellet obtained hereby consists of well-preserved colon crypts without apoptotic features devoid of basal membrane and single cells.

Culture of colonic epithelial cell lines

The human colonic cell lines HT29 and DLD-1 (American Tissue Type Culture Collection; Rockville, MD, USA) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) and passaged twice weekly. For analysis, 10^4 cells were plated in 96-well plates (Nunc, Naperville, IL, USA) in medium containing 10% FCS, 50 IU/ml penicillin, 50 μg/ml streptomycin, and 0.5 mg/ml gentamycine at 37°C in an

atmosphere of 5% CO_2 and relative humidity of 90%. Stimulated cells had either 100 pM TNF-α or the combination of 100 pM TNF-α, 10 pM IFN-γ, and 1 nM interleukin-1β (IL-1β; all cytokines from Sigma-Aldrich) added to the medium and were, in some experiments, co-incubated with the agonistic Fas receptor antibody CH11 (Upstate Technologies, Charlottesville, VA, USA), the mitogen-activated protein kinase inhibitors PD-98059 and SB-203580, the GTPase inhibitor tyrphostin A25, the proteasome inhibitor MG-132, the IAP-inhibitor second mitochondria-derived activator of caspases (Smac)-N7 linked to Antennapedia peptide, or a negative control (SB-202474; all from Calbiochem, San Diego, CA, USA).

Polymerase chain reaction

To investigate the mRNA expression in human primary colonic epithelial cells, mRNA was prepared as previously described [33]. In brief, crypts were transferred directly to Trizol after liberation from the basement membrane. Total RNA was extracted from the colonic epithelium. The integrity and quality of the isolated RNA was determined by evaluation of RNA electrophoresis for each sample.

Reverse transcription (RT) of extracted RNA (100 ng) was performed using RNase H-deficient Superscript II reverse transcriptase and oligo(dT) primers (Life Technologies, Gaithersburg, MD, USA). Aliquots (1 μl) of a diluted RT reaction mixture (20 μl) were used for polymerase chain reaction (PCR). Primers were designed to overlay splice sites in the Hs.127799 gene (BIRC3 gene encoding the cIAP2 protein, UniGene) and in the Hs.123332 gene (BIRC2 gene encoding the cIAP1) using the online Primer3 program. The forward primer for BIRC3 was 5′-CCAAGTGGTTTCCAAGGTGT-3′ and the reverse primer 5′-TGGGCTGTCTGATGT GGATA-3′. The forward primer for BIRC2 was 5′-CCAAGTGGTTTCCAAGGTGT-3′ and the reverse primer 5′-TGGGCTGTCTGATGTGGATA-3′. The forward primer for glutaraldehydephosphate dehydrogenase (GAPDH; Hs.1232332) was 5′-GAGTCAACGGATTTGGTCGT-3′ and the reverse primer 5′-CGGTGCCATGGAATTTGC CAT-3′. Cycling conditions for BIRC3 cDNA amplification was 94°C for 35 s, 59°C for 35 s, and 72°C for 30 s for 28 cycles. The length of the RT-PCR product for BIRC3 was 119 bp. Cycling conditions for BIRC2 cDNA amplification were identical to BIRC3. The length of the RT-PCR product for BIRC3 was 190 bp. The cycling conditions for GAPDH cDNA amplification was 94°C for 35 s, 55°C for 35 s, and 72°C for 30 s for 21 cycles. The length of the RT-PCR product for GAPDH was 175 bp. PCR products were electrophoresed in a 2% agarose gel and subsequently stained with ethidium bromide. Bands were quantified using a laser-equipped fluorescence gel reader (FLA-3000, Fuji-Film, Tokyo, Japan).

The PCR conditions were similar for both cell lines, where 31, 33, and 22 cycles were used for BIRC3, BIRC2, and GAPDH, respectively.

Immunoblotting

Cells were lysed in radioimmunoprecipitation assay buffer [62.5 mM Tris, pH 6.8, 6 M urea, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.003% bromophenol blue, and 5% 2-mercaptoethanol], incubated at 95°C for 5 min and then electrophoresed through a 4–12% BisTris SDS/polyacrylamide gel electrophoresis under reducing conditions [36]. Proteins were then blotted to a Hybond enhanced chemiluminescense (ECL) filter (Amersham Biosciences Europe, Freiburg, Germany) using a graphite electrophoresis unit. Filters were incubated at 25°C for 2 h in blocking buffer [5% skim milk and 0.05% Tween 20 in Tris-buffered saline (TBS)] and subsequently incubated overnight at 4°C in diluting buffer (2% skim milk in TBS) containing the primary antibodies. The primary antibodies used were rabbit anti-human cIAP2 polyclonal antibody at dilution 1:2,000, rabbit anti-human cIAP1 1:1,000 (R&D Systems), and rabbit anti-human GAPDH 1:50,000 (Cell Signalling Technology, Beverly, MA, USA). After washing in blocking buffer, filters were incubated for 1 h at 25°C in a diluting buffer containing 1:10,000 dilution of horse radish peroxidase-conjugated secondary antibody (DakoCytomation, Copenhagen, Denmark). Chemiluminescence reaction was performed according to the manufacturer's instructions using ECL Western blotting reagents (Amersham). Filters were digitally photographed by a LSA 1000 (Fuji-Film) cooled charge-coupled device camera and stripped and reincubated with primary antibodies in the following order: cIAP2, cIAP1, and GAPDH. Between reincubations, stripping was tested by applying secondary antibodies.

Protein from colonocytes was extracted from the phenol-methanol-chloroform phase of Trizol after removal of RNA and DNA phases. The protein phase was dialysed against a 0.5% SDS solution overnight in Slide-A-Lyser chambers (Pierce Biotechonolgy, Rockford, IL, USA) and concentrated by ultracentrifugation through a Microcon centrifugal filter (MilliPore, Billerica, MA, USA).

Expression of cIAP1 and cIAP2 in colonic biopsies

For cIAP1 staining, colonic biopsies were snap frozen in liquid nitrogen within 15 s of sampling and then stored at −80°C until analysis. Approximately 4-μm-thick sections were cut and fixed in methanol and acetone (1:1) for 30 min and dried overnight at 4°C. Subsequently, incubation in blocking buffer (PBS with 2% bovine serum albumin and 0.5% Tween) for

10 min was followed by incubation with polyclonal rabbit anti-human cIAP1 antibodies (1:100; R&D Systems) for 30 min at 21°C, and fluorescein-labeled goat anti-rabbit antibodies (1:10; BD Biosciences Pharmingen, San Diego, CA, USA) for 30 min at 4°C. The specimens were then mounted with antifading solution and the fluorescence monitored using a Leica Diaplan fluorescence microscope (Bensheim, Germany) equipped with a fluorescein isothiocyanate filter. Photomicrographs were taken with a Nikon Coolpix 995 digital camera with fixed exposure conditions.

For cIAP2 analysis, colonic biopsies were fixed in 10% buffered formalin, embedded in paraffin in a standard manner, and stored until analysis. Approximately 5 μm-thick sections were cut, deparaffinized, and rehydrated and pretreated with protease, microwaves, and 3% H_2O_2. The slides were incubated with polyclonal rabbit anti-human cIAP2 antibodies (1:800; R&D Systems) for 30 min. This was followed by horseradish peroxidase-conjugated polymers (Envision, DakoCytomation) for 30 min in room temperature. After washing, the specimens were subsequently incubated with diamino benzidine tetrahydrochloride chromogen. Slides were counterstained with Mayer's hematoxylin.

Electrophoretic mobility shift assay

The preparation of nuclear extracts and performance of electrophoretic mobility shift assay (EMSA) was done as previously described [1].

Cell-death assessment

Cell death was quantitated by measurement of lactate dehydrogenase (LDH) release (Cytotoxicity Detection Kit, Roche Diagnostics, Mannheim, Germany). This method is based on the coupled enzyme reaction in which lactate is converted into pyruvate by LDH with the concomitant reduction of NAD to NADH and H^+ which then reduce 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride to the red-colored formazan. LDH-activity was measured in culture supernatants ("released LDH"), and in the remaining cell-monolayer following lysis ("intracellular LDH") with 0.1% Triton X-100. Cytotoxicity was subsequently calculated as: % cytotoxicity = 100% × "released LDH"/("released LDH" + "intracellular LDH").

Statistics

Nonparametric statistics were applied. The Mann–Whitney test was used to compare medians. The chi-square test was used to compare the expression of cIAP2 in histologic samples. A significance level of 0.05 was applied.

Ethics

The Scientific Ethics Committee of Copenhagen County approved the study. All patients gave their informed consent before participation, and the project fulfilled the Helsinki V declaration.

Results

cIAP1 and cIAP2 expression in colonocytes

The level of IAP mRNA in colonocytes was determined by PCR of cDNA based on RNA extracted from freshly isolated colonocytes. Colonocytes from inflamed UC areas had significantly higher mRNA levels of cIAP2 than controls ($p<0.01$). Patients with UC remission had normal cIAP2 values compared to controls.

To determine the cellular localization of cIAP2 at the protein level, immunohistochemistry was made. Colonic epithelium in uninflamed tissue did not express cIAP2. In inflamed colonic tissue, however, cIAP2 was upregulated in regenerative epithelium, whereas colonic epithelial cells in other areas had either low expression levels of cIAP2 or none at all (Fig. 1). Expression was thus found in areas where epithelial cells had delayed differentiation with lack of goblet cells and large hyperchromatic nuclei, indicating that cIAP2 is expressed in regenerating epithelial cells with high cell turnover. cIAP2 expression was not related to the

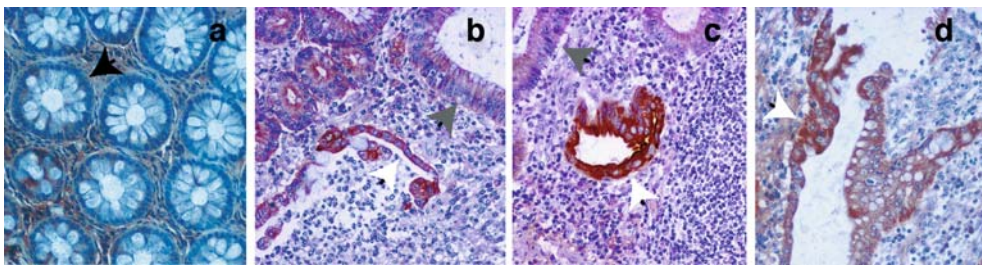

Fig. 1 Microphotographs of control mucosa (**a**) and UC mucosa (**b**–**d**). No staining was seen in control specimen (**a**, *black arrow*). In active UC, cIAP2 expression was limited to regenerative epithelial cells that had high cytoplasmatic expression (**b**–**d**, *white arrows*). Adjacent well-preserved colonic epithelial cells revealed lower or no expression of cIAP2 (**b**–**c**, *grey arrows*). No stromal cells were stained

Table 2 cIAP2 immunohistochemistry results

Samples	Grade of inflammation, median (range)	Epithelial lesions (*n*/*N*)	cIAP2 positive (*n*/*N*)	cIAP2 lesions close to ulceration (*n*/*N*)
Controls	0 (0–0)	0/5	0/5	0/5
Ulcerative colitis	2 (1–3)	5/5	5/5	5/5

Specimens were scored blindly. Grade of inflammation was assessed by conventional histological criteria (see "Materials and methods"). Patients with ulcerative colitis had higher expression of cIAP2 ($p<0.01$).

position along the crypt axis or to surface epithelium. When evaluated blindly, only biopsies from patients with UC showed this pattern of expression ($p<0.01$; see Table 2). cIAP2 protein expression was not detected in biopsies obtained from control subjects.

There was cIAP1 expression in stromal cells, but mRNA and protein cIAP1 levels were identical in UC compared to controls, and no significant epithelial expression was found (data not shown).

cIAP2 regulation upon cytokine stimulation

To further investigate the reason for cIAP2 upregulation in colonic epithelial cells, HT29 and DLD-1 cells were stimulated with either TNF-α or a mixture of TNF-α, IFN-γ, and IL-1β. TNF-α and the cytokine mixture increased cIAP2 mRNA expression in HT29 cells ($p<0.05$), whereas cIAP1 expression remained unchanged (Fig. 2a). Western blot analysis revealed a concomitant upregulation of cIAP2 protein level (Fig. 2c). Contrary to the findings in primary colonocytes, cell lines had a basal expression of cIAP2 on protein level. TNF-α was as effective as the cytokine combination to induce cIAP2 expression (Fig. 2a).

Subsequently, inhibitor assays were performed to determine the possible pathway of cIAP2 induction. Whereas inhibition of MAP-kinase pathways or GTPase inhibition did not alter cIAP expression (Fig. 2d), MG-132, a proteasome inhibitor, dose-dependently downregulated both cIAP1 and 2 expression (Fig. 2b,c). Cytokine-induced cIAP2 induction was additionally downregulated by MG-132 which also inhibited NF-κB activity as shown by EMSA analysis (Fig. 2e).

Effect of proteasome inhibition and inhibition of IAPs on HT29 cell death

To establish whether NF-κB activation and subsequent cIAP2 expression had influence on HT29 cell death, cells were preincubated with MG-132 and stimulated with the agonistic CD95 antibody CH11. MG-132 increased the cell death response to TNF-α alone ($p<0.001$) and to the combination of Fas-agonist and TNF-α ($p<0.0002$ compared to TNF-α and MG-132; see Fig. 3a). This effect was even greater when cells were stimulated by a combination of TNF-α and IFN-γ ($p<0.0002$).

The effect of the cIAP2 upregulation on cell death was subsequently investigated. Cells coincubated with the inhibitor of cIAP2, SmacN7, had increased cell-death response to stimulation with the cytokine mixture and to Fas stimulation ($p<0.008$; see Fig. 3b). The increased cell-death response to

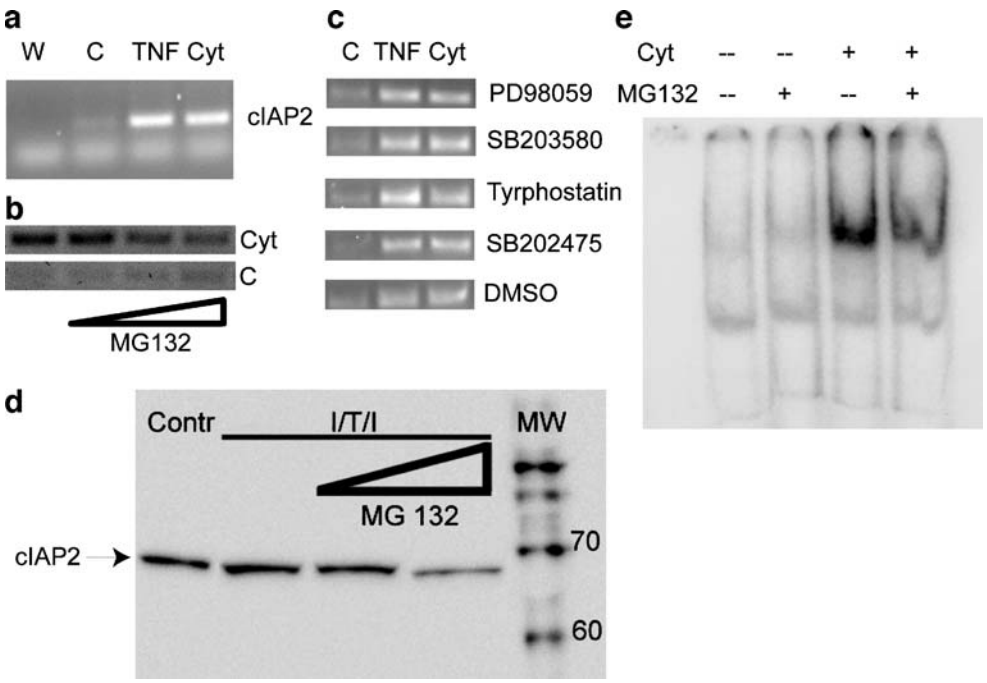

Fig. 2 Analysis of pathways involved in cIAP2 expression in colonic epithelial cell lines. Cytokine stimulation (a combination of IFN-γ, TNF-α, IL-1β; designated Cyt) or TNF-α stimulation (designated TNF) induced cIAP2 expression (**a**, PCR; **d**, Western blot). This upregulation could be inhibited by the proteasome inhibitor MG132 (**b**, PCR; **d**, Western blot). Other MAP-kinase inhibitors and inhibitors of GTPase did not affect cIAP2 expression (**c**, PCR; Western blotting showed similar results, data not shown). A concomitant inhibition of NF-κB was found after MG132 incubation (**e**, EMSA). The notation "*C*" designates control

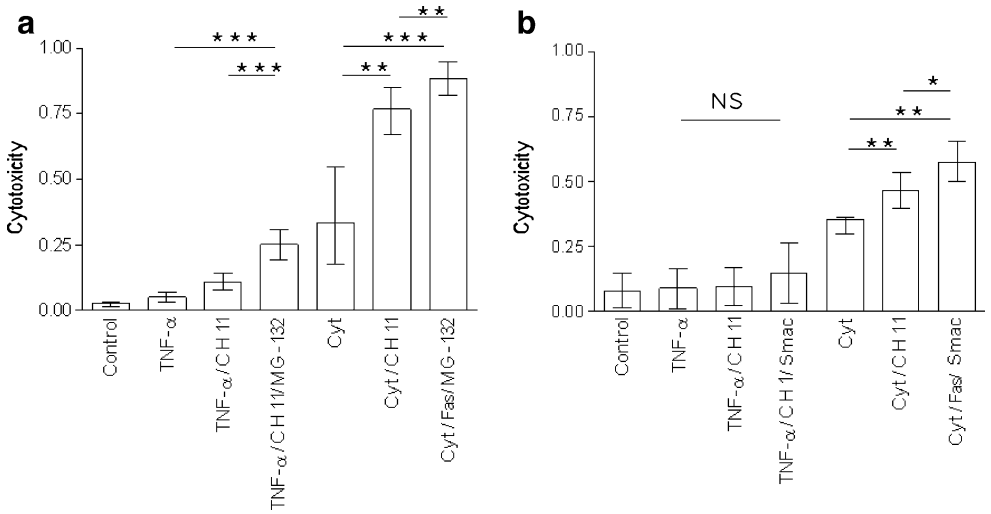

Fig. 3 Histogram showing the cytotoxicity of TNF-α or a combination of TNF-α or IFN-γ, TNF-α, IL1β (designated Cyt) and Fas ligation with the agonistic CH11 Fas receptor antibody. The effect of the proteasome inhibitor MG-132 is depicted in **a**, and the effect of the IAP-inhibitor SmacN7 (designated Smac) is included in **b**. Cytotoxicity is shown as the cytotoxicity rate [dead/(vital cells+dead cells)], and measured by the LDH-release technique. Medians and interquartile are ranges shown. *NS* Nonsignificant; *$p<0.05$; **$p<0.01$; ***$p<0.001$; specific p values are mentioned in "Results"

Fas was seen only after cytokine exposure, suggesting that upregulation of cIAP2 via cytokine stimulation was needed for cIAP2 protection of the cells.

Discussion

We showed that cIAP2, but not cIAP1, is upregulated during mild to moderately active UC, both on mRNA level and on protein level, and that the cells expressing cIAP2 were primarily regenerating epithelial cells. The proinflammatory cytokines IFN-γ, TNF-α, and IL1β, which are of importance in UC, were further shown to upregulate cIAP2 in colonocyte cell lines through activation of NF-κB. Taken together, the current results demonstrate that regenerating colonocytes activate a cytoprotective programme in response to inflammation, which increases the threshold to the cell-death mediator Fas ligand. This response seems to be adequate, as the mucosa during inflammation is invaded by cytotoxic T cells expressing Fas ligand [35]. This can explain why the epithelial lining is often intact in spite of subepithelial accumulation of cytotoxic T cells.

It is well known that the NF-κB pathway is activated in colonocytes in active UC [1]. Activation of the NF-κB pathway is further known to promote survival signaling in a number of cell types, including of course mononuclear immunocompetent cells but also other cell types such as epithelial cells in gastric carcinomas, mesenchymal cells in fibrosarcomas, and mesothelial cells in malignant mesotheliomas [4–6, 12, 38]. In all of these cases, survival appears at least in part due to transcriptional upregulation of IAP proteins as we demonstrated also to be the case during colonic inflammation.

It has been reported that cIAP1 and cIAP2 determines the apoptosis response to Fas ligation in human primary colonocytes [30]. IAPs have recently been assigned another role in colonocytes: Colonic epithelial cells undergo apoptosis when they detach from the basal membrane, a phenomenon called anoïkis. Detachment-induced apoptosis can be inhibited by activating NF-κB in colonic epithelial cells [40]. The resistance to apoptosis has been shown in a number of reports to be due to the activity of IAPs, namely cIAP2 and XIAP [22, 40]. These two IAPs can further be activated by the Ras oncogene, which is not normally activated in epithelial cells but is a known oncogene in colonic carcinomas [20, 21]. When Ras is overexpressed in nonmalignant colonic epithelial cells, these become resistant to detachment-induced cell death as well [21]. Interestingly, bile salts, which previously has been thought to play a role in colon cancer carcinogenesis, also seems to upregulate XIAP through NF-κB activation and thereby render colonic epithelial cells more resistant to apoptosis [37]. Thus, there is ample evidence for a role of IAPs, and especially cIAP2, in the downregulation of apoptotic responses in colonic epithelial cells. Our laboratory is currently investigating the Fas ligand response in primary epithelial cell cultures from patients with active UC.

Activation of cytoprotective pathways goes well along with other modes of regeneration seen during active UC, e.g., that the proliferation is increased [34]. In fact, the mucosa is, during inflammation, not just a hostile milieu but also a milieu that promotes cell survival and generation.

Thus, a wide array of potential proliferation-inducing growth factors are known to be upregulated during inflammation in UC [3, 25]. Many of these growth factors seem to be produced by activated resident cells of the lamina propria, e.g., fibroblasts and myofibroblasts. Although in vitro studies on cell lines have suggested that some of these factors also might decrease cell-death responses, such a mechanism has not been reported in UC [31].

The apoptosis-inhibiting response found in this study could be beneficial by inhibiting apoptotic colonocyte responses during inflammation in UC. It is, however, important to recognize that IAPs are unspecific inhibitors of apoptosis, both by intrinsic and extrinsic pathways [19]. A cytokine-dependent decrease in receptor-mediated apoptosis sensitivity could then be deleterious during chronic inflammatory states by increasing the risk of propagation of DNA-damaged cells where the normal response is either cycle arrest and DNA repair or cell death by apoptosis [8, 28]. The inflammation in UC is accompanied with increased mutagenesis in colonocytes caused by an increased level of reactive oxygen species and dysfunctional DNA repair [13, 23]. These mechanisms might account for part of the increased risk of colorectal cancer found in long-standing and active UC [7]. Interestingly, cIAP2 overexpression has been implicated in tumor initiation in various cancer forms, and p53-mediated apoptosis is, in some cases, known to involve CD95L-CD95 autostimulation [29]. The present results might thus emphasize the importance of inflammation control in prevention of cancer development in UC.

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ORIGINAL ARTICLE

COX-2 localization within plasma membrane caveolae-like structures in human lobular intraepithelial neoplasia of the breast

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Abstract Cyclooxygenase-2 (COX-2) is highly expressed in human intraepithelial neoplasia of the breast and takes part in the molecular pathway implicated in progression of breast cancer. Recently, we demonstrated that COX-2 protein is mainly located in plasma membrane of lobular intraepithelial neoplasia (LIN) cells suggesting a localization in caveolae-like structures. The aim of the present study is to establish subcellular locations of COX-2 and its colocalization with caveolin-1 (CAV-1) to caveolae structures in LIN. To establish a relationship between COX-2 and CAV-1, 39 LINs were studied by immunohistochemistry and confocal microscopy analysis. COX-2 and CAV-1 expression was observed respectively in 79.5 and in 94.9% of LIN studied. A positive correlation was found between membrane COX-2 staining pattern and CAV-1 expression, while no correlation was found between cytoplasm COX-2 staining pattern and CAV-1. Confocal analysis showed that COX-2 localized to plasma membrane was strictly associated to CAV-1 suggesting that an amount of COX-2 protein is placed in caveolae-like structures. Our results show that COX-2 is localized within caveolae compartment and colocalized with CAV-1 protein in LIN lesions. Because caveolae are rich in signaling molecules, this COX-2 compartment may play an important role in diverse breast cancer carcinogenesis processes.

Keywords Lobular intraepithelial neoplasia · CAV-1 · COX-2 · Immunohistochemistry · Confocal microscopy

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Introduction

There is growing evidence that links cyclooxygenase-2 (COX-2) to the development of breast cancer. Liu et al. [16] reported that forced overexpression of COX-2 is sufficient to induce mammary tumorigenesis in transgenic mice. Moreover, a significant reduction in the risk of human breast cancer due to intake of selective COX-2 inhibitors was observed [6].

Recently, we have demonstrated that COX-2 is highly expressed in human ductal intraepithelial neoplasia of the breast and takes part in the molecular pathway implicated in progression of breast cancer [24]. Moreover, our results have demonstrated that COX-2 immunostaining in lobular intraepithelial neoplasia (LIN) shows a prevalent membrane pattern [25] suggesting that COX-2 protein is mainly located in plasma membrane of LIN lesion cells.

It has been reported that COX-2 is localized to endoplasmic reticulum and nuclear envelope [30]. However, recently, Liou et al. [14, 15] have demonstrated in cultured endothelial cells and fibroblasts that a fraction of COX-2 protein is localized to plasma membrane in caveolae-like structures. Caveolins, 21- to 24-kDa proteins, are the major integral membrane component of caveolae [22]. In particular, caveolin-1 (CAV-1) is expressed ubiqui-

tously, and significant levels of CAV-1 protein were demonstrated in normal human mammary epithelial cells [23, 34].

The most active area of research on caveolae/CAV-1 function that is also the most relevant to tumorigenesis focuses on their role as platforms for integrating signal transduction cascades. Caveolae are enriched with membrane receptor-associated signaling molecules and membranous enzymes, anchored to the caveolae via the caveolins [2]. Thus, within the caveolae, a COX-2 interaction with other signaling molecules may be important for the propagation of signal transduction.

The aim of the present study is to recognize subcellular distribution of COX-2 and verify its localization within plasma membrane caveolae-like structures in human LIN of the breast, as the COX-2 compartment in caveolae may participate in specific signaling pathways that are unique to caveolae [22] and thereby contribute to diverse pathophysiological systems such as tumorigenesis [4, 8, 33].

Materials and methods

Clinical material

Immunohistochemical analysis was retrospectively performed on tissue samples that were taken for routine diagnostic purposes. Based on the availability of tissue samples in the archives of the Pathology Service of the Campus Bio-Medico University of Rome, 39 patients with LIN without any invasive component between January 2001 and May 2006 were identified and analyzed. No patient had received chemotherapy, hormonotherapy, or radiation therapy before surgery. Tissue samples were fixed in 10% neutral buffered formaldehyde and embedded in paraffin. Routine hematoxylin and eosin staining was performed on the sections for histopathologic evaluation. LIN histology and LIN grade evaluated at primary diagnosis and extracted from pathology reports were reevaluated by one of the authors (MZ), following Tavassoli criteria published in World Health Organization Classification of Tumours [32]. Based on the quality of the morphologic preservation of all available hematoxylin and eosin-stained slides of the surgical specimen sections, we selected one paraffin block for each case. Consecutive 3-μm sections were recut from each study block. These sections were immunostained for COX-2 and CAV-1 proteins.

Immunohistochemical staining

Immunohistochemistry was performed by the streptoavidin–biotin method. Endogenous peroxidase in the section was blocked by incubating them in 3% hydrogen peroxide. The used antibodies were a mouse monoclonal antibody against COX-2 protein (clone: CX-294, Dakocytomation) and a rabbit polyclonal antibody against CAV-1 protein (clone: N-20, Santa Cruz Biotechnology) at a 1:100 dilution. Sections were incubated with LSAB2 (Dakocytomation). 3–3′-Diaminobenzidine was used for color development, and hematoxylin was used for counterstaining. Negative control slides processed without primary antibody were included for each staining. Macrophages were used as positive internal control for COX-2 staining, while ductal and lobular epithelial cells and blood vessel endothelial cells were used as positive internal control for CAV-1 expression. Slides were examined by two investigators (GP and MZ) without knowledge of the corresponding clinicopathologic data.

Immunostaining was considered positive if appropriate brown staining was seen in membrane or cytoplasm of tumor cell. COX-2 and CAV-1 expression was established calculating the percentage of immunoreactive cells in a total of 1,000 neoplastic cells. LIN lesions with >5% stained cells were recorded as positive [5]. For COX-2 expression, membrane and cytoplasm staining patterns were evaluated separately. Agreement in immunohistochemical evaluation between the two observers was >90%. In cases of disagreement, a final score was determined by consensus after reexamination.

Confocal analysis

Sections were first treated with the antibodies used for immunohistochemical purpose then with tetramethyl rhodamine (TRITC)-conjugated anti-rabbit IgG (Santa Cruz) and fluorescein (FITC)-conjugated anti-mouse IgG (Santa Cruz) both at a 1:200 dilution. After washing with phosphate-buffered saline, coverslips were mounted using an anti-fade solution (AF1, Cityfluor, Cambridge, UK). Negative control slides processed without primary antibodies were included for each staining. Fluorescence images were collected with an E2000U confocal microscope (Nikon, Tokyo, Japan). Acquisition was carried out using the C1 software (Nikon).

Statistical analysis

Spearman's rank correlation test was used to assess relationships between LIN grade, immunohistochemical, and clinicopathological parameters. Values of p of 0.05 were regarded as statistically significant. SPSS software (version 13.00, SPSS, Chicago, IL, USA) was used for statistical analysis.

Results

Patients' characteristics

A total of 39 LIN lesions were studied. The median age of all patients at the time of diagnosis was 57 years (range, 22–83 years), and eight of patients were less than 50 years. Excisional biopsies were performed for calcification in 25, for a mass lesion (5 fibroadenomas, 4 radial scars, and 3 sclerosing adenosis) in 12, and for reduction mammaplasty in 2 cases. Thirteen (33.3%) lesions were LIN1, 20 (51.3%) were LIN2, and 6 (15.4%) LIN3. Data are summarized in Table 1.

Immunohistochemical staining

Among the 39 lesions studied, 31 (79.5%) showed COX-2 expression (Table 2). Macrophages, used as positive internal control, showed only a cytoplasm staining pattern coherently with our recent publications [24, 25]. Membrane COX-2 expression was found in 29 (74.4%) cases, while only 13 (33.3%) lesions showed a cytoplasmic positivity. However, 11 of these latter lesions showed a contemporary membrane immunolabeling; thus, only in two cases cytoplasm staining was present without membrane one (Fig. 1a).

As it concerns CAV-1, 37 lesions (94.9%) showed CAV-1 expression. Immunostaining showed a prevalent membrane pattern associated to a weak cytoplasmic positivity. Stromal tissue around the lesions examined was negative for CAV-1, while ductal and lobular epithelial cells and blood vessel endothelial cells were positive for CAV-1 expression [23], so they were used as positive internal control (Fig. 1b; Table 1).

Correlations between immunohistochemical and patients' characteristics

No statistical significance was found between the age of patients and the expressions of CAV-1 and COX-2. The evaluation of COX-2 expression in different LIN grades showed very similar results to those in our previous analysis performed on another LIN lesion series [25]. A significant correlation was found between COX-2 and LIN grade ($r=-0.394$; $p=0.013$). Furthermore, when membrane and cytoplasm COX-2 staining patterns were separately

Table 1 Patients' characteristics

Characteristics		
Number		39
Age	<50	8 (20.5%)
	≥50	31 (79.5%)
LIN	1	13 (33.3%)
	2	20 (51.3%)
	3	6 (15.4%)
COX-2	Negative	8 (20.5%)
	Positive	31 (79.5%)
CAV-1	Negative	2 (5.1%)
	Positive	37 (94.9%)

Table 2 COX-2 expression in LIN lesion

COX-2 expression	Cases/total	Percentage
Membrane and/or cytoplasm localization	31/39	79.5%
Membrane with or without cytoplasm localization	29/39	74.4%
Membrane without cytoplasm localization	18/39	46.1%
Cytoplasm with or without membrane expression	13/39	33.3%
Cytoplasm without membrane expression	2/39	5.1%

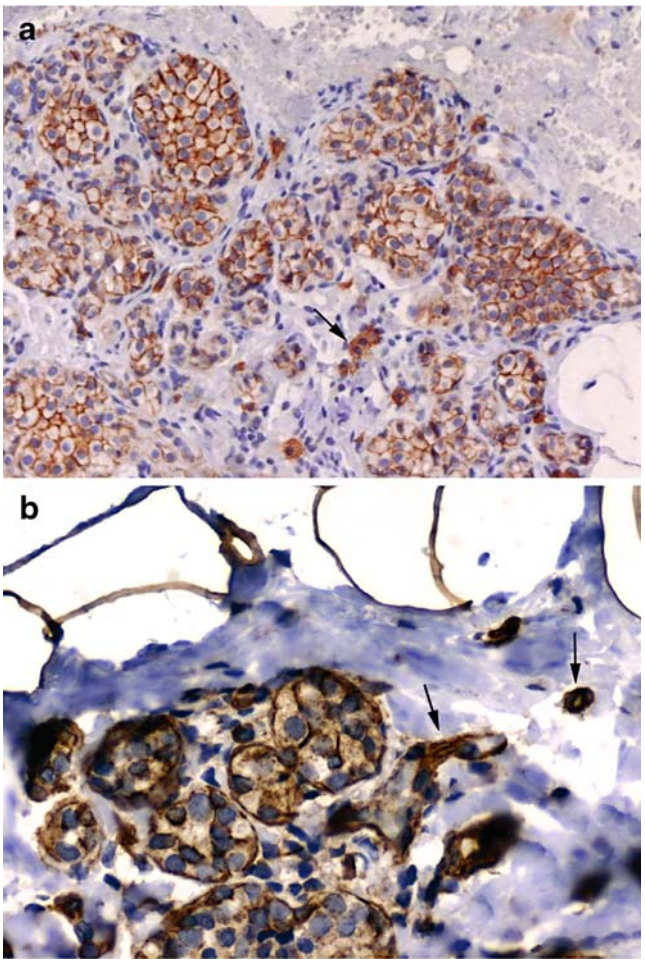

Fig. 1 Immunohistochemical staining. **a** Immunoreactivity with monoclonal antibody anti-COX-2 showed membrane positivity in LIN lesions examined. Tissue macrophages (*arrow*) were used as positive internal control. **b** Immunostaining with polyclonal antibody anti-CAV-1 showed membrane positivity associated to a weak fine granular cytoplasmic staining of LIN cells. Stromal tissue was negative, while blood vessel endothelial cells (*arrows*) were positive for CAV-1 expression; thus, they were used as positive internal control (original magnification: **a** ×200, **b** ×400)

evaluated, a significant correlation was found between membrane COX-2 expression and LIN grade (r=−0.385; p=0.016), while no significant statistical correlation was found between LIN grade and cytoplasm COX-2 expression (r=−0.059; p=0.723; Table 3). Analysis of CAV-1 expression did not provide a significant statistical correlation between this protein and LIN grade (r=−0.04; p=0.81; Table 4).

Relationship between CAV-1 and COX-2 expression

As it concerns the relationship between CAV-1 and COX-2 expression, no significant statistical correlation was found between these two variables (r=0.143; p=0.385). However, when membrane and cytoplasm COX-2 staining patterns were separately evaluated, a positive statistical correlation was found between membrane COX-2 staining pattern and CAV-1 expression (r=0.396; p=0.013), while no significant statistical correlation was found between CAV-1 expression and cytoplasm COX-2 staining pattern (r=−0.306; p=0.058; Table 5).

COX-2 localization in caveolae-like structures

To establish the COX-2 subcellular localization in caveolae-like structures, immunofluorescence double staining experiments between CAV-1 and COX-2 were performed. As previously demonstrated by immunohistochemical experiment, COX-2 and CAV-1 staining showed a prevalent membrane staining pattern. In all LIN lesions examined, the image overlay showed that some portion of the COX-2 localized to plasma membrane was strictly associated to CAV-1 protein, thus suggesting that an amount of COX-2 protein is placed in caveolae-like structures (Fig. 2).

Discussion

COX-2 expression is induced in response to exogenous stimuli such as mitogenic factors and cytokines and occupies a central position in prostanoid biosynthesis: It converts arachidonic acid derived from the upstream enzyme phospholipase A2. By the synthesis of prostaglandin E2, COX-2 affects numerous mechanisms that have been implicated in breast carcinogenesis processes such as angiogenesis [18], immune response [9], and aromatase synthesis induction [35].

Recently, we have demonstrated that COX-2, with a prevalent cytoplasmatic pattern, is highly expressed in human ductal intraepithelial neoplasia of the breast supporting the role of this protein in the early step of breast carcinogenesis and that it takes part in the molecular pathway implicated in progression of breast cancer [24]. Subsequently, we have demonstrated that COX-2 is also expressed in human LIN of the breast. Moreover, when LIN lesions were stratified following Tavassoli classification [32], a significant statistical difference was found between LIN3 vs LIN1–2, thus let hypothesize that this different protein expression pattern could reflect a different biological behavior [25]. Our results have also demonstrated that COX-2 immunostaining in LIN lesions, differently from what was previously established in ductal intraepithelial lesions [24], shows a prevalent membrane pattern, suggesting that COX-2 protein is mainly located in plasma membrane of LIN cells. Furthermore, our results showed a strong correlation between membrane COX-2 staining pattern and LIN grade, while no significant statistical correlation has been found between LIN grade and cytoplasmic COX-2 staining pattern suggesting that membrane COX-2 expression may have a primary role in the evolution of LIN pathology.

The finding that COX-2 expression has a primary role in the first phases of carcinogenesis process is supported by many studies [6, 16, 24, 25]. However, to the best of our knowledge, little is known about the role of a different COX-2 subcellular location in breast carcinogenesis process.

Recent studies have suggested that COX-2 subcellular location is an important determinant for its metabolic

Table 4 LIN grade and CAV-1 expression

LIN grade	Total	CAV-1 expression
LIN1	13	12
LIN2	20	20
LIN3	6	5
Spearman correlation test	r	−0.04
	p	0.81

Table 3 LIN grade and COX-2 expression

LIN grade	Total	COX-2 expression	Cytoplasm COX-2 expression	Membrane COX-2 expression
LIN1	13	12	5	11
LIN2	20	17	6	17
LIN3	6	2	2	1
Spearman correlation test	r	−0.394	−0.059	−0.385
	p	0.013	0.723	0.016

Table 5 Correlation between COX-2 and CAV-1 expression

		CAV-1 expression
COX-2 expression	*r*	0.143
	p	0.385
Membrane COX-2 expression	*r*	0.396
	p	0.013
Cytoplasm COX-2 expression	*r*	−0.306
	p	0.058

Spearman test

activities and cellular functions [15]. Liou et al. [15] have demonstrated, by subcellular fractionation analysis, that a fraction of COX-2 protein is localized to plasma membrane in caveolae structures in human cultured foreskin fibroblasts.

Caveolins, 21- to 24-kDa proteins, are the major integral membrane component of caveolae [22]. In particular, CAV-1 is expressed ubiquitously, although at different levels in different tissues, with the highest levels in adipocytes, endothelial cells, fibroblasts, smooth-muscle cells, and a variety of epithelial cells [28]. In particular, as it regards mammary tissue, CAV-1 mRNA and protein expression were demonstrated in both epithelial [3, 12, 34] and extra-epithelial components of the human mammary glands (myoepithelial cells) [7, 29]. Furthermore, by immunohistochemical analysis, a clear expression of CAV-1 concentrated at the surface membrane has been confirmed in the human normal epithelial cells of mammary gland [23].

To establish a relationship between COX-2 and caveolae-like structures in LIN pathology, in the current study, a series of LIN lesions were analyzed for COX-2 and CAV-1 expression. A positive statistical correlation was found between membrane COX-2 and CAV-1 expression, while no significant statistical correlation was found between CAV-1 and cytoplasmic COX-2 staining. Furthermore, results obtained by confocal analysis demonstrate for the first time a colocalization of COX-2 and CAV-1 protein on plasma membrane suggesting that some portion of the COX-2 is localized to caveolae of human LIN cells.

COX system is known to be a microsomal enzyme system that is mainly located in endoplasmic reticulum and nuclear envelopment [30]. COX-2 has a stretch of hydrophobic residues that serves as the binding site to the luminal endoplasmic reticulum membrane [10, 26]. It is unclear

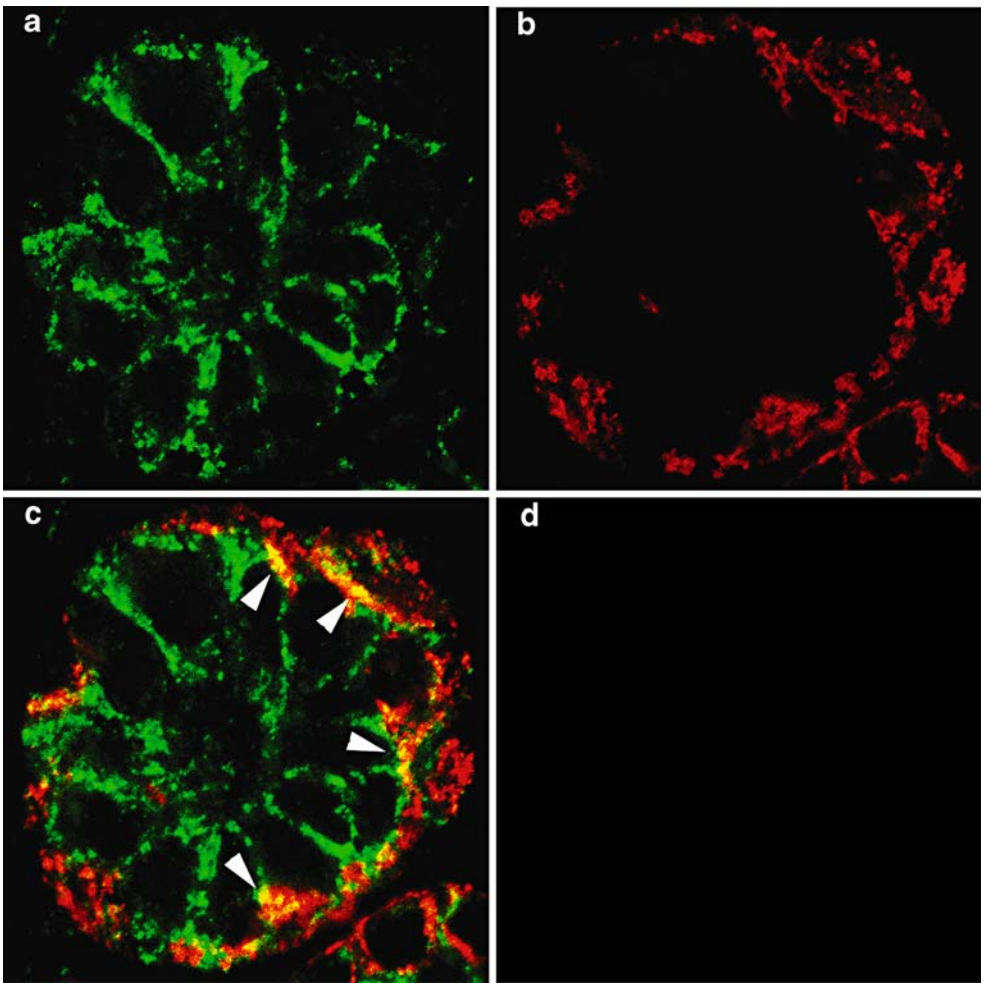

Fig. 2 Confocal analysis. LIN lesions were double-stained with FITC and TRITC and examined by confocal microscope. The figures shown here represent the typical staining pattern for majority of LIN lesions studied. A representative LIN lesion with positive plasma membrane stain for COX-2 (**a**) and CAV-1 (**b**). The image overlay (**c**) of **a** and **b** shows colocalization of CAV-1 and COX-2 on plasma membrane suggesting that plasma membrane COX-2 is located in caveolae-like structures. The image **d** shows the negative control processed without primary antibodies (original magnification ×1,000)

how COX-2 leaves its endoplasmic reticulum residence and migrates to caveolae. One possibility is that COX-2 retention in endoplasmic reticulum is regulated by a posttranslational mechanism, and an alteration of the control mechanism allows COX-2 exit. Once COX-2 comes into the cytosol, it may bind CAV-1 in the cytosolic caveolae-like vesicles, which have been shown to transport proteins to the plasma membrane [19, 21].

The COX-2 compartment in caveolae, which is segregated from the general prostaglandin synthesis factory in endoplasmic reticulum and nuclear envelopment, may participate in specific signaling pathways that are unique to caveolae [22] and thereby contribute to diverse pathophysiological systems such as tumorigenesis [4, 8, 33].

It has been suggested that type IIA and type V secretory phospholipase A2, which are expressed on plasma membrane, are functionally coupled to COX-2 [1, 20]. Functional coupling of secretory phospholipase A2 with COX-2 has been shown to play a major role in the delayed phase of prostaglandin E2 synthesis [11], which affects numerous mechanisms that have been implicated in breast carcinogenesis processes [9, 18, 35].

Furthermore, caveolae are enriched with diverse signaling mediators such as nonreceptor tyrosine kinases [17], components of the Ras-mitogen-activated protein kinase pathway [13, 31], receptor tyrosine kinase (epidermal growth factor receptor, Her2/neu) [3], and plasma membrane estrogen receptor [27]. Because these signaling mediators are specifically involved in the development of breast cancer, COX-2 plasma membrane localization may represent a new frontier for studying its tumorigenic activity.

In conclusion, results of current study represent the first evidence of a colocalization of COX-2 with CAV-1 protein in human LIN lesion suggesting that a portion of COX-2 protein is localized in caveolae-like structures in breast cancer pathology. Further investigations on the regulatory characteristics of COX-2 expression in LIN lesion, such as the sorting mechanism of COX-2 to the caveolae, and on the difference between the microsomal and plasma membranous COX-2 activity may provide us with important clues to the physiology and pathology of COX-2 expression in LIN.

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ORIGINAL ARTICLE

Biochemical and ultrastructural evidence of endoplasmic reticulum stress in LGMD2I

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Abstract Limb girdle muscular dystrophy type 2I (LGMD2I) is due to mutations in the fukutin-related protein gene (*FKRP*), encoding a putative glycosyltransferase involved in α-dystroglycan processing. To further characterize the molecular pathogenesis of LGMD2I, we conducted a histological, immunohistochemical, ultrastructural and molecular analysis of ten muscle biopsies from patients with molecularly diagnosed LGMD2I. Hypoglycosylation of α-dystroglycan was observed in all *FKRP*-mutated patients. Muscle histopathology was consistent with either severe muscular dystrophy or myopathy with a mild inflammatory response consisting of up-regulation of class I major histocompatibility complex in skeletal muscle fibers and small foci of mononuclear cells. At the ultrastructural level, muscle fibers showed focal thinning of basal lamina and swollen endoplasmic reticulum cisternae with membrane re-arrangement. The pathways of the unfolded protein response (UPR; glucose-regulated protein 78 and CHOP) were significantly activated in LGMD2I muscle tissue. Our data suggest that the UPR response is activated in LGMD2I muscle biopsies, and the observed histopathological and ultrastructural alterations may be related to sarcoplasmic structures involved in FKRP and α-dystroglycan metabolism and malfunctioning.

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Keywords FKRP · Glycosylation · LGMD2I · Muscular dystrophy · UPR

Introduction

Limb girdle muscular dystrophy type 2I (LGMD2I) is an autosomal recessive muscular dystrophy caused by mutations in the gene coding for fukutin-related protein (*FKRP*) [6]. LGMD2I is clinically heterogeneous [4, 6, 9, 22, 28], and it is allelic to MDC1C (congenital muscular dystrophy type 1C) with onset in the neonatal period [5].

The function of FKRP in skeletal muscle is largely unknown; however, indirect evidence suggests that FKRP may be a putative glycosyltransferase involved in α-dystroglycan (α-DG) processing [6, 13]. Muscle biopsies of patients affected by either LGMD2I or MDC1C show a variable reduction of α-DG glycosylation [4–6]. α-DG glycosylation is essential for proper interaction of dystroglycan with its extracellular matrix ligands (such as laminin α2) [16] and thus to anchor the muscle fibers to the basement membrane [17].

In the literature, there is a large debate about the subcellular localization of FKRP. Initially, it was hypothesized that FKRP was a Golgi-resident protein required for post-translational modification of dystroglycan and that mislocalization of the mutant protein may underlie MDC1C [13]. Later, it was suggested that FKRP localizes in rough endoplasmic reticulum (ER) [14, 21, 27] and that protein mislocalization is not a common mechanism of disease in LGMD2I/MDC1C [10, 27]. More recently, data indicate that FKRP is present at the muscle cell surface, associates with the dystrophin-glycoprotein complex, and has a unique role in α-DG processing pathway [2].

Here, we have analyzed the histopathological and ultrastructural changes in LGMD2I skeletal muscles in order to further characterize the pathogenesis of LGMD2I.

Material and methods

Patients Muscle biopsies from ten patients affected by LGMD2I were selected from our muscle biopsy database. Both FKRP-mutated alleles were identified in all patients (Table 1). Clinical and genetic findings in all but one patient have been described in detail [4]. All, but patient number 5857, who was asymptomatic, showed proximal muscle weakness. The Gardner-Medwin and Walton functional scale was used to grade muscle function in the patients [15].

At the time of diagnosis, open muscle biopsy was obtained after written informed consent.

Muscle pathology and immunohistochemical studies Thirteen serial sections, 10 μm thick, were obtained from each muscle biopsy. The first section was stained with ATPase at pH 4.3 and the total number of fibers (at least 1,000) counted on photographic enlargement and used to calculate the percentage of fibers immunolabelled with the different antibodies. The fibers area was estimated using the Scion Image Beta 4.03 software.

Twelve different monoclonal antibodies were used as follows: collagen IV (1:50) (Chemicon, Temecula, CA), perlecan (1:100) (MAB1948, Chemicon), laminin α2 directed against the 80-kDa fragment (1:1,000; MAB1922, Chemicon) and against the entire molecule (1:500; NCL-merosin, Novacastra, Newcastle upon Tyne, UK), α-dystroglycan (1:200; IIH6) [12], α-sarcoglycan (1:100; Novocastra), dystrophin (1:100; Novocastra), neonatal myosin heavy chain (1:100; NCL-MHCn; Novocastra), macrophages (CD68; clone EBM11; Dako, Carpinteria, California, USA), CD4 positive helper/inducer T cells (clone MT310; Dako), CD8 positive cytotoxic/suppressor T cells (clone DK25; Dako), and major histocompatibility complex (MHC) class I molecules (W6/32; Dako).

The intensity of reaction was examined by visual inspection by the same observer and graded as follows: absent or normal (−), slight increase or reduction (+), moderate increase or reduction (++), severe increase or reduction (+++).

The severity of the dystrophic process and muscle histopathology was scored as previously described [7].

α-DG immunoblot was done as previously described using the IIH6 antibody [4].

Electron microscopy Small muscle specimens were fixed after surgery in 2.5% glutaraldehyde and postfixed in 1% OsO_4. After dehydration in graded ethanol, the specimens were embedded in Araldite. Thin sections were stained in uranyl acetate and lead citrate and observed in a Philips 410T transmission electron microscope.

Table 1 Clinical and molecular data in LGMD21 patients

Patient, sex	FKRP protein change	Age at onset (years)	Age at biopsy (years)	Disease duration at biopsy	Clinical grading at biopsy/at last evaluation (years)
960, M	Leu276Ile Pro462Ser	2	2	0	4/8 (17[a])
6661, F	Pro358Leu (homozygous)	6	6	0	1/1 (8)
5342, M	Leu276Ile (homozygous)	7	42	35	5/5 (42)
1191, M	Leu276Ile (homozygous)	13	26	13	4/8 (42)
888, F	Pro358Leu Pro462Ser	14 (at 4 years high CK)	4	–	0/8 (24)
899, M	Leu276Ile (homozygous)	22	23	1	1/5 (42)
879, F	Val160Phe Pro358Leu	35	39	4	4/6 (61)
3285, F	Leu276Ile (homozygous)	49	54	5	3/4 (63)
1475, F	Arg244His (homozygous)	50	58	8	1/4 (64)
5857, M	Leu276Ile (homozygous)	Asymptomatic	48	–	0/0 (48)

[a] deceased

Table 2 Muscle pathology and immunohistochemical data in LGMD2I

Patient	Muscle type	Type I fibers (%)	Fibro-fatty tissue	Degenerating fibers (%)	Neonatal myosin (%)	IIH6	MHC class I[a]	CD68	CD4+/CD8+	Pathology severity score
960	Quadriceps	80	+++	0.7	8	–	±	+++	++/±	Active
6661	Quadriceps	68	+	0.1	0.1	+	±	++	−/−	Mild
5342	Quadriceps	75	++	0.1	4	+	±	++	+/+	Severe
1191	Quadriceps	41	+	0.8	4	+	+	++	N.D./±	Moderate
888	Quadriceps	65	+	0.7	2	–	+	+	+/±	Moderate
899	Quadriceps	48	±	0	0.7	++	+	++	−/+	Moderate
879	Biceps	51	±	0.1	0	+	±	±	±/±	Mild
3285	Triceps	35	+	0	0	++	+	+	±/−	Moderate
1475	Biceps	48	±	0	0	++	++	±	++/+	Mild
5857	Quadriceps	34	–	0.4	0	++	++	++	++/+	Mild

N.D. Not determined
[a] Muscle membrane

Unfolded Protein Response studies RNA was extracted from frozen muscle biopsies from 10 patients and 11 normal controls using SV Total RNA Isolation (Promega, Madison, WI, USA) and reverse transcribed using an ImProm-II Reverse Transcription System (Promega) according to the manufacturer's instructions. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed using an ABI Prism 7900 (Applied Biosystems, Foster City, CA, USA) and SYBR Green detection (Invitrogen, Carlsbad, CA, USA). The sequence of primers for GRP78 and CHOP were as published [1, 29]. As an internal control, the extent of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression in the same tissue was determined. Relative GRP78 and CHOP expressions were given as GRP78/GADPH, and CHOP/GADPH ratios and normalized values were subjected to a 2-ΔΔCt formula to calculate the fold change between controls and patients. All reactions were carried out in triplicate to reduce variation.

Results

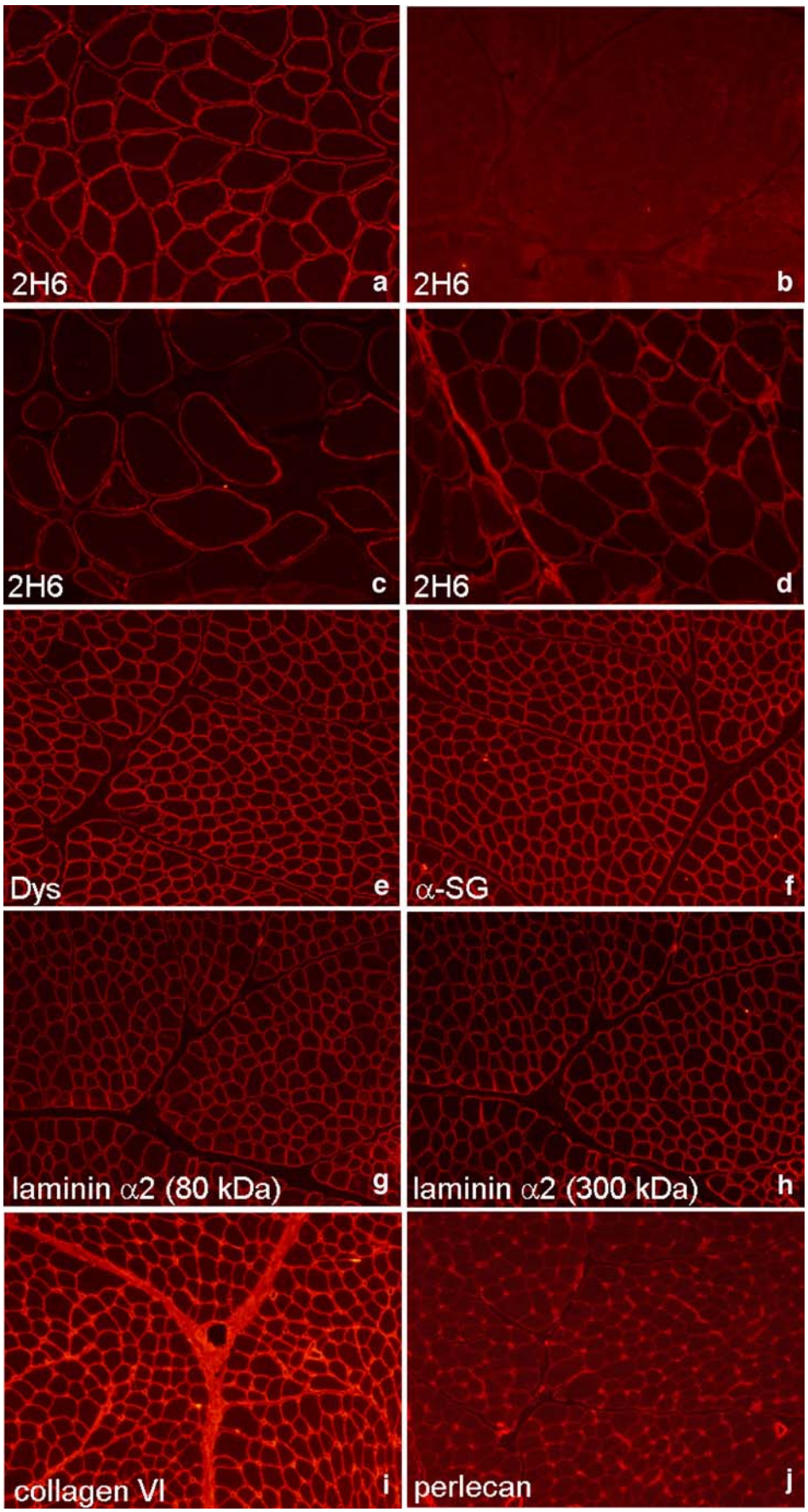
Patients Five male and five female patients affected by LGMD2I were studied. Age at onset was on average 22 years (range 2–50 years), and average age at biopsy 30 years (range 2–58 years). Mean disease duration at biopsy ranged from 0 to 35 years. Clinical severity at biopsy ranged from 0 (asymptomatic) to 5 (unable to rise from the floor) according to the Gardner Medwin and Walton scale (Table 1).

α-DG studies Since mutations in the *FKRP* gene result in α-DG hypoglycosylation, we used an antibody directed against a glycosylated epitope of the protein (IIH6) to study muscles biopsies from ten LGMD2I patients and found a depletion in α-DG glycosylation in all biopsies studied (Table 2; Fig. 1). However, comparison between patients showed a complete deficiency in only two of them (numbers 960 and 888) (Fig. 1b), while a variable reduction of α-DG glycosylation ranging from slight to moderate was observed in the remaining patients (Fig. 1c, d). Interestingly, a mosaic-like pattern was also noted in most cases, with some fibers showing clear, strong α-DG labeling at the sarcolemma and others a marked depletion of the protein (Fig. 1c).

α-DG immunoblotting using the IIH6 antibody was done in seven of the ten LGMD2I patients studied and confirmed a depletion in glycosylated α-DG in all. In five patients (patients 960, 1191, 888, 899, 979) only traces of glycosylated α-DG, in patient #5857 about 5%, and in patient #1475 about 20% of controls glycosylated α-DG were detected.

Extracellular matrix and dystrophin-associated protein studies To determine whether modifications of α-DG glycosylation may influence the expression of extracellular matrix proteins, laminin α2, perlecan, and collagen IV were studied in patients' muscle. No significant differences were observed in patient compared with control muscle (Fig. 1). Moreover, to investigate the dystrophin-associated complex, dystrophin and α-sarcoglycan were also studied; their expression in LGMD2I muscle biopsies proved normal compared to controls (Fig. 1).

Muscle pathology According to the severity of the pathological pictures (regeneration, degeneration, and fibrofatty replacement) muscle biopsies were graded as mild, moderate, severe, and active muscular dystrophy (Table 2).


2H6
a
2H6
b
2H6
c
2H6
d
Dys
e
α-SG
f
laminin α2 (80 kDa)
g
laminin α2 (300 kDa)
h
collagen VI
i
perlecan
j

◀ **Fig. 1** α-Dystroglycan immunofluorescence in LGMD2I patients' muscle biopsies. **a** Control, **b** patient number 888, **c** patient number 5342, and **d** patient number 1475 muscle biopsy stained with the IIH6 antibody directed against a glycosylated epitope of α-dystroglycan. α-Dystroglycan was normal in the control and completely negative in patient number 888. Muscle biopsy of patient number 5342 shows a marked reduction of immunostaining but a few scattered fibers are α-dystroglycan-positive in a mosaic-like pattern. α-Dystroglycan of patient number 1475 is mildly reduced. **e**–**j** Muscle biopsy of patient number 888 stained with antibodies directed against various membrane and extracellular matrix proteins. All proteins showed normal reaction in the patient's muscle biopsy

To study muscle regeneration, neonatal myosin was immunostained in patients and controls (Table 2). Neonatal myosin-positive muscle fibers were observed in six patients' biopsies, ranging from <1 to 8% of the total number of fibers. These regenerating fibers were either clustered in small groups of 10–15 elements (in patient numbers 960, 888, 1191, and 5342) or scattered in the muscle biopsies. Degenerating fibers, including hyaline and necrotic fibers and fibers undergoing phagocytosis, were less than 1% in all biopsies studied, and fibrofatty replacement, assessed by visual inspection, ranged from severe to absent (Table 2).

In most patients in our series, muscle histopathology was relatively benign and showed mild (40%) or moderate myopathic features (40%) with fiber size variability, a mild increase in perimysial connective tissue, increased number of central nuclei, and scattered foci of inflammatory cells (Fig. 2b,d). In one patient (number 960; Table 1) muscle pathology was consistent with an active, aggressive muscular dystrophy resembling Duchenne dystrophy (DMD-like; Fig. 2a) and in another (number 5342; Table 1) with a severe, advanced dystrophy. Interestingly, this latter patient was the only one to be biopsied 35 years after onset of symptoms (Fig. 2c).

Muscle infiltrate characterization To characterize the small foci of inflammatory cells observed in the LGMD2I muscle biopsies, a panel of different antibodies specific for T helper/inducer, T cytotoxic/suppressor and phagocyte markers was used (Table 2). A mild or moderate increase in MHC class I was observed in all biopsies. Mononuclear cells, either scattered or organized in small aggregates, were localized in the interstitium and in the perivascular space, and sometimes they surrounded and invaded single muscle fibers. Macrophages formed the most abundant mononuclear cell subset and were seen either surrounding or invading muscle fibers or localized in the perimysium or endomysium. The T cells were predominantly of the CD4+ type (T helper), although a few CD8+ cells were observed.

Electron microscopy Ultrastructural studies were performed on biopsies from three patients (numbers 5342, 3285, and 5857). In all cases, the basal lamina of muscle cells showed abnormalities. In normal muscle, the basal lamina appears as a uniformly fibrillar dense structure overlying the sarcolemma. In LGMD2I, the basal lamina appeared thinner than normal, deranged and often disrupted in non-

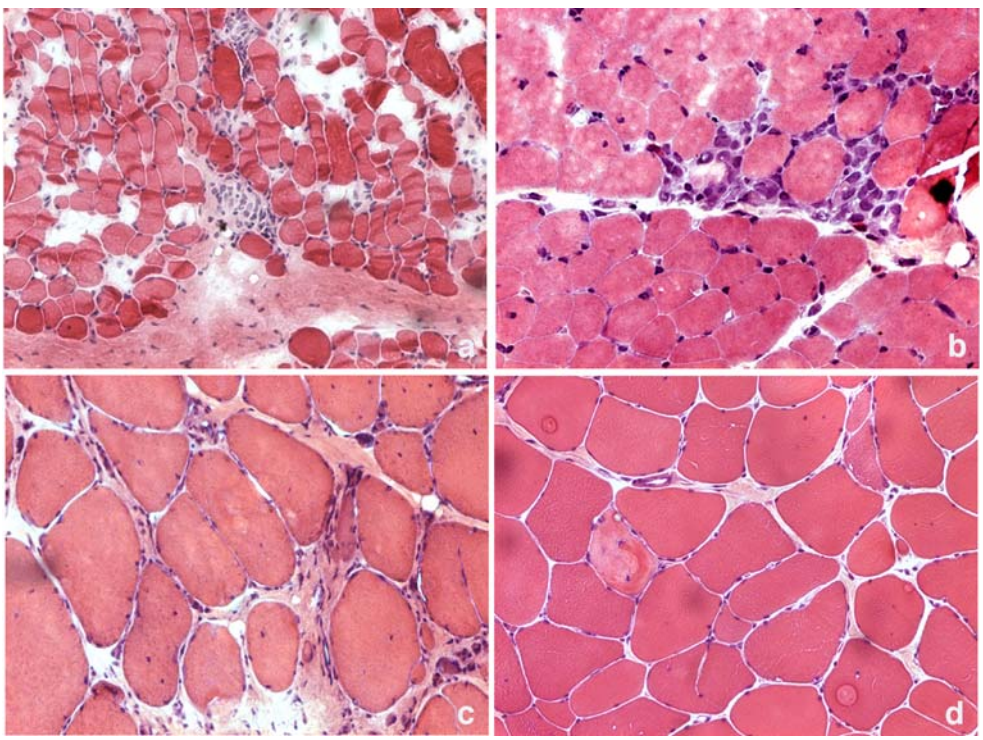


Fig. 2 Muscle histopathology in LGMD2I patients. **a** Patient number 960, **b** patient number 888, **c** patient number 5342, and **d** patient number 1191 muscle biopsy stained with hematoxylin and eosin. **a** Aggressive muscular dystrophy, **b** a small focus of mononuclear cells invading necrotic muscle fibers, **c** an advanced, long-lasting muscular dystrophy, and **d** mild myopathy

necrotic muscle fibers and focally resulting in a skein-like structure (Fig. 3a). In the sarcoplasmic compartment, a severe loss of myofilaments, dilated sarcoplasmic reticulum, and T system profiles were observed. T tubules were often swollen and assembled asymmetrically with supernumerary junctional sarcoplasmic reticulum components (Fig. 3b). The main ultrastructural feature was a marked accumulation of lipid droplets (Fig. 3c) both in the intermyofibrillar region and in the subsarcolemmal area, where it often appeared to be associated with many mitochondria (Fig. 3d).

Unfolded protein response studies In order to verify whether mutant FKRP and/or hypoglycosylated α-DG may elicit an unfolded protein response (UPR), we used quantitative RT-PCR to study the expression of the GRP78 chaperone protein, whose function is to increase the folding capacity of the endoplasmic reticulum, and the expression of CHOP, a proapoptotic transcription factor.

mRNA expression level of GRP78 in LGMD2I patients (GRP78/GADPH ratio±SD, 2.05±1.08) showed a significant increase as compared to controls (GRP78/GADPH ratio±SD, 1.0±0.3), +2-folds ($p<0.007$; Fig. 4).

The expression of CHOP was also up-regulated in LGMD2I patients but to a lesser extent: +1.5-folds compared to controls ($p<0.05$; Fig. 4). The CHOP/GADPH expression ratios were 1.74±0.62 in LGMD2I patients vs 1.18±0.61 in controls.

Moderate variability was observed in the expression level of both GRP78 and CHOP in LGMD2I patients' muscle biopsies (Fig. 4).

Discussion

The molecular mechanisms underlying the wide clinical spectrum due to *FKRP* gene mutations are poorly understood. We studied muscle biopsies from ten patients affected by LGMD2I to further characterize the pathogenesis of the disease and suggest that the activation of an endoplasmic reticulum stress response may partly explain differences in disease severity. Diagnosis in our cohort of patients was based on the identification of both *FKRP*-

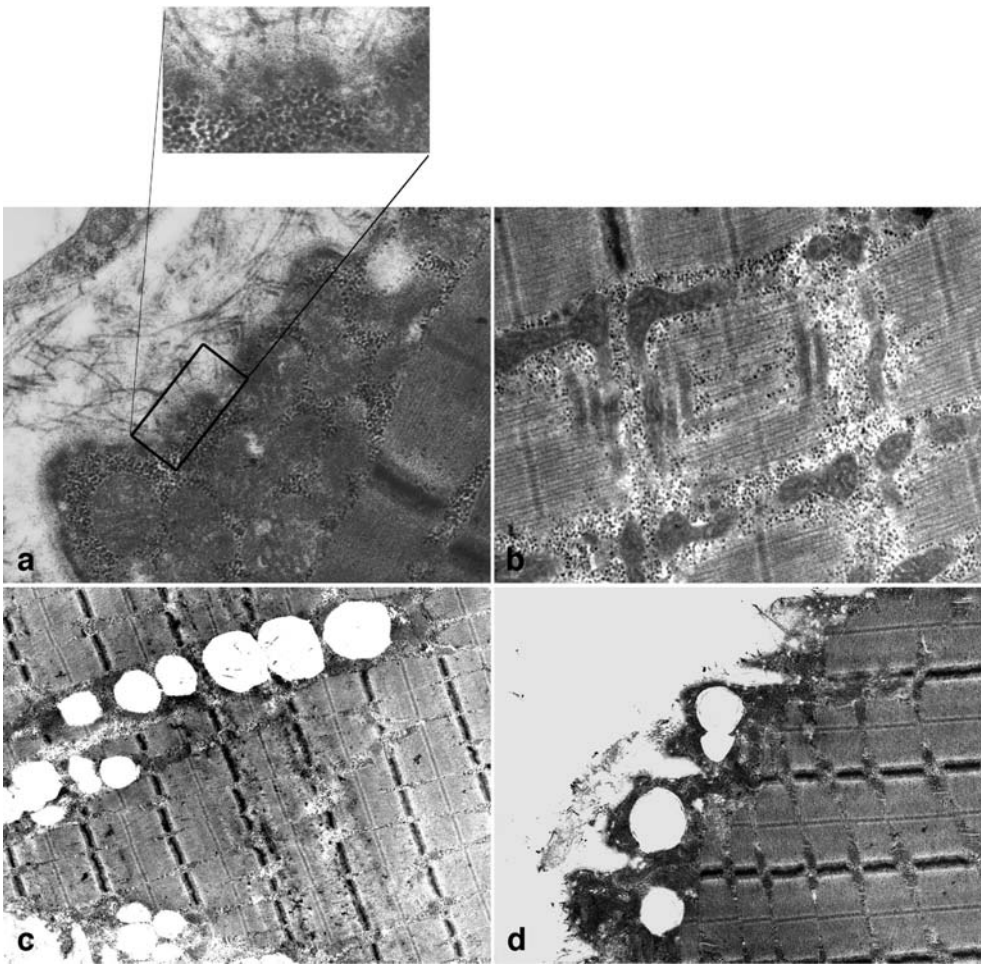


Fig. 3 Transmission electron microscopy in LGMD2I patients. **a** and **c** Patient number 5342, **b** patient number 3285, and **d** patient number 5857. In **a**, the myofiber basal lamina focally features a skein-like structure, and an irregularly-textured appearance (in the *enlargement details* of the deranged basal lamina). Triad reduplication (**b**) is present in the sarcoplasm together with many lipid droplets (**c**) which appear to be mitochondria-associated in the subsarcolemmal region (**d**)

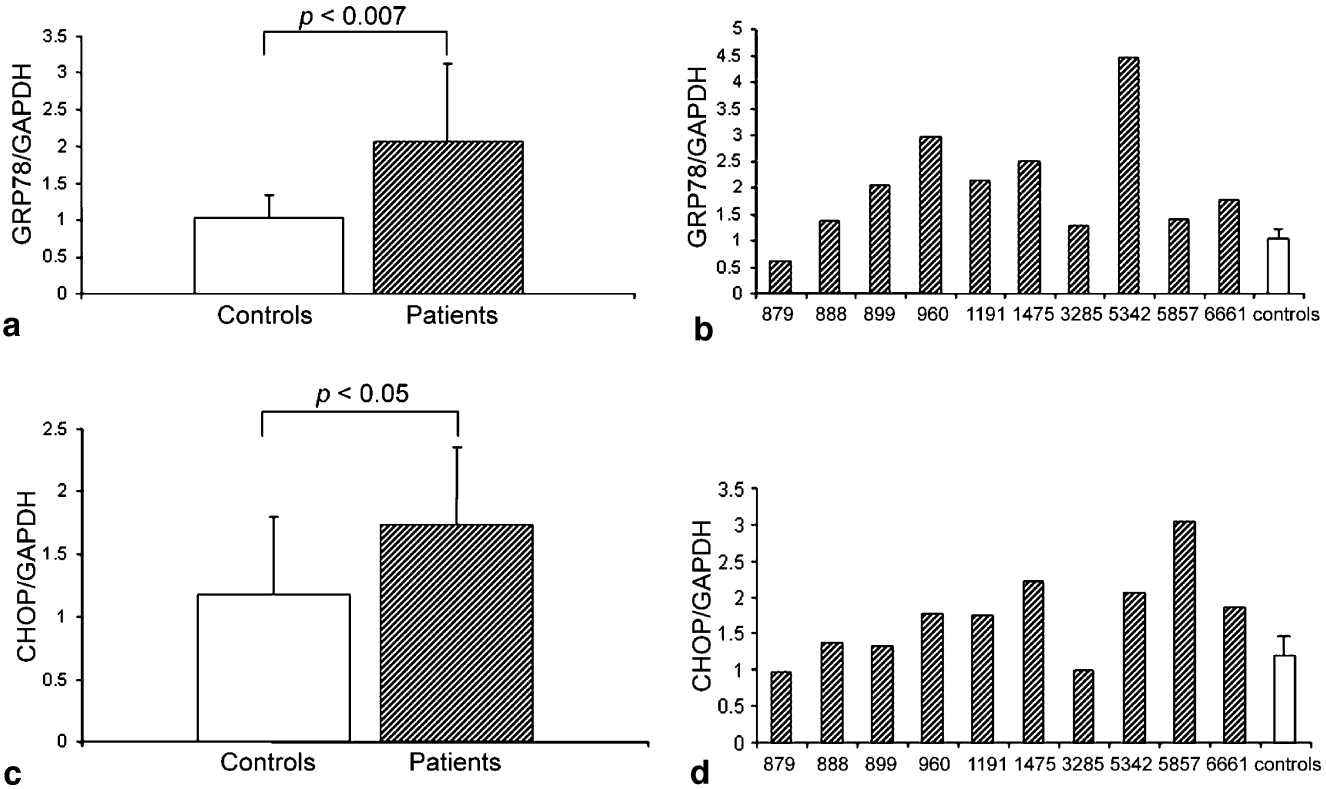


Fig. 4 Up-regulation of GRP78 and CHOP in LGMD2I. Quantitative reverse transcription PCR (RT-PCR) shows significant transcriptional up-regulation of GRP78 and CHOP in LGMD2I patients. The expression of each experimental gene was quantified in relation to GADPH control gene. Quantitation of RT-PCR products is shown with standard deviation and *p* values vs control. **a** and **c** Mean GRP78 and CHOP expression values in patients and controls; **b** and **d** expression values for GRP78 and CHOP for individual patients

mutated alleles in all. The histopathological spectrum in this cohort was not specific, but a variable reduction of α-DG glycosylation was observed in all patients' muscle biopsies, confirming the role of FKRP in α-DG processing in LGMD2I. α-DG is a heavily glycosylated protein that has a pivot role in stabilizing the functional network between the muscle fiber membrane and the extracellular matrix. The interactions between α-DG and its extracellular ligands (laminin, perlecan, neurexin) seem to be mediated by the sugar chains of α-DG itself. Thus it is likely that abnormal glycosylation of this protein may result in derangement of the basal lamina as already observed in other muscular dystrophies due to defective glycosylation of α-DG [19]. Indeed, our ultrastructural studies showed a focally disorganized and thinner basal lamina in LGMD2I.

Various hypotheses have been so far put forward to explain the differences in disease severity in FKRP-related muscular dystrophies (the level of α-DG hypoglycosylation [5, 6, 13], the type and the effect of the *FKRP* gene mutation on the protein function [13], the loss of Golgi localization, and the accumulation of the protein in ER [18]), but none alone fully explain clinical variability.

We hypothesized that mutant FKRP, retained in the ER, may trigger an endoplasmic reticulum stress response and thus modulate clinical phenotype.

Protein glycosylation is an organized and ordered process. Newly synthesized proteins are sequentially modified by glycosyltransferases during transfer from the ER to the Golgi apparatus [11, 23]. Recently, it has been suggested that transportation of mutant FKRP is altered in the cells and leads to ER retention [18]. It is conceivable that mutant FKRP and/or hypoglycosylated dystroglycan may be misfolded in the ER and detrimental to the cell. Misfolded proteins may then trigger an intracellular signalling pathway from the ER to the nucleus, known as the UPR, resulting in transcriptional up-regulation of several ER-resident proteins [11, 23] whose function is to re-establish cell homeostasis. It is interesting to note that by blocking glycosylation, the UPR can be experimentally activated in the yeast *Saccharomyces cervisiae*, the best model system in which UPR has been studied [8].

In order to verify the hypothesis that UPR is activated in LGMD2I, we studied GRP78 and CHOP expression in patients' muscle biopsies. We showed that GRP78, an ER chaperone protein that increases protein folding activity induced by ER stress, is significantly up-regulated in LGMD2I. Moreover, we also showed that CHOP, a pro-apoptotic factor, member of the C/EBP family (C/EBP-homologus protein) also induced by ER stress [20], is up-regulated in LGMD2I, albeit it to a lesser extent. Both

GRP78 and CHOP up-regulation cope with the accumulation of unfolded or misfolded proteins: GRP78 by increasing the folding activity of the ER and CHOP by activating the ER-associated caspases [25].

Moreover, the up-regulation of class I MHC molecules we observed in our cohort of patients may also contribute to the activation of the UPR in LGMD2I. It has recently been shown that overexpression of class I MHC molecules may induce an ER stress response and may mediate muscle-fiber damage and dysfunction in the absence of lymphocytes through a nonimmune pathway involving both Grp78 and CHOP up-regulation [24]. Skeletal muscle fibers do not constitutively express class I MHC molecules, but their up-regulation is an early and consistent feature of human inflammatory myopathies [24]. Interestingly, we observed mild, secondary inflammation in most of our LGMD2I biopsies studied mainly constituted by macrophages, by a few CD4 and CD8 T lymphocytes, and by a variable, but constant, up-regulation of class I MHC molecules.

Lastly, our electron microscopy studies on patients' muscle biopsies provide indirect evidence suggesting that UPR activation occurs in LGMD2I. These revealed rough endoplasmic reticulum proliferation and modifications, as dilated cisternae triad reduplication, and accumulation of lipid droplets.

Expansion of the ER and elevated levels of phospholipid biosynthesis under UPR induction occur in mammalian cells [26] and in yeast [3] and are probably direct to reducing the concentration of unfolded proteins to prevent aggregation.

Unfortunately, we did not find direct correlations between the degree of UPR activation and severity of clinical manifestations suggesting that clinical phenotype in LGMD2I is the result of multiple molecular mechanisms.

The link between the UPR, lipid biosynthesis and biogenesis of the ER in LGMD2I needs further study, but our preliminary data suggest that the histopathological and ultrastructural alterations observed in LGMD2I may be related to the sarcoplasmic structures involved in FKRP and/or α-DG metabolism and malfunctioning.

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ORIGINAL ARTICLE

Interaction between dendritic cells and nerve fibres in lymphoid organs after oral scrapie exposure

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Abstract In transmissible spongiform encephalopathies (TSEs), the infectious agent, called PrPsc, an abnormal isoform of the cellular prion protein, accumulates and replicates in lymphoid organs before affecting the nervous system. To clarify the cellular requirements for the neuroinvasion of the scrapie agent from the lymphoid organs to the central nervous system, we have studied, by confocal microscopy, the innervations within Peyer's patches, mesenteric lymph nodes and the spleen of mice in physiological conditions and after oral exposure to prion. Contacts between nerve fibres and PrPsc-associated cells, dendritic cells (DCs) and follicular dendritic cells (FDCs), were evaluated in preclinical prion-infected mice. Using a double immunolabelling strategy, we demonstrated the lack of innervation of PrPsc-accumulating cells (FDCs). Contacts between nerve fibers and PrPsc-propagating cells (DCs) were detected in T-cell zones and cell-trafficking areas. This supports, for the first time, the possible implication of dendritic cells in the prion neuroinvasion process.

Keywords Prion · Dendritic cells · Innervation

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Introduction

Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are invariably lethal, transmissible neurodegenerative diseases that affect humans and animal species. Prion infections cause characteristic lesions in the central nervous system (CNS). According to the protein-only hypothesis, the causative agent, called prion, is an abnormal conformer of a host membrane glycoprotein. Expression of this normal cell protein (PrPc) is essential to TSE pathogenesis, as the basic pathogenic mechanism involves post-translational conversion of PrPc into the pathogenic conformer, scrapie PrP (PrPsc) [47].

Prions colonize the lymphoid compartment of infected organisms in most TSEs. After intracerebral, oral or intraperitoneal inoculation, prion is detected in many sites of the lympho-reticular system (LRS), including the spleen, lymph nodes, Peyer's patches and tonsils [34]. The accumulation of TSE agents in lymphoid tissues is essential for efficient neuroinvasion [19, 39, 45]. Indeed, it highlights the role of prion-associated cells and the peripheral nervous system, innervating infected lymphoid organs in prions neuroinvasion.

Follicular dendritic cells are stromal-differentiated cells present in the germinal centres of activated lymphoid follicles. Follicular dendritic cells (FDCs) are greatly implicated in prion disease because they accumulate PrPsc

after scrapie infection [30]. Moreover, mice devoid of FDCs and lacking an organized micro-architecture show reduced or impaired prion accumulation in lymphoid organs after peripheral exposure [46]. Immunologic disappearance or de-differentiation of mature and functional FDCs inhibit peripheral prion pathogenesis [35, 40]. However, how TSE agents initially arrive in the FDCs network and spread from the FDCs to the peripheral nervous system remains unclear. TSE agents are unlikely to be transferred directly across an FDC–nerve synapse [34], as such physical contacts between them are rare and each cell type occupies a distinct and separate anatomical site [12, 16]. For example, within the spleen, sympathetic nerves are associated with blood vessels, whereas FDCs are located in the periphery of the white pulp within B-cell follicles. FDCs form immobile networks; thus, a mobile intermediate cell seems necessary to transfer TSE agents to the peripheral nervous system.

The enteric nervous system is an important component of the autonomic nervous system, and it regulates intestinal motility and secretions through stimuli from sympathetic nerves and parasympathetic nerves [15]. Detailed chronological analysis of the distribution of disease-specific PrP within the nervous system of orally inoculated rodents indicates that the scrapie agent subsequently spreads from the gut-associated lymphoid tissue (GALT) to the CNS through the enteric nervous system [6, 7, 27]. Indeed, TSE agents are thought to reach their initial CNS target sites by spreading in a retrograde direction along efferent fibres of both sympathetic and parasympathetic nerves [6, 37]. Parasympathetic nerves, unlike sympathetic nerves, enter the CNS independently of the spinal cord, indicating that TSE agents can enter the CNS through several routes [3]. Once disease-specific PrP enters the spinal cord; it spreads along it in both anterograde and retrograde directions [5]. The neuroinvasion of scrapie in sheep [26, 51], CWD in mule deer [48], sporadic CJD [21] and vCJD in humans [25] from the GALT also seems to occur through the enteric nervous system. Confirmation of the involvement of sympathetic nerves in TSE pathogenesis was provided by experiments demonstrating that neuroinvasion was impaired after depletion of sympathetic nerves [23]. The transmission of TSE agents within the peripheral nervous system and into the CNS is dependent on the expression of PrPc [8, 22]. These findings suggest that innervation of secondary lymphoid organs is a rate-limiting step in the pathway to neuroinvasion [21].

Dendritic cells are migrating cells capable of binding and carrying prion [28]. Interestingly, DCs are sufficient to induce scrapie infection directly via the peripheral nervous system [2]. Anatomical connections between migratory bone-marrow-derived DCs and the peripheral nervous system have been described in Peyer's patches of healthy mice [12, 33]. DCs could function as a bridge between the gut lumen, the TSE replicative machinery and the peripheral nervous system. It is tempting to speculate that DCs could transport prions to the peripheral nerves in lymphoid organs, thereby enabling the process of neuroinvasion.

We examined the topography of the peripheral nervous system in lymphoid tissues, considered to be the likely site of initial neuroinvasion of the infectious prions. We demonstrate contacts between nerve fibres and DCs in preclinical scrapie infected lymphoid organs. These observations support their possible implication in the neuroinvasion process and permit to localise some crucial sites where prions could invade the nervous system.

Materials and methods

Animals

C57Bl/6 mice were maintained with food and drink ad libitum under standard conditions, in compliance with our institution's ethical guidelines. Twelve mice were infected with 139A scrapie strain by oral inoculation of 200 μl of a 10% brain homogenate and were euthanised at the preclinical stage, 105 days post inoculation. Peyer's patches (PP), mesenteric lymph nodes (MLN) and spleens (S) were dissected and available for examination. Twelve age-matched normal brain inoculated control mice were also euthanised at day 105, and lymphoid organs were dissected for examination.

Detection of resistant prion protein

To confirm the presence of scrapie infection in the mice, brains and lymphoid organs were fixed in 4% formalin and embedded in paraplast. Endogenous peroxidase activity was inhibited on sections (7 μm) by a 30-min incubation in 0.3% H_2O_2 in phosphate-buffered saline (PBS). They were pretreated according to the following steps: incubation in 4 M guanidine thiocyanate at 4°C for 2 h, hydrated autoclaving (immersion in citrate buffer pH 6.0) for 10 min at 121°C, immersion in 98% formic acid for 5 min (RT) and in proteinase K at 37°C for 15 min (10 μg/ml). PrPsc detection was performed using the monoclonal antibody SAF 83 (kindly provided by J.Grassi), 1/100 for 2 h at room temperature. The antibody had been pre-incubated with a biotinylated antibody (GAM-biot, Pharmingen, 1/100) at room temperature for 10 min. The enzyme-conjugated streptavidin (AEC, Zymed) and peroxidase-conjugated biotin-avidin complex (ABC, Zymed) were used for light microscopy observations.

Counter-staining was performed by immersion in Papanicolaou solution at room temperature.

Cryosections

The lymphoid organs were immersed in tissue-TEK OCT embedding medium (Sakura, Zouterwoude, The Netherlands) and snap-frozen. Sections (10 μm) were cut at −20°C with a microtome (Microtom HM 500 OM, France), deposited on glass slides coated with poly-L-lysine (Sigma, St. Louis, USA), air-dried, fixed in acetone for 10 min at 4°C and stored at −20°C until use.

Immunofluorescent stainings

After re-hydration, cryosections were incubated with antibodies directed against immune cells and nerve fibers. Incubation with antibodies was performed at room temperature in the dark for 1 h.

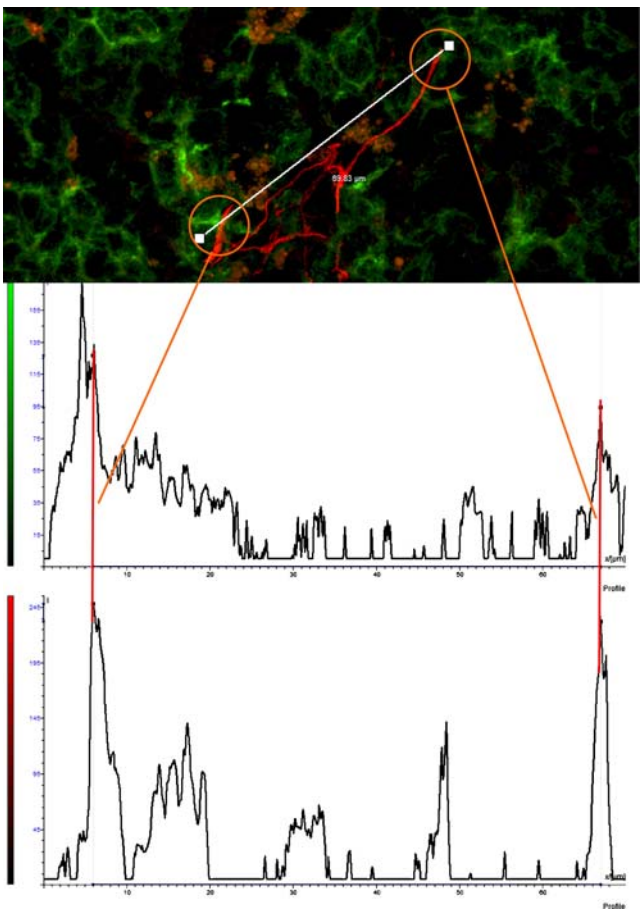

Fig. 1 Confocal analysis of co-localisation of dendritic cells and nerve fibers in scrapie infected mice. Imaging (*left*) and spectral (*right*) analysis of close connections between DCs (*green*) immunolabelled with anti-CD11c antibody and nerve fibres (*red*) detected with anti-NF 150 kDa antibody. The spectral study was realised along the *white bar* on the picture. This picture was one of the confocal images. *Bar* 25 μm

Immune cells were stained with the following antibodies:

1. *Dendritic cells*, anti-CD11c-FITC (1/400) (eBioscience, USA)
2. *Macrophages*, anti-CD11b-PE (1/200) (BD Biosciences, Belgium)
3. *Immature B cells*, anti-IgD-FITC (1/200) (BD Biosciences)

Nerve fibers were labelled with antibodies directed against:

1. *Intermediate neurofilaments*, rabbit anti-mouse *NF H* 1/100 (200 kDa), *NF M* 1/800 (150 kDa) and *NF L* 1/200 (68 kDa; Chemicon, USA).
2. *Schwann cells*, rabbit anti-cow *GFAP* 1/250 (Dako, USA).
3. *Synapses*, rabbit anti-mouse *Synaptophysin* 1/100 (Chemicon)
4. *Catecholaminergic nerve fibers*, rabbit anti-mouse *tyrosin hydroxylase* 1/400 (Chemicon).

A rhodamine-conjugated goat anti-rabbit secondary antibody (Molecular Probe, Leiden, the Netherlands), diluted 1/6,000, was secondary applied on cryosections.

To test the general specificity of the antibodies used, samples were incubated with irrelevant antibodies. Negative controls were obtained by incubating samples with only secondary antibodies.

Confocal analysis

All samples were observed with a Leica (Germany) SP2 confocal microscope. Cryosections of 10 μm were scanned on their best fluorescent zone (5–7 μm), which was divid-

Table 1 Innervation of lymphoid zones and lamina propria in infected and control Peyer's patches

	Villi	Crypts	IFR	SED	Vessels	GC
NF 68 kDa	++	++	+	−	−	−
NF 150 kDa	−	−	+	−	−	−
NF 200 kDa	−	−	−	−	++	−
GFAP	+++	+++	++	+	−	−
TH	++	++	+	+	−	−
Synapto	++	++	−	−	−	−

+++ Strongly innervated; ++ fairly innervated; + slightly innervated; − no positive nerve fibre. *IFR* Interfollicular regions; *SED* subepithelial domes; *GC* germinal centres

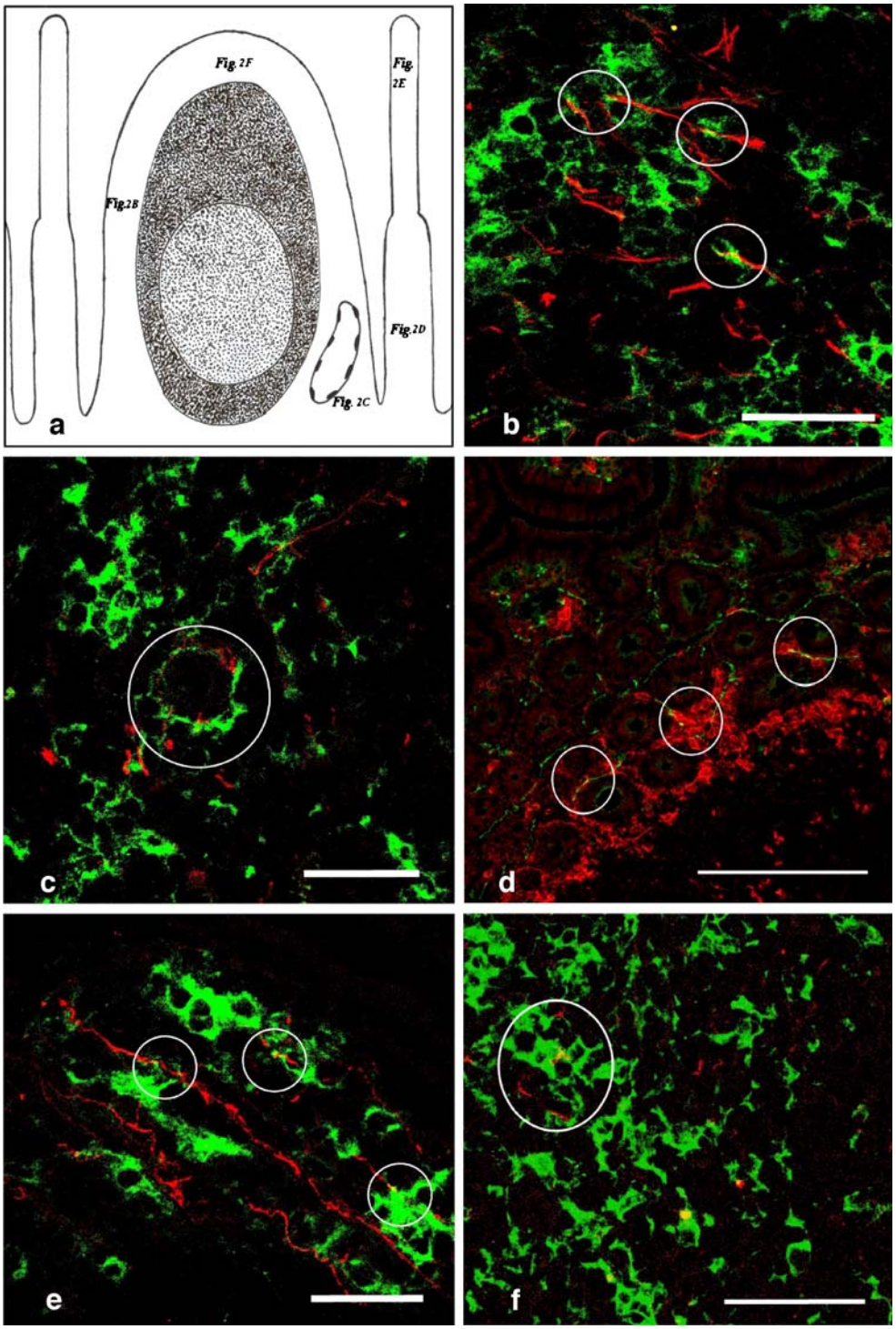

Fig. 2 Neuroimmune connections within scrapie-infected Peyer's patches. Double immunolabellings of dendritic cells highlighted with antibody directed against CD11c and nerve fibres labelled with antibodies raised against: NF 68 kDa (interfollicular region, **b**); NF 200 kDa (vessel walls, **c**); Synaptophysin (crypts, **d**) and GFAP (villi and SED, **e** and **f**) in transverse sections of scrapie-infected Peyer's patches. The localisation of illustrated neuro-DC interfaces in the organ was summarised in the **a**. The numerous close contacts in yellow are surrounded by a *white ring*. *Bars* 40 μm (**b**); 40 μm (**c**); 80 μm (**d**); 25 μm (**e**); 50 μm (**f**)

ed in 15 sections. Each section was analysed. Virtual colors were attributed for detection channels (green for immune cells and red for nerve fibers) and the merge (yellow). Two analytical methods were used to highlight connections between dendritic cells and nerve fibers: image analysis and spectral analysis of color channel (Fig. 1). These combined approaches allowed us to confirm contacts.

Results

Neuro-dendritic cell interfaces in scrapie infected Peyer's patches

Peyer's patches can be histologically separated in two regions: lymphoid and non-lymphoid compartments. Lymphoid areas include the suprafollicular domes, the inter-

Table 2 Neuroimmune contacts between enteric nervous system and dendritic cells in infected and control Peyer's patches

	Villi	Crypts	IFR	SED	Vessels
NF 68 kDa	+	+	+	−	−
NF 150 kDa	−	−	+	−	−
NF 200 kDa	−	−	−	−	+
GFAP	+	+	+	+	−
TH	+	+	+	−	−
Synapto	+	+	−	−	−

+ Proven contact; − absence of contact. *IFR* Interfollicular regions; *SED* subepithelial domes

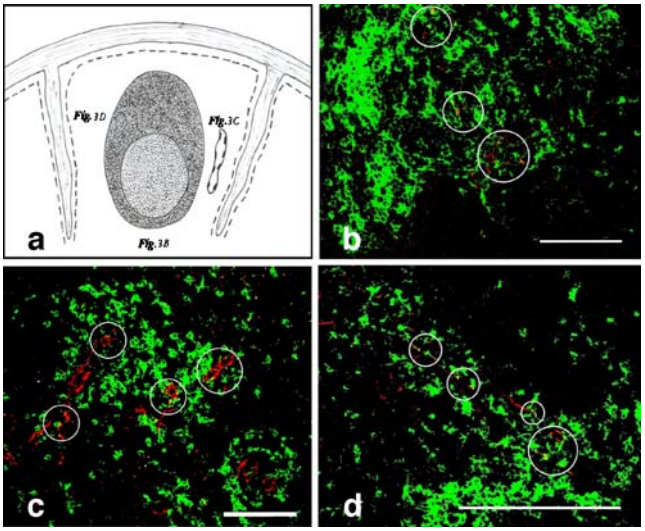

Fig. 3 Interfaces between dendritic cells and nerve fibres in scrapie-infected mesenteric lymph nodes. Double immunolabellings, replaced in their tissular location in the **a**, of dendritic cells highlighted with antibody directed against CD11c (*green*) and nerve fibres (*red*) labelled with antibodies raised against: NF 150 kDa (T-cell zone, **b**), NF 200 kDa (vessel walls, **c**) and GFAP (interfollicular region, **d**) in sections of scrapie-infected mesenteric lymph nodes. Neuroimmune contacts, in *yellow*, were highlighted by a *white circle*. *Bars* 60 μm (**b**); 45 μm (**c**); 100 μm (**d**)

follicular regions (IFR) or T-cell zones, the lymphoid follicles and draining vessels. Non-lymphoid compartments involve lamina propria of villi and crypts.

As described in a previous study on healthy mice using NF antibodies and GFAP [12], the lamina propria is strongly innervated compared with the lymphoid compartments. Our present study on scrapie infected mice, based on additional nerve markers, confirms these observations by highlighting the absence of innervation in the germinal centres (Table 1).

The region IFR is the most innervated lymphoid compartment. In this area, NF 68 kDa, GFAP, TH and NF 150 kDa positive fibres were found in contact with dendritic cells. In the blood vessel walls, DCs established contacts only with NF 200 kDa positive nerve fibres. GFAP, TH, synaptophysin and NF 68 kDa markers were co-localised with DCs within the crypts and in the villi. In the subepithelial domes, above the lymphoid follicles, neuro-DCs interfaces were observed only with the anti-GFAP antibodies (Fig. 2). We obtained the same results in control mice and in mice orally inoculated with scrapie (Table 2).

Table 3 Innervation of lymphoid zones in infected and control mesenteric lymph nodes

	IFR	T Zones	Vessels	Medulla	GC
NF 68 kDa	+	+	−	−	−
NF 150 kDa	−	+	−	−	−
NF 200 kDa	−	−	+	+	−
GFAP	++	++	−	+	−
TH	+	+	−	+	−
Synapto	+	−	−	−	−

++ Fairly innervated; + slightly innervated; − no positive nerve fibre. *IFR* Interfollicular regions; *T Zone* T-cell zones; *GC* germinal centres

Neuro-dendritic cell interfaces in scrapie infected mesenteric lymph nodes

Lymph nodes are classically subdivided in three regions: cortex, paracortex and medulla. The cortex includes lymph follicles and the interfollicular areas. Paracortex is a thymus dependent zone (T-cell zone).

Within the IFR, nerve fibres were fairly to slightly positive to NF 68 kDa, GFAP, TH and synaptophysin labellings. In the T-cell zones, some GFAP, TH and NF 150 kDa-positive nerve endings were observed. Once again, along the blood vessels, the innervation was detected only with the anti-NF 200 kDa antibodies. A slight innervation of the medulla was observed with NF 200 kDa, GFAP and TH immunolabellings. Germinal centres did not contain any nerve fibres (Table 3). In comparison to Peyer's patches, the lymph node appeared less innervated, but the innervation of corresponding lymphoid areas was similar.

Proven contacts between dendritic cells and nerve fibres, NF 150 kDa/GFAP and TH positive, were highlighted in the T-cell zone. Along blood vessels, DCs established contacts with NF 200 kDa positive nerve fibres, like in the Peyer's patches, as they did with synaptophysin, GFAP and

Table 4 Neuroimmune contacts between peripheral nervous system and dendritic cells in infected and control mesenteric lymph nodes

	IFR	T Zones	Vessels	Medulla
NF 68 kDa	−	−	−	−
NF 150 kDa	−	+	−	−
NF 200 kDa	−	−	+	−
GFAP	+	+	−	−
TH	+	+	−	−
Synapto	+	−	−	−

+ Proven contact; − absence of contact. *IFR* Interfollicular regions; *T Zones* T-cell zones

TH positive fibres within the IFR. In contrast, nerve endings and DCs were not in contact in the medulla (Fig. 3). Similar results were obtained on both control and orally scrapie-inoculated mice (Table 4).

Neuro-dendritic cell interfaces in scrapie-infected spleen

The T-cell zones that encircle the central arterioles were slightly to fairly innervated, except with the NF 200kDa marker. Vessel walls were labelled by anti-NF 200 kDa and NF 68 kDa antibodies. The marginal zones that surround the lymphoid follicles and the T-cell zones were positive for antibodies against NF 68 kDa, NF 150 kDa and GFAP. No nerve fibre was identified within the germinal centres (Table 5).

Neuroimmune contacts between the peripheral nervous system and the dendritic cells were detected in different areas of the white pulp. In the T-cell zones, established connections between NF 68 kDa, GFAP, synaptophysin positive fibres and DCs were highlighted. In contrast to Peyer's patches and mesenteric lymph nodes, DCs were in contact with NF 68 kDa and NF 150 kDa positive fibres in the vessels of the white pulp. Within the marginal zones,

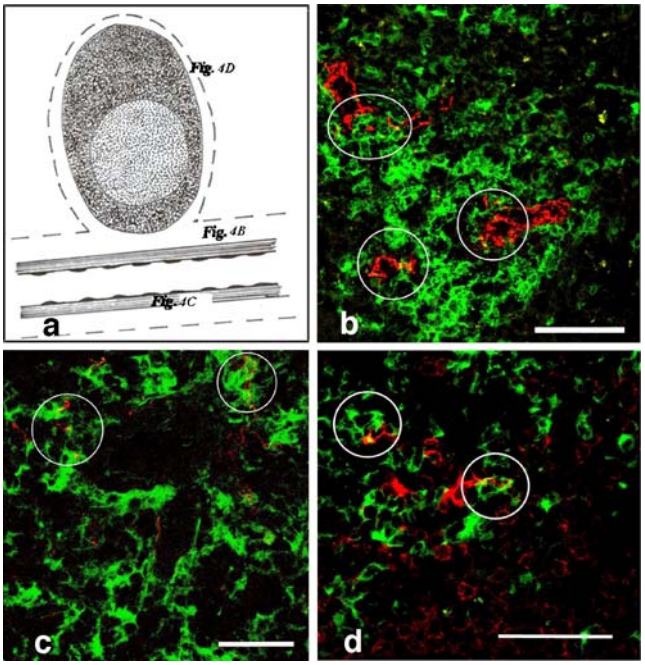

Fig. 4 Neuroimmune connexions within scrapie-infected spleens. Double immunolabellings, replaced in their tissular location in **a**, of dendritic cells highlighted with antibody directed against CD11c (*green*) and nerve fibres (*red*) labelled with antibodies raised against: NF 68 kDa (T-cell zone, **b**), NF 200 kDa (vessel walls, **c**) and GFAP (marginal zone, **d**). Interactions in *yellow* are surrounded by a *white ring*. *Bars* 40 μm (**b**); 30 μm (**c**); 40 μm (**d**)

dendritic cells established connections with GFAP and NF 150 kDa nerve fibres (Fig. 4). As in the other studied lymphoid tissues, the neuroimmune interfaces were highlighted in the same areas in mice inoculated with scrapie or normal brain (Table 6).

Discussion

The oral route is considered the most likely mode of inoculation in natural TSEs such as scrapie [24], BSE or

Table 5 Innervation of lymphoid zones in infected and control spleens

	T Zones	Vessels	Marginal Zones	GC
NF 68 kDa	+	+	+	−
NF 150 kDa	++	−	++	−
NF 200 kDa	−	+	−	−
GFAP	+	−	+	−
Synapto	+	−	−	−

++ Fairly innervated; + slightly innervated; − no positive nerve fibre. *T Zone* T-cell zones; *GC* germinal centres

Table 6 Neuroimmune contacts between peripheral nervous system and dendritic cells in infected and control spleens

	T Zones	Vessels	Marginal zones
NF 68 kDa	+	+	−
NF 150 kDa	−	−	+
NF 200 kDa	−	+	−
GFAP	+	−	+
Synapto	+	−	−

+ Proven contact; − absence of contact. *T Zones* T-cell zones

vCJD [11]. The sequence of BSE and scrapie agent propagation after oral intake suggests that the agent enters the gut mucosa through Peyer's patches and then invades the mesenteric lymph nodes, followed by the LRS unrelated to the digestive tract (e.g. the spleen) [36]. Two mechanisms of prions dissemination are suspected: the blood spreading [34], which emerged with new cases of vCJD after transfusion and the propagation via nerve fibres, studied here. This last one is initiated during the preclinical period [37, 52]. However, the exact location of prion invasion in the nervous system, inside the lymphoid organs, remains as yet unclear.

Several studies have suggested that neuroinvasion occurs from the FDC network [35], but follicular dendritic cells and the peripheral nervous system seem to be anatomically separated. Except in a recent study on bovines where we described nerve endings in contact with follicular dendritic cells [13] and studies focusing on cats [15], pigs [4] and sheep [27], other studies failed to reveal nerve fibres in germinal centres. In this study, we obtained similar results within murine scrapie-infected lymphoid organs and controls: neuroimmune connections appeared to be localized in the same areas and involved the same cell types. Thus, prion infection did not induce a neuro-FDC interface. Even if abnormal germinal centre reactions were reported [38], it has previously been described that prion do not induced histopathological changes [10, 14] or immunotoxic effects [1].

By morphological analysis in confocal microscopy, we pointed several hypothetical sites of neuroinvasion in lymphoid organs. All of them are associated with the peripheral nervous system and migrating dendritic cells. In scrapie-infected Peyer's patches, DCs and nerve fibres were connected in the lamina propria of villi and crypts, in the IFR and the suprafollicular domes. Villi [29] and the suprafollicular dome [20, 41] are suspected to be the site of prion entry within the gut. Thus, they could also be the first site of prion neuroinvasion. Neuro-DC interfaces in the T cell and the marginal zones of spleen as well as in the T-cell zone and the IFR of mesenteric lymph nodes could be a second site of neuroinvasion.

The mechanism of peripheral neuroinvasion by prions from these sites remains unclear. Three components seem to be implicated: the way by which nerve fibres acquire prions, the effect of infectious prions on peripheral neurons and nerve fibres and the transport of prions along nerve fibres to the CNS.

Prions could infect the nervous system via some mechanisms. Authors have argued that exosomes are the clue for prion dissemination [18, 44, 53]. Cells, especially dendritic cells, may exploit the nature of endosome-derived exosomes to transmit the infectious prion protein, bypassing cell–cell contact. Others have proposed that prions are transported directly via small intercellular projections known as tunnelling nanotubes [9]. These exchange processes could provide a mechanism for the efficient spread of proteins from dendritic cells to new host cells [54]. In addition, recent study suggested that the expression of Muscarinic Acetylcholine receptor M2 on dendritic cells could provide a way of prion neuroinvasion [33]. Neuro-immune synapses were suspected since a long time [49], but little proof was presented. Our study shows connections between dendritic cells and nerve endings or nerve fibres in lymphoid organs. These observations are in favour of a synaptic and a neuritic communication between immune and nerve cells. The mechanism of neuroinvasion, within the described potential sites could involve one or more components.

Contrary to brain's neurons, peripheral neurons seem to be infected [50] but not affected by the presence of prion. It implies that the peripheral nervous system (PNS) accumulates prions [32] without a lack of function or death. Moreover, neuropathology of the PNS did not change prion pathogenesis [31]. These surprising phenomena could be imputed to different accumulation mechanisms involving intracellular trafficking and vesicular compartment [43]. Membrane composition and the type of neurotransmitters released [42] could also induce different levels of neuron sensibility to prions.

Authors estimated that the velocity of transport of infectivity in the PNS does not correspond to either fast or slow axonal transport, indicating that PrPsc transport in the PNS may occur through another mechanism [22]. A domino effect fashion via PrPc-paved chain of Schwann cells was proposed. We demonstrate, in this report, the presence of numerous contacts between dendritic cells and GFAP-positive fibres, thus Schwann cells. This is in favour of the involvement of Schwann cells in prion pathogenesis.

Peripheral nerve fibres seem to be static elements, which are not influenced by scrapie infection. Mobile cells are necessary to bring them in contact with infectious prion. Here, we demonstrate connections between nerve fibres and dendritic cells, in healthy and scrapie-infected mice, at strategic sites in lymphoid organs implicated in prion diseases.

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CASE REPORT

Hemorrhagic pulmonary oxalosis secondary to a noninvasive *Aspergillus niger* fungus ball

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Abstract We report a case of hemorrhagic pulmonary oxalosis secondary to a noninvasive *Aspergillus niger* fungus ball. A patient with cavitary lung disease and hemoptysis developed progressive lung infiltrates and intractable metabolic acidosis leading to death. At autopsy, aspergillomas were identified in both the right upper and middle lobes surrounded by a large rim of necrotic and hemorrhagic parenchyma. Microscopic examination showed extensive crystal deposition and vascular thrombosis. Fungal growth was limited to the intraluminal mycelia, and no tissue or blood vessel invasion was present. Remote crystal deposits were also localized in the absence of fungal organisms to the contralateral lung and to the tubules of both kidneys. The crystals were birefringent in polarized light and stained with colloidal iron. X-ray powder diffractometry and electron impact ionization mass spectrometry identified the crystals as calcium oxalate monohydrate. Furthermore, non-necrotizing granulomatous lesions were identified in the lungs, liver, and spleen, consistent with sarcoidosis, and may have predisposed this patient to developing pulmonary aspergillomas.

Keywords Pulmonary oxalosis · *Aspergillus niger* · Fungus ball · Calcium oxalate monohydrate · Metabolite · X-ray powder diffractometry · Electron impact ionization mass spectrometry · Crystal deposition · Sarcoidosis

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Introduction

Aspergillus is a dichotomously branching septate hyphal mold that causes a variety of diseases in both immunocompetent and immunocompromised hosts [2, 4]. Whereas the term aspergillosis denotes either allergic or tissue-invasive disease as a result of *Aspergillus*, the term aspergilloma describes the mycelial growth of the fungus in a cavitary space [1, 4]. A variety of *Aspergillus* spp. cause disease, including *A. fumigatus* (~85% of cases), *A. flavus* (~10%), *A. niger* (~2%), *A. terreus* (~2%), and *A. nidulans* (~1%) [2].

A. niger and, to a lesser degree, other *Aspergillus* species release oxalic acid ($C_2O_4H_2$), a side product of the tricarboxylic acid cycle, as a mycotoxin. The oxalic acid complexes with free calcium ions in tissue and blood to form calcium oxalate that is deposited primarily as monoclinic calcium oxalate monohydrate ($CaC_2O_4{\cdot}H_2O$, whewellite) crystals when the solubility product is exceeded. Oxalate crystal deposition can be locally toxic, causing hemorrhage and tissue necrosis [3, 4, 9].

In the present study, we present the case of a 61-year-old male who died from complications of both hemorrhagic pulmonary and renal oxalosis caused by a pulmonary *A. niger* fungus ball. We illustrate the diagnostic and therapeutic

challenges of noninvasive *Aspergillus* infection and discuss the patient's possible infectious predisposition because of pre-existing sarcoidosis.

Clinical history

A 61-year-old man was admitted to the hospital with progressive shortness of breath, chest pain, and a 2-week history of multiple daily episodes of hemoptysis, each productive of 5–15 ml of bright red blood. During the preceding several months, he had experienced recurrent fevers and a weight loss of 11 kg. His medical history included type 2 diabetes mellitus, hypercholesterolemia, and diverticulosis. He had never smoked and reported no recent travel.

Three months before admission, he had been hospitalized for a persistent, productive cough with one episode of hemoptysis. Computed tomography (CT) examinations performed during the first hospitalization showed small pulmonary right upper lobe cavitary changes (Fig. 1a), bilateral bronchiectasis, and mild splenomegaly. Bronchoalveolar lavage and sputum specimen cultures obtained during the first admission were positive for rare colonies of *A. niger*. A serum galactomannan immunoassay was negative. He was started empirically on rifampicin, ethambutol, isoniazid, and clarithromycin for mycobacterial coverage, but the treatment was discontinued 2 weeks before the second admission when all cultures returned negative.

A transbronchial right upper lobe lung biopsy performed on day 2 of the second hospitalization showed intraluminal mycelial growth of septate hyphae with pigmented conidia, consistent with an *A. niger* fungus ball, and diffuse oxalate crystal deposition but no evidence of tissue or blood vessel invasion. Empirical treatment was started with intravenous vancomycin, levofloxacin, metronidazole, voriconazole, and liposomal amphotericin B. Chest CT examination showed dense consolidation within the right upper and lower lobes and marked interval increase in the right upper lobe cavitary lesions with adjacent ground glass opacity (Fig. 1b). Unfortunately, the patient's condition deteriorated rapidly, and the patient died on day 5 of the second admission with acute respiratory and renal failure with uncontrolled metabolic acidosis. An autopsy was conducted.

Materials and methods

Fresh tissue samples were fixed in buffered formalin and embedded in paraffin. Routine 5-μm sections were stained with hematoxylin and eosin. Special histological stains were performed according to standard protocols established at the Department of Pathology and Laboratory Medicine, Massachusetts General Hospital, Boston, MA. For immunohistochemical detection of *Aspergillus* spp., rabbit polyclonal IgG at 1:50 dilution was used (CP 106, BIOCARE, Concord, CA) on heat-treated paraffin tissue sections, followed by horseradish peroxidase–diaminobenzidine chemistry and alkaline hematoxylin counterstaining. For crystal extraction, tissue was cleared at 20°C in 7% (m/v) aqueous NaOCl for 24–48 h. The cleared solution was then filtered through a 0.45-μm vacuum-driven filter unit (Millipore, Billerica, MA). The crystalline residual was washed extensively with H_2O, transferred into a 1.5-ml reaction tube, and dried at 37°C. Recovery and purity of the isolated crystals were monitored by microscopic examination of air-dried smears on glass slides in polarized light. X-ray powder

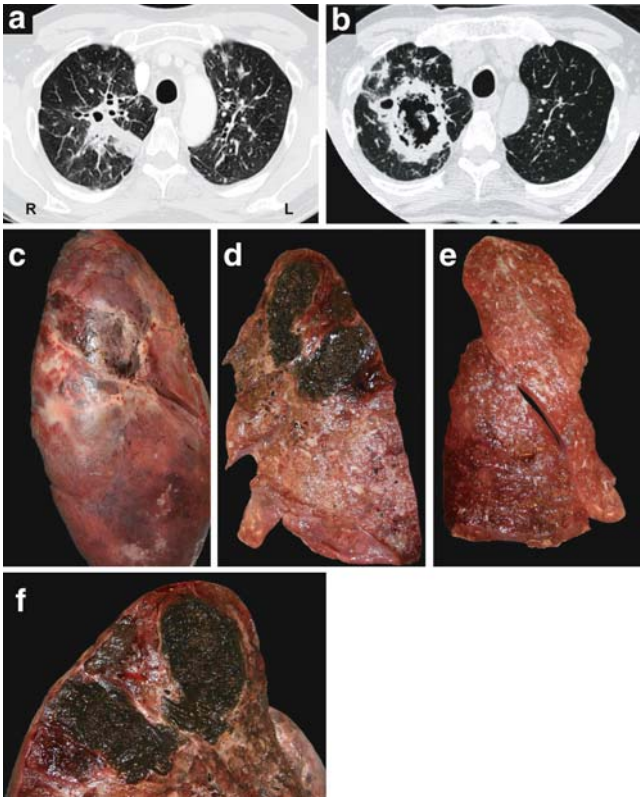

Fig. 1 **a**, **b** Images of transverse chest CT scans through the level of the aortic arch acquired 3 months (**a**) and 5 days (**b**) before the patient's death, respectively, demonstrating the rapid expansile progression of a cavitary lesion in the right upper lobe rimmed by X-ray dense tissue. **c**–**f** Gross autopsy photographs of the intact (**c**) and sectioned (**d**, **f**) right lung showing two communicating cavitary lesions predominantly affecting the upper lobe, each measuring approximately 6 cm in greatest dimension. The lesions are composed of *A. niger* mycelia admixed with dark black, hemorrhagic, and necrotic debris, walled by a thin rim of fibrous tissue, and surrounded by congested, focally infarcted, and hemorrhagic lung parenchyma. The inferior lesion extends partially into the middle lobe. The remaining parenchyma displays moderate consolidation. **e** Representative gross section of the left lung exhibiting moderate consolidation and predominantly inferior lobe congestion. No cavitary lesions were present in the left lung

diffractometry (XRD) was carried out on a Scintag XDS 2000 instrument (Scintag, Cupertino, CA) equipped with a 2.2-kW copper $K_{\alpha 1}$ X-ray source (λ=1.5406 Å) and a vertical theta–theta goniometer. Measured diffraction data were electronically searched against the International Centre for Diffraction Data PDF database (http://www.icdd.com). Electron impact ionization (EI) mass spectra were acquired on a JEOL AX-505H magnetic sector instrument with an electron beam energy of 70 eV (JEOL, Peabody, MA).

Results

Gross pathologic findings At autopsy, the right and left lungs weighed 2,200 and 1,500 g, respectively. Small serosanguineous pleural effusions were present bilaterally. The pleural surface of the right lung showed a circumscribed area of dark red discoloration, surrounded by consolidation of the upper lobe and middle lobes (Fig. 1c). Sectioning of the formalin-inflated lungs revealed two contiguous cavities involving the right upper and middle lobes, each approximately 6 cm in the greatest dimension (Fig. 1d). The lesions contained dark black, friable, hemorrhagic, and necrotic tissue (Fig. 1f). The cavities were surrounded by a rim of tan-gray fibrous tissue and an outer layer of congested, focally hemorrhagic and infarcted lung parenchyma. The remaining right lung parenchyma displayed moderate consolidation and congestion. Gross examination of the left lung revealed moderate parenchymal consolidation and predominantly inferior lobe congestion (Fig. 1e). These findings were suggestive of an evolving acute bilateral bronchopneumonia. No cavitary lesions were present in the left lung.

The remainder of the general autopsy revealed mild biventricular cardiac hypertrophy, serosanguineous ascites, moderate splenomegaly, and mild nephrosclerosis. Neuropathological examination showed mild hypertensive vascular changes in the basal ganglia. All other gross findings were essentially within normal limits.

Microscopic findings Microscopic examination of the right upper and middle lobe cavities revealed abundant intraluminal mycelia with septate hyphae and pigmented conidia. A representative section illustrates the transition from the intraluminal mycelium via a zone of necrotic and hemorrhagic tissue (but free of fungal organisms) to congested and hemorrhagic yet partially viable pulmonary parenchyma (Fig. 2a, left to right). Examination with polarized light revealed abundant deposition of birefringent crystals, predominantly in the necrotic transition zone between the intraluminal mycelium and congested pulmonary parenchyma (Fig. 2b). Crystal deposition was frequently accentuated in the walls of local small- to medium-sized blood vessels containing intraluminal thrombi (Fig. 2b, inset). Extensive histopathological sectioning and examination of the cavitary lesions did not reveal tissue invasion by fungi.

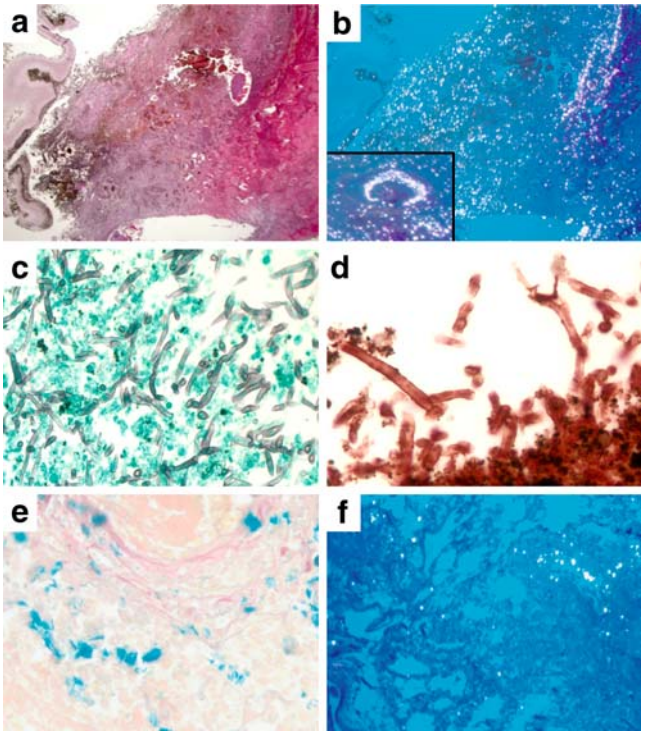

Fig. 2 **a** Hematoxylin–eosin stained representative pulmonary autopsy tissue section taken from a cavitary edge showing (from *left* to *right*) the transition from a well demarcated intraluminal mycelium via a zone of necrotic and hemorrhagic tissue (free of fungal organisms) to congested and hemorrhagic yet mostly viable pulmonary parenchyma. **b** Tissue section identical to **a** but visualized in polarized light revealing abundant deposition of birefringent crystals, predominantly in the necrotic transition zone between intraluminal mycelium and congested pulmonary parenchyma. Note accentuation of crystal deposition in walls of local small- to medium-sized blood vessels (**b**, *inset*). **c** A Gomori methenamine silver stain highlights abundant intracavitary hyphae in the patient's lung lesions. **d** Immunohistochemical identification of the fungal hyphae as *Aspergillus* spp. Note the brown staining reaction of the hyphae, the acute angle branching, and the presence of dark black fungal pigment. **e** The deposited crystals are stained by Hale's colloidal iron reaction (*blue*). **f** Small amounts of birefringent crystals, mostly as intraluminal deposits, are also present in the contralateral (left) lung. However, these deposits were not associated with fungal organisms, hemorrhage, or necrosis. Original magnifications: **a**, **b**, 20× (*inset*, 80×), **c**, **e**, 400×, and **d**, 840×, **f**, 40×

Abundant intracavitary hyphal forms of *A. niger* were seen in the patient's pulmonary cavitary lesions by Gomori methenamine silver staining (Fig. 2c). Immunohistochemically, the fungi reacted with antibodies against *Aspergillus* spp. (Fig. 2d). The morphological appearance (2–5 μm diameter septate hyphae with acute angle branching and pigmented conidia) was consistent with *A. niger*, an impression subsequently confirmed by microbiological culture and speciation of lung tissue samples. The deposited

crystals were stained by Hale's colloidal iron reaction (Fig. 2e). Small numbers of birefringent crystals were also observed in the contralateral (i.e., left) lung, distant from the aspergillomas on the right (Fig. 2f). However, there was no evidence of fungal organisms, hemorrhage, or necrosis.

Both kidneys revealed tubular deposition of crystals (Fig. 3). However, there was no tissue response to the crystals, such as inflammation, hemorrhage, infarction, or necrosis. Furthermore, no crystal deposits along or within blood vessel walls were present in the kidneys.

Numerous scarred non-necrotizing granulomas with occasional multinucleated giant cells were identified in autopsy tissue sections of the lungs, spleen, and liver (Fig. 4). No prominent lymphocytic inflammation was associated with the granulomas. Furthermore, special stains for microorganisms were negative. Taken together, these findings were judged to be consistent with a diagnosis of sarcoidosis.

Analytical chemical findings The chemical composition of the crystalline deposits was analyzed by XRD. We first purified the crystals by clearing lung and renal tissue specimens in aqueous sodium hypochlorite followed by filtration. We then examined air-dried crystal smears on glass slides by XRD. As shown in Fig. 5a, the most intense d-spacing peaks (2θ angles in parentheses) were observed at 5.9116 Å (14.9738°), 3.6377 Å (24.4500°), 2.9626 Å (30.1406°), 2.8335 Å (31.5481°), 2.3520 Å (38.2337°), and 1.9767 Å (45.8688°). The corresponding d-spacing diffraction peaks (2θ angles in parentheses) of a crystalline calcium oxalate monohydrate standard (monoclinic $P2_1/n$ space group), as listed in the International Centre for Diffraction Data PDF database, are expected to appear at 5.93 Å (14.927°), 3.65 Å (24.365°), 2.966 Å (30.104°), 2.840 Å (31.473°), 2.355 Å (38.182°), and 1.978 Å (45.835°). Based on excellent agreement between the experimentally determined d-spacing values and the database entries, the crystals were unambiguously identified as calcium oxalate monohydrate ($CaC_2O_4{\cdot}H_2O$, whewellite).

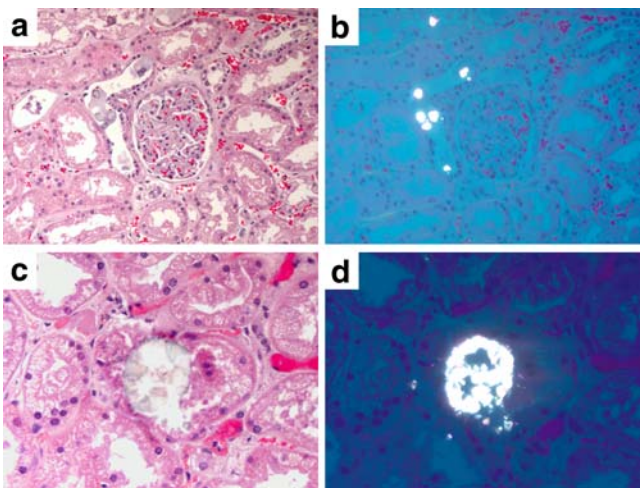

Fig. 3 **a**, **c** Photomicrographs of renal autopsy tissue sections showing intratubular calcium oxalate crystals (hematoxylin–eosin stain). Note the absence of a tissue reaction to the crystals. The tubular epithelium exhibits mild postmortem changes. **b**, **d** Examination in polarized light demonstrates the birefringent property of the crystals. Original magnifications: **a**, **b**, 200×, **c**, **d**, 400×

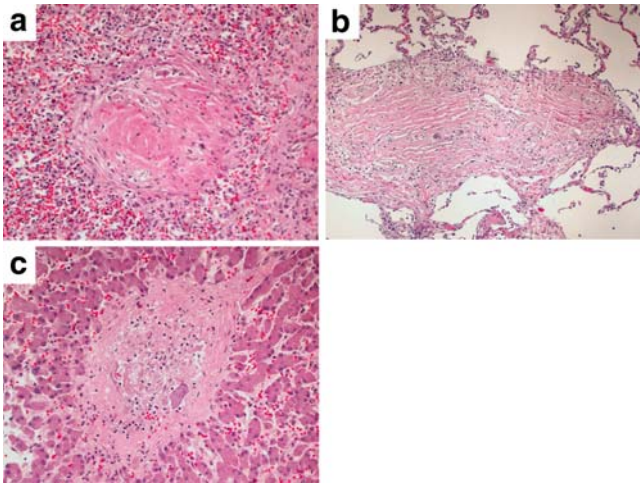

Fig. 4 Photomicrographs of autopsy tissue sections from the patient's spleen (**a**), left lung (**b**), and liver (**c**) showing scarred non-necrotizing granulomas and occasional giant cells without significant lymphocytic inflammation (hematoxylin–eosin stain). These findings are consistent with sarcoidosis. Original magnifications: 100×

The crystalline material was also analyzed by EI mass spectrometry. As shown in Fig. 5b, we readily detected the singly charged molecular cation radical of oxalic acid $\left([C_2O_4H_2{}^{\bullet}]^+\right)$ with a monoisotopic mass of 90 amu. The peak pattern surrounding the major peak at m/z=90 is consistent with reference EI spectra of oxalic acid (Spectral Database of Organic Compounds, http://www.aist.go.jp; NIST Chemistry WebBook, http://webbook.nist.gov) and likely the result of a combination of isotope contributions and gas phase reactions. This finding indicated that free oxalic acid was present among the crystalline material. It should be noted that the components of calcium oxalate salt (free calcium cations and free oxalate anions) are not detectable by EI mass spectrometry.

Discussion

A small number of cases of pulmonary *A. niger* aspergillomas with associated oxalosis have been reported in the predominantly older literature. However, most of these were cases of tissue-invasive aspergillosis [3, 6, 7, 10].

In the present paper, we describe a case of fatal hemorrhagic pulmonary oxalosis secondary to an intracavitary, noninvasive *A. niger* infection. At autopsy, the

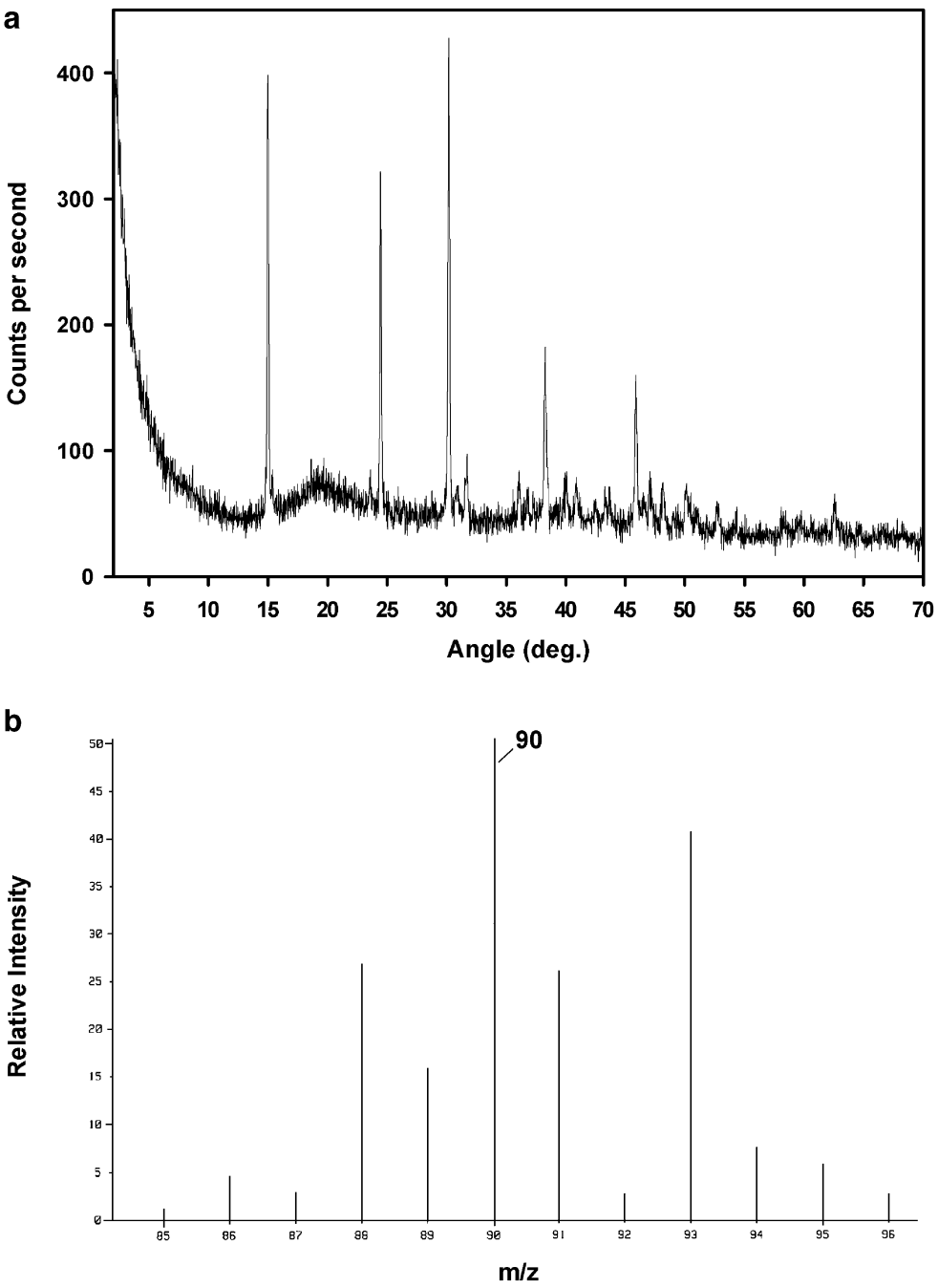

Fig. 5 **a** X-ray powder diffractometry experiment of crystals isolated from the patient's right cavitary lung lesion. The abscissa denotes the 2θ diffraction angle in degrees. The ordinate indicates the measured scattered X-ray intensity in counts per second for a given 2θ angle. Note the sharp diffraction maxima. **b** Electron impact ionization mass spectrum of crystals isolated from the patient's right cavitary lung lesion. The abscissa denotes the measured mass to charge ratio (m/z). The ordinate indicates the relative intensity of ions detected at a given m/z. The largest peak at m/z=90 corresponds to the singly charged molecular cation radical of oxalic acid ($[C_2O_4H_2{}^{\bullet}]^{+}$) with a monoisotopic mass of 90 amu. The peak pattern surrounding the major peak is likely the result of a combination of isotope contributions and gas phase reactions

right upper and middle lobes showed two large communicating cavities containing friable, dark black, partially hemorrhagic material (Fig. 1). Microscopic examination, immunohistochemistry, and microbiological culture identified the lesions as aspergillomas as a result of *A. niger*. There was massive deposition of birefringent crystals in the hemorrhagic and infarcted lung tissue surrounding the fungus balls (Fig. 2). The high concentration of tissue-toxic crystals in and around small pulmonary blood vessels likely caused the extensive hemorrhagic necrosis and rapid expansion of the aspergillomas observed radiographically (Fig. 1a,b) and the rapid clinical deterioration leading to death, all in the absence of fungal invasion of tissues.

Aspergillus hyphae are 2–5 μm wide, frequently septate, and branch at acute angles (Fig. 2) [2]. Often difficult to detect on hematoxylin–eosin stains, hyphae are best visualized by silver stains or by immunohistochemical detection (Fig. 2c,d). Sporulation is rarely observed in tissue, except in air-filled spaces, such as lung cavities or paranasal sinuses. In the absence of sporulating forms, the hyphae cannot be reliably distinguished on morphologic grounds from other pathogenic molds, including *Scedosporium* or *Fusarium*.

The association of *A. niger* infection with tissue crystal deposition is well documented [1–3]. *A. niger*, and to a lesser degree other *Aspergillus* species, release oxalic acid ($C_2O_4H_2$) as a mycotoxin [1, 3, 8]. Oxalic acid is formed as a side product of the tricarboxylic acid cycle by enzymatic hydrolysis of oxaloacetate by oxaloacetate acetylhydrolase (EC 3.7.1.1). The released oxalic acid complexes with calcium ions present in the surrounding tissue and blood to form calcium oxalate molecules that crystallize once the solubility product is exceeded, primarily as monoclinic calcium oxalate monohydrate ($CaC_2O_4{\cdot}H_2O$, whewellite).

Histologic examination of autopsy tissue specimens revealed massive deposition of calcium oxalate crystals in hemorrhagic and infarcted lung tissue surrounding the pulmonary *A.niger* fungus balls (Fig. 2b). It is interesting to note that increased amounts of crystal deposits were seen surrounding and within blood vessel walls in the periphery of the cavitary lesions (Fig. 2b, inset). Oxalate crystal deposition can be locally toxic, causing confined tissue necrosis and hemorrhagic infarction [3, 9]. The deposition of crystals in and around small- and medium-sized arteries may explain the significant degree of hemorrhagic infarction associated with the spatial expansion of the aspergillomas despite the absence fungal tissue invasion.

Examination of both kidneys revealed marked tubular deposition of calcium oxalate crystals (Fig. 3). It is interesting to note that there was no significant inflammatory, hemorrhagic, or necrotizing tissue reaction in the kidneys. Furthermore, there was no clinical evidence of decreased renal function despite substantial crystal burden. It is possible that the renal oxalate deposits developed within a relatively short period before the patient's death, not allowing for the establishment of a histologic response. Alternatively, the local milieu of the kidney may not predispose to or protect against the development of an inflammatory response to oxalate crystals.

We employed analytical chemical methods to establish the molecular identity of the deposited crystals. First, we analyzed crystals isolated from autopsy tissue samples by XRD (Fig. 5a). Crystalline materials have unique and characteristic positions and intensities of X-ray powder diffraction lines (d-spacings). Comparison of our measured data with a comprehensive database of known standard samples (International Centre for Diffraction Data PDF database) unambiguously identified the crystals as calcium oxalate monohydrate ($CaC_2O_4{\cdot}H_2O$, whewellite) in a monoclinic space group. Thus, the presence of significant amounts of other known crystalline forms of calcium oxalate, i.e., di- or trihydrate, was essentially excluded. We next examined the crystalline material by EI mass spectrometry, which can detect molecular cation radicals formed by the impact of electrons possessing low kinetic energy. We readily detected the predicted singly charged molecular cation radical of oxalic acid $\left([C_2O_4H_2{}^{\bullet}]^{+}\right)$ with a monoisotopic mass of 90 amu (Fig. 5b). This finding indicates the presence of free oxalic acid that may be the result of direct release by the fungus before calcium complexation and/or chemical equilibrium conditions between the crystalline salt and the soluble acid.

$CaC_2O_4{\cdot}H_2O$ is poorly soluble at physiologic pH with a solubility product at 37°C of 2.8×10^{-9} mol^2/l^2, corresponding to a saturation concentration of 53 μM. It is interesting to note that the patient's plasma free oxalate concentration determined on hospital day 3 (2 days before death) was normal at 14 μM (normal, <27 μM). This finding suggests that complexation of free oxalate with calcium and subsequent crystallization may primarily occur in confined spaces (e.g., the lungs and the renal tubules) where the local concentration exceeds the solubility product. As a result, the serum free oxalate concentration may be inhibited from rising despite significant fungal burden.

We found that the calcium oxalate crystals stained with Hale's colloidal iron reaction (Fig. 2e). In this reaction, crystals are exposed to aqueous ferric chloride in acetic acid. Under these conditions, ferric ions replace calcium ions as complex partners with the carboxylate groups of oxalate. Subsequent reaction of the bound ferric ions with acidified potassium ferrocyanide results in formation of ferric hexacyanoferrate, a complex of dark blue color (Prussian Blue). The result is of interest because it has been proposed that the adsorption of ferric iron ions available in tissue onto oxalate crystals may contribute to the pathophysiology of crystal deposition in vivo through generation of reactive oxygen species via the Fenton reaction [3].

The patient had experienced several months of progressively productive cough, dyspnea, and weight loss with intermittent hemoptysis. An initial finding of rare colonies of *A. niger* in bronchoalveolar lavage and sputum cultures had been considered to represent saprophytic colonization and had been judged as nondiagnostic for infection in the absence of immunosuppression. Specifically, the patient's differential blood count and the number of circulating $CD4^+$ T cells were within normal limits, and the patient was not taking steroids or other immunosuppressive medications.

Examination of autopsy tissue samples from the lungs, the spleen, and the liver revealed non-necrotizing granulomas with scattered multinucleated giant cells and a paucity of lymphocytic inflammation (Fig. 4). In the absence of microorganisms, these findings were considered most consistent with sarcoidosis. Chronic fibrocystic pulmonary sarcoidosis has been associated with an increased risk of pulmonary aspergilloma [5, 11, 12]. Furthermore, the patient also had type 2 diabetes mellitus, a condition that has been found to predispose to *Aspergillus* infection [4].

Disease manifestations of *Aspergillus* spp. are diverse and depend on the complex interactions between fungus

and host [4]. Within a given tissue or organ, pathology can range from invasive disease to benign, noninvasive saprophytic colonization. Whereas there was no evidence of invasive fungal infection in the present case, the patient ultimately succumbed to massive pulmonary hemorrhage and subsequent multiple organ failure caused by *Aspergillus*-induced calcium oxalate crystal deposition.

In conclusion, we describe an uncommon case of noninvasive chronic cavitary pulmonary aspergillomas as a result of *A. niger*. While clinically not overtly immunocompromised, the patient may have been predisposed to infections by underlying fibrocystic pulmonary sarcoidosis and type 2 diabetes mellitus. In association with the pulmonary aspergillomas, massive deposition of calcium oxalate monohydrate crystals was identified. The chemical identity of the crystals was elucidated by a combination of histological and analytical chemical techniques, including XRD and EI mass spectrometry. The marked hemorrhage and necrosis in the periphery of the rapidly expansile pulmonary lesions were due to the local toxicity of oxalate crystal deposition, rather than direct tissue invasion by fungal hyphae.

This case illustrates the diagnostic challenges posed by noninvasive aspergillomas and sheds new light on the pathophysiology of this disorder. Surgical treatment, which might have been definitive therapy, was precluded in this case by the advanced clinical deterioration at the time of presentation. However, the present case raises questions as to whether the diagnosis of pulmonary aspergilloma as a result of *A. niger* should optimally prompt an early aggressive surgical approach.

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CASE REPORT

Adenocarcinoma of the minor duodenal papilla with intraepithelial spread to the pancreatic duct

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Abstract It is extremely rare to encounter tumors arising exclusively in the minor duodenal papilla. We report a 60-year-old male patient with a polypoid type of adenocarcinoma of the minor papilla. Preoperative examinations, including computed tomography (CT) and magnetic resonance cholangiopancreatography (MRCP), suggested pancreas divisum and showed a series of stones in the dorsal pancreatic duct. The patient underwent subtotal stomach-preserving pancreaticoduodenectomy (SSpPD). On histology, an adenocarcinoma was located in the minor papilla, which was limited to the mucosa, without invasion of the duodenum, sphincter muscles of the minor papilla, or the underlying pancreas. The carcinoma cells, together with dysplastic and hyperplastic epithelium of the pancreatic duct, extended peripherally within the pancreatic duct. No cystic dilatation of the pancreatic duct was observed. The ventral pancreatic duct was short and narrow; there was evidence of chronic pancreatitis in the dorsal pancreas, whereas the ventral pancreas was almost normal, suggesting the existence of pancreas divisum. Although it is well known that adenocarcinoma of the duodenal papilla is sometimes accompanied by intraepithelial spread in the pancreatic duct, an adenocarcinoma arising in the minor papilla in this case with pancreas divisum was more extended than our thoughts.

Keywords Adenocarcinoma · Minor duodenal papilla · Intraepithelial spread · Pancreas divisum

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Introduction

Tumors of the minor duodenal papilla are rare. Most reported cases have been benign tumors, including carcinoid [12, 15, 19, 22, 23], somatostatinoma [3, 13, 20], adenoma [21], adenomyoma [4], and gangliocytic paraganglioma [11, 14]; only one case of adenocarcinoma has been previously reported [24]. Tumors in the minor papilla may be underestimated because of the difficulty in determining the origin of tumors that involve the papilla because the papilla may be overgrown even by small tumors, and the histological appearance of periampullary tumors is similar [1].

We present a case of primary adenocarcinoma of the minor duodenal papilla that exhibited wide intraepithelial spread in the pancreatic duct. Moreover, the existence of pancreas divisum was also suspected because of the distribution of pancreatitis on microscopic examination.

Clinical history

A 60-year-old male patient complained of transient abdominal pain for 2 months. His general practitioner performed gastroduodenoscopy and found a polypoid tumor of the duodenum. The patient was referred to the Department of Hepatobiliary Pancreatic Surgery at the National Cancer

Center Hospital East for further evaluation and treatment. The patient had no history of alcohol abuse.

Material and methods

The surgical specimen was fixed in 10% buffered formalin and submitted entirely for histology. The paraffin-embedded tissue were sectioned and stained with hematoxylin and eosin (H&E). Subsequently, tissue samples were stained immunohistochemically with the following monoclonal antibodies: cytokeratin (CK) 7 (1:100; Dako), CK20 (1:50; Dako), Muc-2 glycoprotein (1:100; Novocastra laboratories), and Muc-5AC glycoprotein (1:50; Novocastra laboratories).

Results

On physical examination, there were no abnormal abdominal findings. Other than slightly elevated γGTP (57 IU/l: normal 10–47) and blood glucose (107 mg/dl: normal 69–104) levels, all other laboratory tests, including the hematological profile, renal function, pancreatic enzymes, liver enzymes, electrolytes, and tumor markers (carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA)) were within normal limits.

On gastroduodenoscopy, the stomach appeared normal, but there was a villous polypoid tumor, about 3 cm in diameter, with a stalk in the second portion of the duodenum (Fig. 1). The major duodenal papilla was identified about 2 cm distal to the tumor. Computed tomography (CT) demonstrated a solid 40×35-mm tumor with expansive growth that occupied the lumen of the descending portion of the duodenum and showed slight attenuation with contrast medium. Tumoral extension toward the underlying pancreas was not detected. The CT also showed a series of pancreatic stones within the duct of the head of the pancreas. The distal side of the pancreatic duct was dilated and the parenchyma of the distal pancreas showed mild atrophy. Neither lymph node involvement nor distant metastasis was detected. Magnetic resonance cholangiopancreatography (MRCP) (Fig. 2) and the coronal view of magnetic resonance image (MRI) showed the relationship between the stones and the pancreatic duct more clearly. The series of stones was about 3 cm long; one end of the stones appeared to be positioned near the stalk of the tumor, and the other end was within the main pancreatic duct. The stones were suspected to be located within the dorsal pancreatic duct. MRCP also revealed a short ventral pancreatic duct; a communication between the dorsal and ventral pancreatic duct was not clearly identified. These findings suggested the existence of pancreas divisum.

Pathological examination of the preoperative biopsy specimen revealed a well-differentiated adenocarcinoma. With a presumptive diagnosis of adenocarcinoma of the duodenum or minor duodenal papilla and chronic pancreatitis, the patient had surgery. At laparotomy, because the tumor had a thick and broad stalk on palpation, it was suspected that the tumor might have invaded the duodenum or the underlying pancreas. Consequently, a subtotal stomach-preserving pancreaticoduodenectomy (SSpPD) was performed.

Macroscopic examination showed a villous polypoid tumor, 50×30×25 mm in size; the stalk was 15 mm in diameter. The tumor was located about 2 cm proximal from the major papilla, which was normal in size and shape. The cut surface showed that the dorsal pancreatic duct was

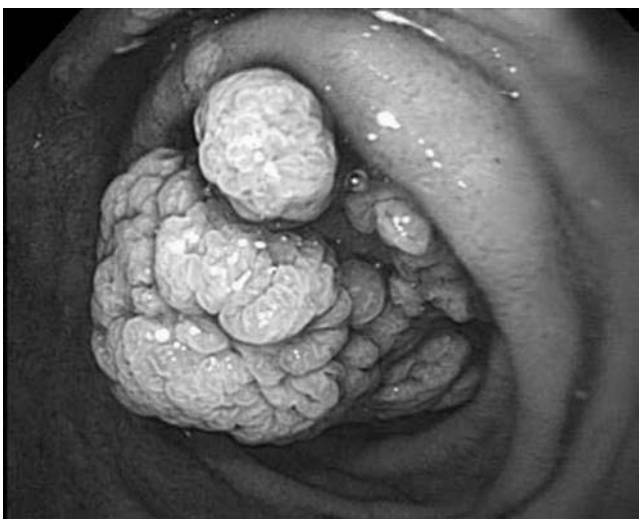

Fig. 1 Gastroduodenoscopy showing a villous polypoid tumor with a stalk in the second portion of the duodenum

Fig. 2 Magnetic resonance cholangiopancreatography (MRCP) showing a short ventral pancreatic duct (*white arrow*); the communication between the dorsal and ventral pancreatic ducts is not clearly identified. A series of stones (*white arrow head*) is seen in the dorsal pancreatic duct

obstructed by a series of stones, as had been demonstrated by the preoperative examinations (Fig. 3).

Microscopic examination showed that the main exophytic tumor was composed of eosinophilic tall columnar cells with oval and pseudostratified nuclei that were arranged in well-formed tubular pattern (Fig. 4a). The tumor was diagnosed as a well-differentiated tubular adenocarcinoma. The main tumor was limited to the mucosa and regarded as in-situ carcinoma without stromal invasion. At the tumor's stalk, muscular bundles similar to the sphincter of Oddi, which encircled the dorsal pancreatic duct, were present. These findings suggested that the tumor had arisen from the minor papilla and not from the duodenum. The adenocarcinoma cells spread through the sphincter bundles and the dorsal pancreatic duct; they replaced the normal pancreatic duct epithelium with intraductal carcinoma peripherally (Fig. 5a). Intraductal adenocarcinoma with micropapillary projection was observed in almost all areas of the dorsal pancreas, predominantly around the dorsal pancreatic duct. At the frontal edge of the intraductal spread, dysplastic epithelium and hyperplastic epithelium were observed (Figs. 5 and 6). At the cut end of the pancreas, the epithelium of the main pancreatic duct and the other branched ducts showed hyperplastic changes, which were considered to be reactive changes caused by tumor spread.

On immunohistochemical staining, both the main polypoid adenocarcinoma and the intraductal lesions showed the mixed positive pattern of CK7 and CK20. Muc-5AC was also multifocally positive both in the main tumor and the intraductal components. Muc-2 was negative except small number of cells in the base of the main tumor. The expression pattern for CK7, CK20, and Muc-5AC in the main tumor was maintained even in the ductal spreading area of the pancreas (Fig. 4).

Chronic pancreatitis was found in the dorsal pancreatic parenchyma, with infiltration of inflammatory cells and fibrocollagenous tissue; the ventral pancreas was almost normal (Fig. 5a). The ventral pancreatic duct was short and narrow, and the epithelium of the ventral pancreatic duct did not include any carcinoma, dysplastic cells, or hyperplastic cells. These findings supported the presence of pancreas divisum. It is surprising to note that intraductal spread of carcinoma cells was observed in the small branches of the uncinate process, which anatomically belongs to the ventral pancreas, which was unaffected by pancreatitis (Figs. 5a and 6). About half of the uncinate process had carcinoma in situ. Neither lymphovascular invasion nor lymph node metastasis was observed.

The postoperative course was uneventful and the patient was discharged on the 13th postoperative day.

Discussion

The minor duodenal papilla is situated in the anterior duodenal wall, about 2 cm proximal to the major papilla [2, 16]. It primarily drains pancreatic fluid from the dorsal pancreas to the duodenum in the embryo [7].

Tumors of the minor duodenal papilla are uncommon, and few cases have been reported. Most reported cases have been submucosal benign tumors, such as carcinoid [12, 15, 19, 22, 23] and somatostatinoma [3, 13, 20]; only a single case of adenocarcinoma of the minor papilla has been previously reported [24]. Yamano et al. [24] reported a 77-year-old male with an ulcerating tumor, in which the dorsal pancreatic duct epithelium was partially replaced by carcinoma cells from the minor papilla; however, details of the pathological findings were not described. In contrast to our case, their case also had an intraductal papillary

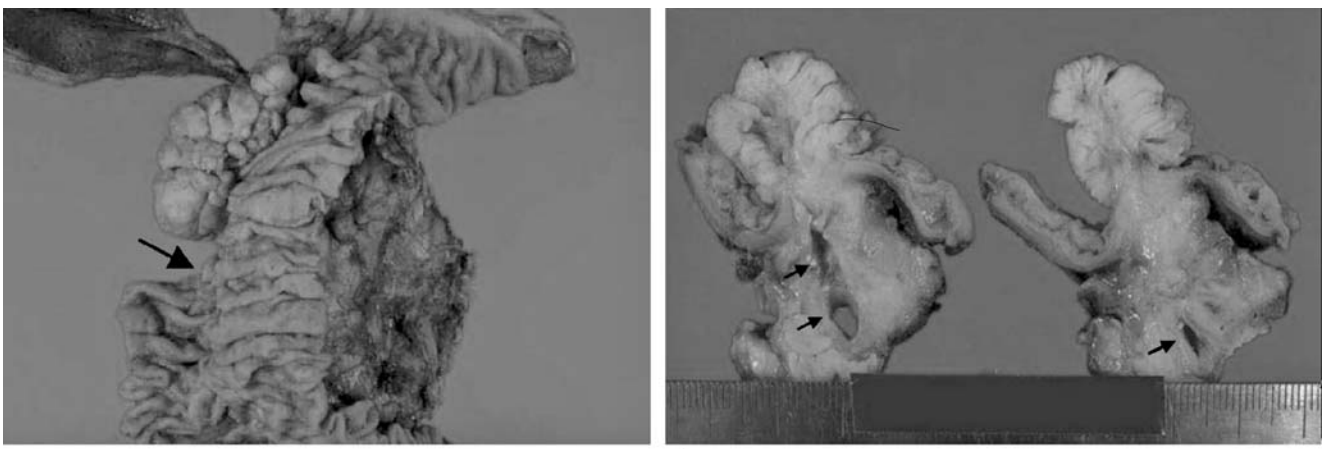

Fig. 3 **a** An exophytic polypoid tumor arising from the minor papilla. The major papilla (*arrow*) is normal in size and shape. **b** Cut section shows that the dorsal pancreatic duct was obstructed with a series of stones. (*arrows*; dorsal pancreatic duct with stones removed)

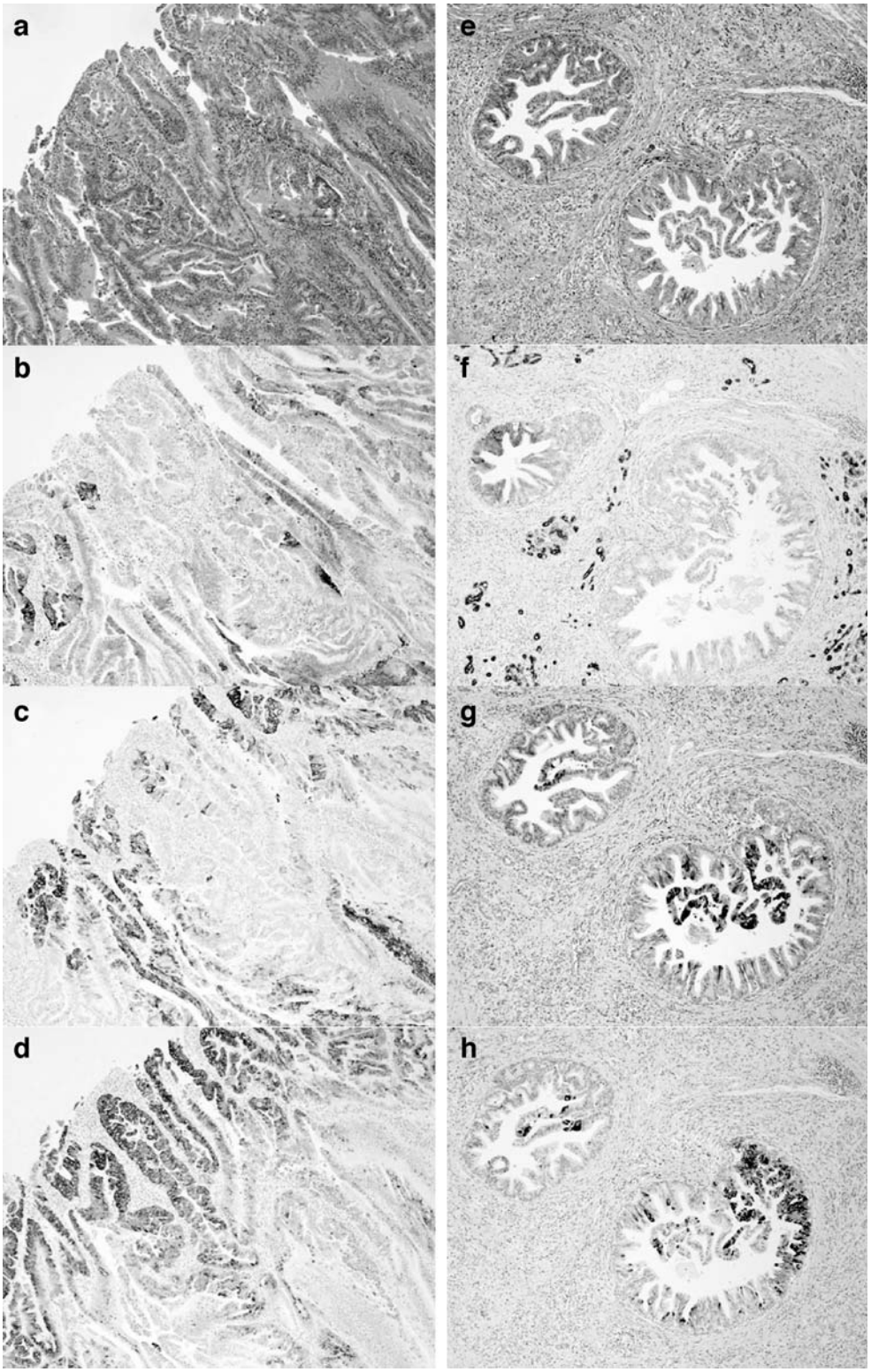

Fig. 4 **a**–**d** In-situ carcinoma of the main polypoid lesion (H&E, **a**) showing immunohistochemical positivity for CK7 (**b**), CK20 (**c**), and Muc-5AC (**d**) (×40). **e**–**h** Carcinoma in situ observed in the dorsal pancreatic duct (H&E, **e**) showing immunohistochemical positivity for CK7 (**f**), CK20 (**g**), and Muc-5AC (**h**) (×40)

adenoma with mucin hypersecretion in the cystically dilated branch duct in the head of the pancreas. Although the two tumors differed in their gross type, both cases had intraductal spread of the cancerous component. In the present case, however, carcinoma in situ had spread close to the cut end margin and also to the tiny peripheral branches without cystic dilatation.

In addition, our case had calcified stones located in the dorsal pancreatic duct. The majority of cases of pancreatic stones are secondary to chronic pancreatitis; however, the

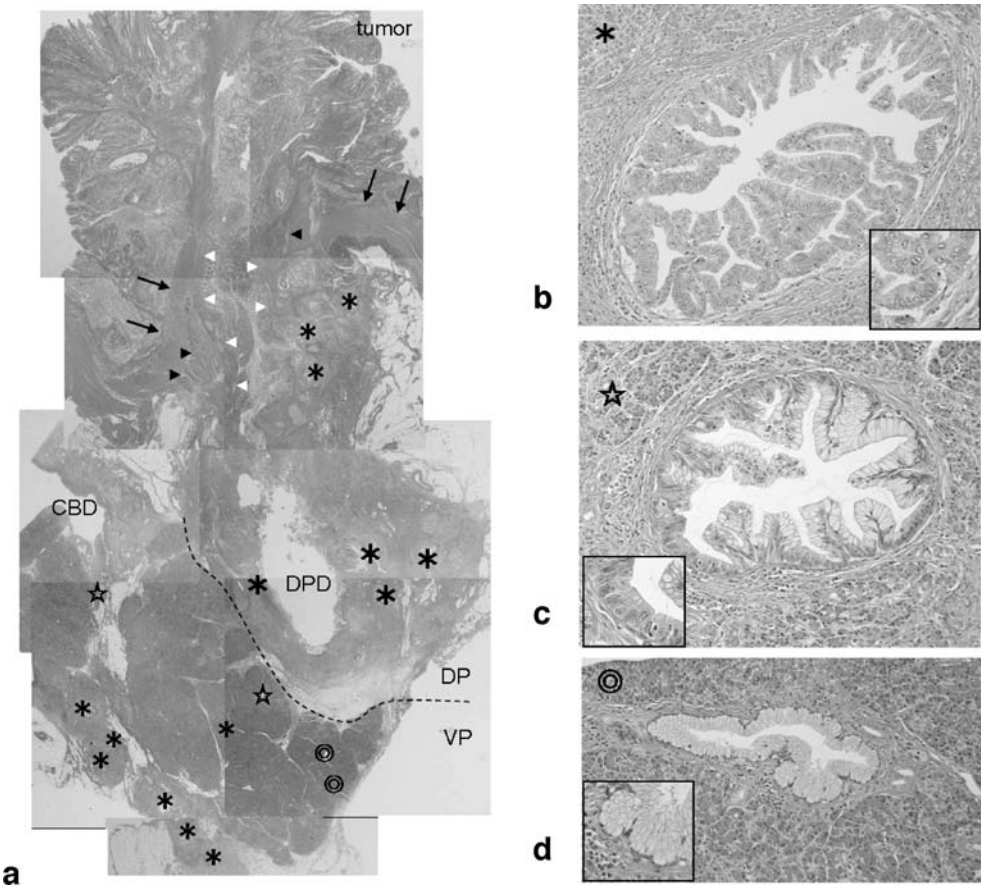

Fig. 5 **a** At the stalk of the tumor, muscular bundles (*black arrow* heads), which encircle the dorsal pancreatic duct (*white arrow heads*), are present. The carcinoma in situ has spread through the sphincter bundles to the branched duct around the dorsal pancreatic duct (DPD); dysplastic and hyperplastic epithelium are present peripherally (*black arrows*, muscular layer of the duodenum; *, carcinoma in situ; ☆, dysplastic epithelium; ◎, hyperplastic epithelium; *CBD*, common bile duct). Chronic pancreatitis is evident in the dorsal pancreas (DP), while the ventral pancreas (VP) appears almost normal. **b** Carcinoma in situ (*) (H–E×100). **c** Dysplastic epithelium (☆) (H–E×100). **d** Hyperplastic epithelium (◎) (H–E×100)

size, number, and distribution of stones vary by the type of pancreatitis [5, 10, 18]. In alcoholic chronic pancreatitis, there are numerous irregular small stones throughout the pancreas. On the other hand, in obstructive pancreatitis, the stone is usually large, solitary, and inside the lumen of the pancreatic duct. The pathogenesis of the stone is considered to be because of the stasis of pancreatic flow [17, 25]. With respect to stones in patients without a history of alcohol abuse, stagnation of pancreatic fluid as a consequence of the duct obstruction by a tumor might lead to development of stones, as in the present case.

The MRCP and MRI findings and the distribution of pancreatitis, which mainly affected the dorsal pancreas, suggested the existence of pancreas divisum. Pancreas divisum is a common congenital anomaly of the pancreas, which results from an abnormal fusion between the ventral and dorsal

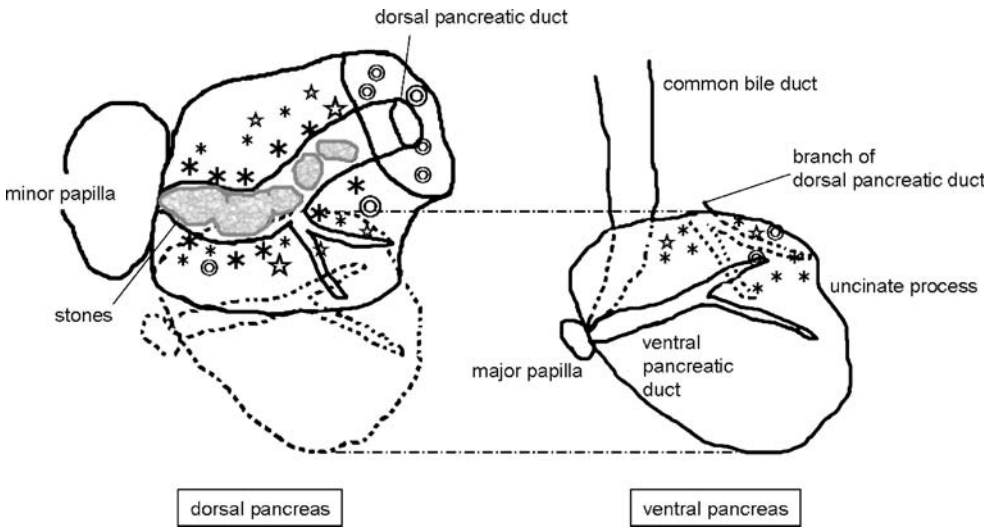

Fig. 6 Schematic distribution of carcinoma in situ (*), dysplastic epithelium (☆), and hyperplastic epithelium (◎). The dorsal and ventral pancreases are depicted separately for convenience. Carcinoma in situ is located predominantly near the dorsal pancreatic duct, and the dysplastic and hyperplastic epithelium is observed surrounding the carcinoma in situ. The branches of the uncinate process, which belongs to the ventral pancreas, are partially involved with cancerous spread

pancreatic ducts during fetal development [8]. It is divided into complete and incomplete types. In the complete type, there is no communication between the two ducts, whereas in the incomplete type, an inadequate communication exists between the two ducts. Pancreas divisum is strongly associated with pancreatitis, especially in alcoholic patients. Irrespective of the type of pancreas divisum, pancreatitis often occurs only in the dorsal pancreas, as was observed in this case. An interaction between a poorly functioning minor duodenal papilla and the increased flow of pancreatic juice caused by alcohol or food intake is thought to cause pancreatitis [8]. The presence of this anomaly in our patient may have promoted stasis of the pancreatic juice and the formation of the pancreatic stones.

In the present case, most of the intraductal carcinoma was limited to the dorsal pancreas, though some carcinoma was observed in the branches of the uncinate process, which is anatomically classified as being part of the ventral pancreas. In complete pancreas divisum, intraductal carcinoma in the dorsal pancreas never spreads to the ventral pancreas. Because intraductal components were observed in the uncinate branches, minor peripheral communications must have existed between the two ducts in our patient.

Pathologically, adenocarcinoma of the major papilla is classified into two types: intestinal and pancreatobiliary type, based on the epithelium of its origin [1, 9]. The former is derived from intestinal (duodenal) mucosa covering the papilla, whereas the latter is associated with pancreatobiliary epithelium lining the common channel and duct systems within the papilla. This classification is also supported by immunohistochemical staining such as cytokeratin and apomucin [26]. CK20 and Muc-2 are associated with the intestinal type, whereas CK7 and Muc-5AC expression is relatively specific for the pancreatobiliary type. Immunohistochemical staining in the present case showed the mixed positive expression for CK7 and CK20. This finding indicated that the tumor might arise from the transitional area between the intestinal mucosa covering the minor papilla and the dorsal pancreatic ductal epithelium. Moreover, the mixed positive pattern for CK7 and CK20 was observed uniformly from the main polypoid adenocarcinoma to the minute intraductal lesions, suggesting that the character of both components was equivalent. This fact supported that the intraductal components were extended from the main lesion.

On microscopic examination, the morphological feature of the intraductal carcinoma component was similar to that seen in intraductal papillary mucinous neoplasm (IPMN) of the pancreas or pancreatic intraepithelial neoplasia (PanIN). IPMN generally demonstrates grossly visible cystic lesions or exophytic masses (usually >1 cm in diameter) along with grossly visible papillae and luminal mucin production [6]. In the present case, the lesion suggesting IPMN was not detected in either radiological or macroscopical examination, and moreover, mucin was not observed on the cut surface of the specimen. In addition, the main polypoid tumor conclusively involved the minor papilla. The intraductal component, which was observed even in the tiny peripheral branches (<5 mm) with lowering the grade of the lesion distally, was in direct continuity with the main tumor. Taken together, it is consistent that the association of IPMN or PanIN was less likely, and the tumor originated from the minor papilla, then extended into the distal pancreatic duct.

The pathogenesis of our case likely involved the following factors: (1) the patient had a congenital, incomplete type of pancreas divisum that had been asymptomatic; (2) the tumor arose in the minor papilla and obstructed the dorsal pancreatic duct; (3) stagnation of the pancreatic fluid from the dorsal pancreas caused pancreatitis and the formation of calcified stones; (4) on the other hand, the intraductal tumor extended widely into the peripheral ducts, and some tumor components traveled through small communications between the dorsal and ventral pancreatic ducts, and eventually reached to the branches of the uncus, which was unaffected by pancreatitis.

It is interesting to note that, in this case, the intraductal component of the adenocarcinoma of the minor papilla extended along the pancreatic duct more than expected; it extended even beyond the minor communication between the dorsal and ventral pancreatic ducts of pancreas divisum, which was detected incidentally.

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CASE REPORT

Isolated vasculitis of the female genital tract: a case series and review of literature

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Abstract To provide a clinicopathologic review of vasculitis confined to the female genital tract, we describe three cases, and we searched PubMed from 1965 to 2006 with analysis of all relevant articles. We identified 118 additional cases in the literature of whom 108 had isolated necrotizing vasculitis similar to classical polyarteritis nodosa (PAN-type), and 10 presented isolated giant cell arteritis (GCA-type) of the female genital tract. In most cases, arteritis was discovered surprisingly. The mean age of these patients was 48.6 years for the PAN-type and 64.1 for the GCA-type. Vasculitis affected a single organ in 88 (81.5%) cases of which 71 (65.7%) involved the cervix in the PAN-type, whereas it was limited in the myometrium in five (50%) cases in the GCA-type. There was no progression to a systemic vasculitis in 99.1% of the cases. A comparable favorable outcome was reported after surgery in all cases with a mean follow-up of 40.8 months. We conclude that isolated vasculitis of the female genital tract is a distinct condition, with two different patterns but a similar favorable outcome after resection. Its knowledge is needed to avoid aggressive evaluation and therapy.

Keywords Vasculitis · Female genital tract · Polyarteritis nodosa · Giant cell arteritis

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Introduction

Vasculitis apparently confined to one organ is well described in the skin, kidney, central nervous system, peripheral nervous system, appendix, pancreas, gallbladder, breast, or epididymis. However, vasculitis confined to the female genital tract is rare, with about a hundred reported cases in the literature. Moreover, the follow-up in these cases was short, with different patterns of histological lesions being reported and systemic involvement sometimes being apparent at the time of operation or at subsequent follow-up.

We report a series of three cases of vasculitis limited to the female genital tract with a prolonged follow-up. We have reviewed their clinical and pathological features and all those reported in the literature to analyze whether vasculitis of the female genital tract may represent a distinct and limited condition and to describe its characteristics.

Materials and methods

Three cases of necrotizing vasculitis of the female genital tract were diagnosed by pathological examination, between 1993 and 1997, at our institution. All pathological specimens were reviewed with an extensive analysis of the entire uterus, fallopian tubes, and ovaries. Paraffin sections were stained by hematoxylin and eosin. The number of vessels

involved and their sizes were determined. The type of parietal infiltrative cells was analyzed. A complete clinical and biological workup was conducted in 2006 for these patients. An extensive literature review was carried out by searching the PubMed Medline database. All relevant publications from 1965 to 2006 in several languages were included. We performed analysis of all relevant articles. We used the 1990 American College of Rheumatology criteria for the classification of polyarteritis nodosa (PAN) [25], giant cell arteritis (GCA) [17], and Wegener granulomatosis [24] and the microscopic polyangiitis definition according to the 1994 Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis [19].

Clinical history

The clinical characteristics and histological features of our three patients with necrotizing vasculitis apparently confined to the female genital tract are reported in Table 1.

Patient 1 A 49-year-old woman complained of pelvic pain and vaginal bleeding. The patient did not present declining general health. No abnormality was found on examination. A full blood count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were within the normal ranges. Ultrasound investigation showed an enlarged polymyomatous uterus. A total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed.

Histopathology showed necrotizing vasculitis lesions on two artery walls (sizes 360 and 480 μm) only in the uterine cervix. There was severe inflammatory cell infiltration in and around the vascular wall (lymphocytes, neutrophils, and eosinophils) and fibrinoid necrosis of part or the entire vessel (Fig. 1a). Vascular lumina were narrowed, but no thrombus was seen. Examination of the myometrium also found many benign leiomyomas.

The patient had no further treatment and recovered within 2 weeks. Antineutrophil cytoplasmic antibody (ANCA) tests have always remained negative. The patient has remained well with a follow-up of 118 months.

Patient 2 A 54-year-old woman presented with a sudden right pelvic and lumbar pain. There was no sign evoking a systemic vasculitis. Vaginal examination showed a right adnexal mass and a congestive cervix. Laboratory data including a urinalysis, a full blood count, the CRP rate, renal and liver function tests were normal. Ultrasound investigation showed a large pelvic mass with calcifications inside (72×70 mm) spreading from the right fallopian tube to the top of the bladder. A total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed.

Table 1 Clinical characteristics and histological features of our three patients with isolated necrotizing vasculitis of the female genital tract

Number	Age (years)	Symptoms	Type of vasculitis	Site of vasculitis	Number of involved arteries	Size of involved arteries	Pattern	Fibrinoid necrosis	Thrombi	Infarcts	Aneurysms	Infiltrate	Follow-up (months)	Associated lesions
1	49	Menorrhagia, pelvic pain	PAN	Cervix	2	360 to 480 μm	Diffuse	Yes	No	No	No	Lymphocytes, neutrophils, eosinophils	118	Leiomyoma
2	54	Lumbar and pelvic pain	PAN	Myometrium, mesovarium	2	840 μm both	Diffuse	Yes	Yes	No	No	Lymphocytes, neutrophils	170	Right ovarian teratoma
3	72	Fever, ESR=101, CRP=6	PAN	Fallopian tubes, ovaries, myometrium, isthmus, cervix, mesovarium	Numerous	324 to 1,320 μm	Diffuse	Rare	No	No	No	Lymphocytes, rare neutrophils, few giant cells	129	Mucinous cystadenoma

ESR Erythrocyte sedimentation rate (mm/h), *CRP* C-reactive protein (mg/dl), *PAN* polyarteritis nodosa

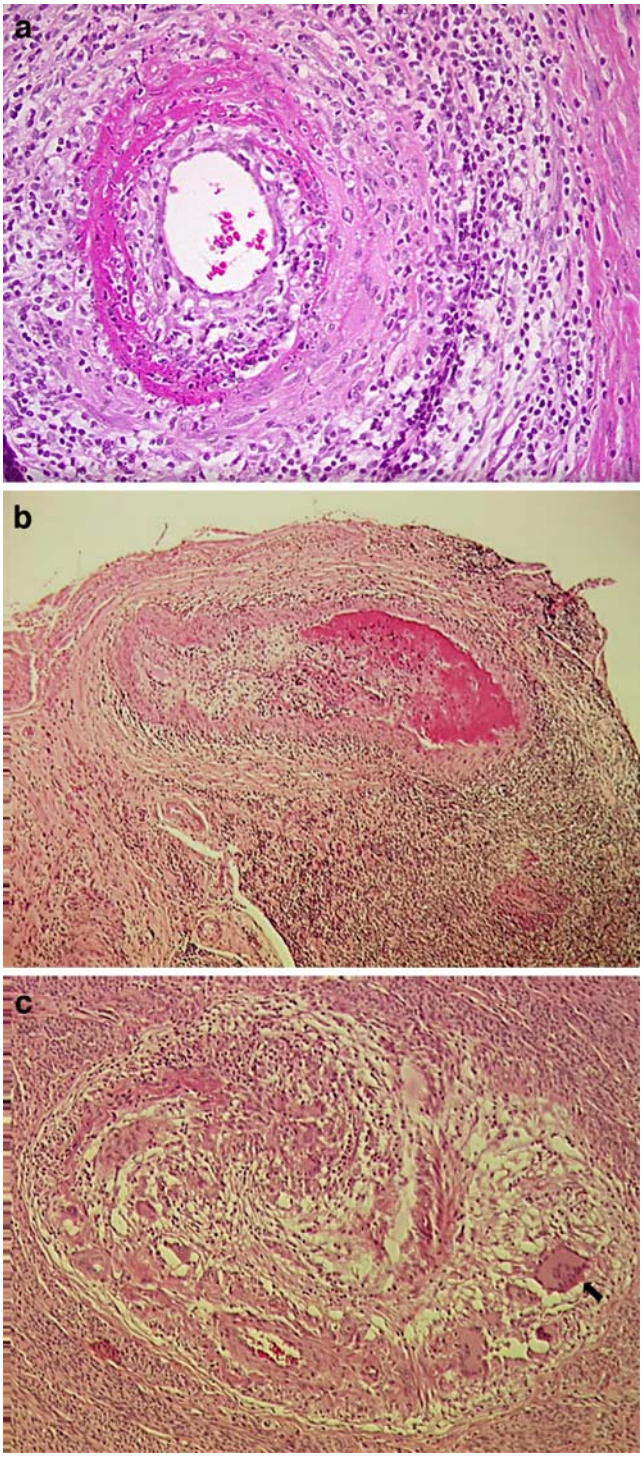

Fig. 1 **a** Inflammatory infiltrate and fibrinoid necrosis of a 480-μm arteriole of the cervix. Patient 1, hematoxylin and eosin ×400. **b** Transmural mixed inflammation and fibrinoid change in an 840-μm artery of the mesovarium, with destruction of the wall and luminal thrombus. Patient 2, hematoxylin and eosin ×200. **c** Mixed inflammation of a 1,320-μm artery wall, in the uterus, including multinucleated giant cells (*arrow*). Patient 3, hematoxylin and eosin ×400

Histopathology showed a mature cystic ovarian teratoma, containing hair and calcifications. Analysis of the myometrium and mesovarium showed necrotizing vasculitic lesions affecting two different 840-μm arteries. Fibrinoid necrosis had spread all through the vessel wall with very severe inflammatory cell infiltration (lymphocytes and neutrophils). One of the arteries was obstructed by a fibrin thrombus (Fig. 1b).

The patient had no further treatment. She recovered within 10 days. With a 170-month follow-up, she has developed no clinical or biological disorder evoking systemic vasculitis. ANCA tests always remained negative.

Patient 3 A 72-year-old woman was admitted because of a 2-month history of isolated fever (37.7 to 38.5°C). The patient was in a good general health. There was no recent infection. Physical examination revealed an isolated enlarged left ovary, whereas she presented neither pelvic pain nor vaginal bleeding. The ESR was 101 mm after the first hour, and CRP was 7.7 mg/dl. Antinuclear antibodies were positive at 1/200 (both Hep-2 and rat liver cells) with no anti-DNA. ANCA test, cryoglobulinemia test, and serologies for hepatitis B and C viruses were negative. Other laboratory data including a full blood count, blood cultures, renal and liver tests were normal or negative. A computed tomography (CT) scan of the pelvis showed a 45-mm cystic mass in the left adnexa. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed.

Histopathology showed a mucinous cystadenoma with no evidence of malignancy associated with widespread vasculitis affecting the ovaries and fallopian tubes, the myometrium, the isthmus, the cervix, and the mesovarium. Many arteries (324 to 1,320 μm) showed inflammatory cell infiltration composed mainly of lymphocytes and neutrophils with fibrinoid necrosis of the vessel wall. One giant cell was found (Fig. 1c) but remained isolated despite an extensive pathological examination. The internal elastic lamina was disrupted or totally destroyed. No vascular thrombosis was seen.

No further treatment was performed. Fever disappeared, and ESR and CRP eventually normalized. ANCA tests have always remained negative. With a 129-month follow-up, she has developed no clinical or biological manifestation that would have evoked systemic vasculitis.

Results and discussion

In addition to our three patients, our literature review found a total figure of 118 reported cases of vasculitis confined to the female genital tract [1–7, 9–13, 15, 18, 20–23, 26, 28, 29, 31, 32, 35–41]. Pathological features showed that 108 had isolated necrotizing vasculitis of the female genital tract

Table 2 Clinical characteristics and histological features of 118 patients with isolated vasculitis of the female genital tract

Author (reference)	*N*	Age	Type	Site	Size of involved arteries	Follow-up	Associated lesions
Ansell [2]	10	43.5 (34–52)	PAN	CVX	Medium	33 (0–180) months	CIN (10%)
Fayemi [9]	2	60 (57–63)	PAN	CVX	Medium	6 months–?	Leiomyoma (50%), perineuritis (100%)
Lin [26]	1	34	PAN	CVX, MM	Small to medium	4 years	None
Crow [6]	4	49.7 (18–78)	PAN	CVX	Small	6 weeks	None (50%), leiomyomas (25%), CIN (25%)
Wilansky [39]	11	45.3 (22–62)	PAN	CVX (54%), EXT (27%), OV (9%), FT (9%)	Small	56 (12–96) months	CIN (9%), bladder carcinoma (9%), cervical carcinoma (9%)
Fetissoff [10]	1	53	PAN	CVX	Medium	3 years	CIN
Tranbaloc [38]	1	57	PAN	CVX	Small	1 year	CIN
Padwell [32]	3	43 (37–49)	PAN	CVX	Small to medium	?	Chronic cervicitis (100%), leiomyomas (67%), endometrial polyp (33%)
Womack [40]	1	36	PAN	CVX, OV	Small	15 months	Endometriotic benign cyst
Lauritzen [23]	2	45 (35–55)	PAN	CVX	Small	4 (1–7) years	CN (50%), adenomatous hyperplasia (50%)
De Mascarel [7]	1	52	PAN	CVX	Small	13 months	Leiomyomas
Ilbery [18]	1	35	PAN	OV	Small to medium	18 months	None
Julie [20]	1	40	PAN	CVX	Small	?	CIN
Neises [31]	1	44	PAN	CVX	Small to medium	?	?
Kaya [21]	1	48	PAN	OV	Small to medium	9 months	Leiomyomas, benign ovarian cyst
Abu-Farsakh [1]	1	40	PAN	EXT	?	10 months	Leiomyomas
Francke [12]	11	57 (37–74)	PAN	EXT (36%), CVX (18%), CVX+IS (27%), IS (9%), OV (9%)	Small to medium	89.4 (12–156) months	Leiomyomas (36%), CIN (18%), endometrial adenocarcinoma (18%), benign ovarian cysts (18%), endometriosis (9%)
Pilch [35]	3	53.7 (46–68)	PAN	CVX (67%), EXT (33%)	Small to medium	29.3 (4–48) months	Leiomyoma (67%), endometrial hyperplasia (33%)
Woywodt [41]	5	41 (23–53)	PAN	CVX	Small to medium	28.8 (12–60) months	CIN (20%), leiomyomas (20%)
Chatelain [5]	1	50	PAN	CVX	Small (600)	3 months	Adenomyosis
Ganesan [13]	41	50.1 (22–80)	PAN	CVX (63%), OV (10%), EXT (10%), MM (7%), IS (7%), FT (3%),	Small=less than 2 mm (60%), medium=2 to 10 mm (33%), any (7%)	3 (1–23) years	Leiomyomas (46%), adenomyosis (30%), CIN (12%), cancer (12%), endometrial polyp (12%), benign ovarian cyst (7%), ovarian teratoma (2%)
Fraenkel-Rubin [11]	1	51	PAN	CVX, OV, FT	Medium	6.5 years	Leiomyomas
Kini [22]	1	62	PAN	CVX, MM	Medium	11 months	Benign ovarian cyst
Heazell [15]	1	49	PAN	CVX	?	?	Leiomyoma
Pisal [29]	1	34	PAN	CVX	Medium	18 months	CIN
Banerjee [3]	1	44	PAN	CVX	small	2 years	None

Table 2 (continued)

Author (reference)	*N*	Age	Type	Site	Size of involved arteries	Follow-up	Associated lesions
Pirozynski [36]	2	72.5 (66–79)	GCA	MM	?	2.5 (2–3) months	None (50%), leiomyomas (50%)
Martinelli [29]	1	69	GCA	MM	?	?	None
Bell [4]	1	64	GCA	FT, OV	?	17 years	Fibromas
Marrogi [28]	3	59.3 (46–73)	GCA	CVX (33%), MM (33%), EXT (33%)	?	54 (6–144) months	Leiomyomas (67%), endometrial hyperplasia (33%)
Abu-Farsakh [1]	1	77	GCA	MM	?	22 months	Endometrial adenocarcinoma, adenomyosis
Ganesan [13]	2	54 (53–55)	GCA	CVX (50%), PM (50%)	Small (50%), medium (50%)	10 (1–19) years	Endometrial carcinoma, leiomyoma (50%), tuberculous salpingitis (50%)

N Number of reported cases, *Age* mean age in years (range), *FU* mean follow-up (range), *CIN* cervical intraepithelial neoplasia, *PAN* polyarteritis nodosa, *GCA* giant cell arteritis, *CVX* cervix, *OV* ovaries, *EXT* extensive, *IS* isthmus, *MM* myometrium, *FT* fallopian tubes, *PM* parametrium

(INVF) and 10 presented isolated giant cell arteritis of the female genital tract (IGCAF; Table 2). The histological patterns in INVF are similar to those described in systemic PAN. These consist of a severe inflammatory cell infiltration in and around the vessel (mainly neutrophils, and eosinophils and lymphocytes) with fibrinoid necrosis of part of or throughout the artery wall. The internal elastic lamina is often disrupted. The vasculitic process is often segmental (only a segment of the artery may be affected along its length) and focal (a normal, unaffected artery may lie side by side with a severely affected one), with coexisting acute and healed vascular lesions. As well as the necrotizing patterns, systemic PAN may also induce luminal thrombi, as seen in case 2 from our series. In IGCAF, arteritis affects the small- to medium-sized vessels, with predominance of giant cells, lymphocytes, macrophages, and less common eosinophils. There is disruption of the internal elastic lamina with luminal narrowing or even thrombus.

The mean age of the patients with INVF was 48.6 years (18–80). In two cases, bleeding related to a vasculitis-induced ulcer [1] or which could have been related to vasculitic lesions of the left ovary [18] was the presenting symptom. However, in most cases of INVF, as in our observations, arteritis was discovered by routine histological examination of specimens obtained for apparently different local gynecological lesions. Indeed, other histological lesions are associated in 82 (75.9%) of 108 reported cases (Table 2), the most frequent being benign leiomyomas (35 cases, 32.4%) [6, 7, 11–13, 32, 35, 41].

One of our patients (case 3) presented with fever and a raised ESR of 101 mm/h that normalized after surgery. She had no systemic symptoms at the time of diagnosis or later with a 10-year follow-up. Patalano and Sommers [33] described vasculitic lesions in the female genital tract in 6 (35%) of 17 necropsied cases of systemic PAN. In the literature, to our knowledge, there are only nine cases of necrotizing vasculitis of the female genital tract revealing a systemic PAN [8, 13, 14, 16, 27, 30, 34, 38]. The mean reported ESR was 96.9 mm/h (range 50–140), and in six cases, the ESR was above 100 mm/h [8, 14, 27, 30, 34, 38]. This contrasts with the low value of ESR found in INVF (mean 16.6, range 2–80, but mentioned in only 24 cases). No ANCA was found in these reported cases. In the literature, there was no progression of INVF to a systemic disease over a mean follow-up of 39.2 months (range 2–276) except a single patient who was reported to have developed a PAN 8 years after hysterectomy [13].

In most cases of INVF, the extent of vasculitic lesions is limited. A single organ being involved was encountered in 88 (81.5%) cases, of which 71 (65.7%) cases involved the cervix [2, 5–7, 10, 12, 13, 20, 23, 32, 35, 38, 39, 41], 10 (9.3%) cases, the ovary [11–13, 18, 21, 39, 40], 10 (9.3%) cases, the myometrium [1, 13, 22, 26, 28, 36, 28], 4 (3.7%) cases, the isthmus [12, 13], 2 (1.9%) cases, the fallopian tubes [13, 39], and 1 (0.9%) case, the parametrium [13] (Table 2). Furthermore, involvement of a single vessel was observed in 29 (26.9%) among the 108 cases of INVF. In contrast, in systemic PAN revealed by gynecological involvement (nine cases), extensive vasculitis lesions of the whole female genital tract were found in five cases (55.6%) [14, 16, 27, 34, 38]. Nevertheless, extension of the vasculitic lesions in the female genital tract cannot predict the outcome in an individual. For example, five cases of INVF with extensive vasculitic patterns never developed a systemic disease [13] and the single patient who developed

a PAN 8 years after INVF had vasculitis initially confined to the small arteries of the cervix [13].

As well as these PAN-type gynecological vasculitides, 48 cases of GCA have been reported with gynecological involvement. In all the cases, GCA of the female genital tract had unexpectedly been discovered upon pathological examination of surgically removed genital organs for different gynecological lesions. More than three out of four patients had signs suggestive of systemic GCA or polymyalgia rheumatica. In contrast, only ten of these patients could be considered as having isolated GCA of the female genital tract (IGCAF) [1, 4, 13, 28, 29, 36]. None presented systemic vasculitis symptoms at the time of diagnosis. Their mean age was 64.1 years (range, 46–79), which is older than for INVF (48.6 years). ESR was raised in four cases [1, 29, 36], with a mean value of 59.5 mm/h (range, 46–67), normal in three cases [13, 28], and not mentioned in three cases [4, 28]. The vasculitic process was confined to the myometrium in five (50%) cases [1, 29, 36], the cervix in two (20%) cases [13, 28], the parametrium in one (10%) case, but was extensive in two (20%) cases [4, 28]. This is different from INVF, which affected only the cervix in two thirds of cases. As in systemic forms, the pathological features may occasionally show some overlap between IGCAF and INVF: fibrinoid necrosis of the media was (infrequently) seen in IGCAF [36], and giant cells were found in a small number of INVF cases [13] as in one of our patients in whom one giant cell was found but remained isolated despite an extensive pathological examination (case 3). In all cases of IGCAF, a favorable outcome was observed after isolated surgery, with a mean follow-up of 59.2 (range, 2–204) months.

In conclusion, vasculitis of the female genital tract rarely occurs in systemic vasculitides. However, isolated vasculitis of the female genital tract is a distinct condition with 121 reported cases, including our series. These cases can be split into 111 PAN-type cases and 10 GCA-type cases. The condition is revealed by gynecological symptoms not due to vasculitis, diagnosed upon pathological findings identical to those of classical PAN or GCA and cured after isolated resection. Its recognition is important for preventing heavy evaluation or medication.

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LETTER TO THE EDITOR

Small intestinal CD4+ T-cell lymphoma: a rare distinctive clinicopathological entity associated with prolonged survival

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Dear Editor,

Intestinal T-cell lymphomas are rare and heterogeneous. Most of them are localised high-grade large cell lymphomas and carry a poor prognosis. The WHO classification only recognises one distinct entity of intestinal T-cell lymphoma, called "enteropathy-associated T-cell lymphoma" [5]. This lymphoma arises in pre-existing celiac disease. The neoplastic cells infiltrate the overlying epithelium, mimicking normal intraepithelial lymphocytes (IELS). They are CD3+, CD5−, CD7+, CD8−/+ and also express the homing receptor CD103 antigen, typically seen in normal and malignant intraepithelial T lymphocytes. CD4 is always negative.

In 1999, Carbonnel et al. [1] reported four patients with clinico-pathological features suggesting a new distinct entity defining extensive small intestinal CD4+ T-cell lymphoma.

We had the opportunity to diagnose an additional case of this T-cell low grade lymphoma in a patient without any evidence of celiac disease, associated with prolonged survival.

In January 2004, a 23-year-old man was admitted in our hospital with a 4-month history of diarrhoea, weight loss (−4 kg) and massive abdominal distension.

Laboratory findings revealed marked eosinophilia (absolute eosinophil count, 6,185 per mm^3) and polyclonal hypergammaglobulinemia (16.7 g/l, N<16 g/l) with normal IgM, IgA and IgE levels. An abdominal computed tomography (CT) scan revealed multiple enlarged mesenteric and retroperitoneal lymph nodes.

Histological examination of the ileal biopsies showed an abundant and diffuse pleomorphic infiltrate, predominantly located in the lamina propria. This infiltrate was composed mainly of small- to medium-sized cells, sometimes cleaved, with inconspicuous nucleoli and plasma cells (Fig. 1a). Crypt architecture was preserved. The villi were normal or slightly shortened. There was no increase in IELs. There was no lymphoepithelial lesion. Numerous polymorphonuclear eosinophils and extensive epithelioid granulomatous reaction were associated with the lymphoid infiltrate (Fig. 1b). Gastric and colonic biopsies were normal. A few days later, the patient developed sub-acute small bowel obstruction, and a laparoscopic ileal resection was performed (Fig. 1c). Mesenteric and ileal lymph nodes were

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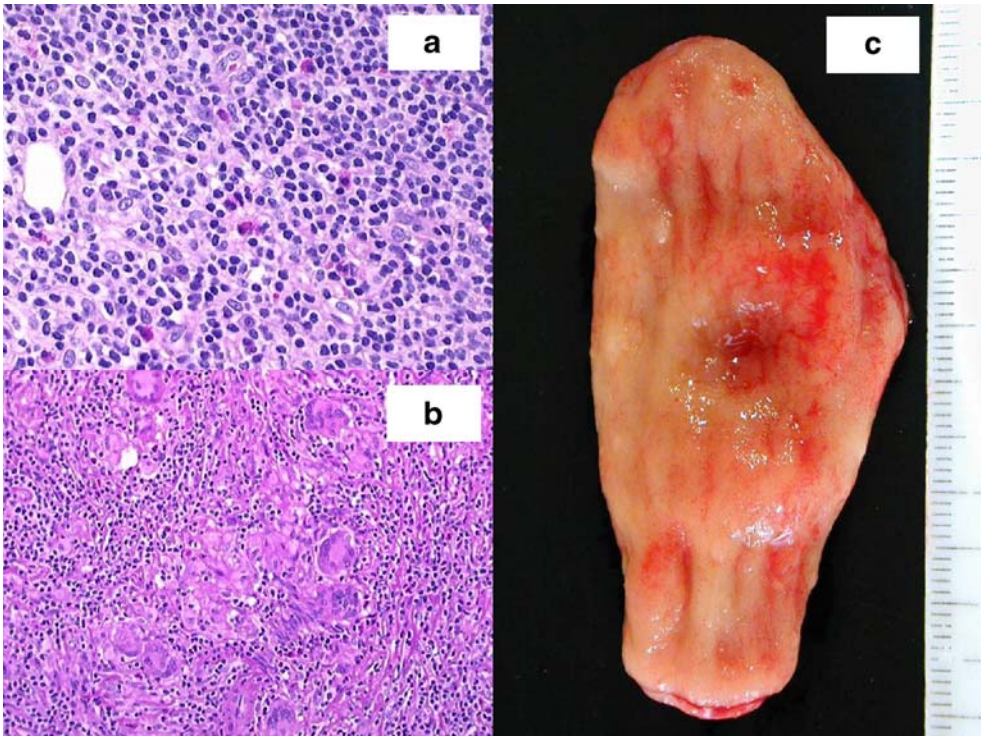

Fig. 1 Neoplastic cells consist of small to medium size cells. Note the presence of eosinophils (hematoxylin and eosin, magnification ×400; **a**). Epithelioid histiocytes with giant cells, forming small clusters and granulomas of different size are intermingled with the neoplastic cells. The granulomas show no central necrosis (hematoxylin and eosin, magnification ×200; **b**). Macroscopic view of surgical ileal resection: the affected bowel segment is oedematous. The tumour presents as a main ulcerating mucosal mass with multiple small nodules all over (**c**)

also resected. In the resected ileal tissue, the infiltrate predominated in the lamina propria, but involved the entire intestinal wall. The mesenteric and ileal lymph nodes architecture was replaced by sheets of small lymphocytes showing the same morphologic features as the neoplastic cells observed in the surgical ileal resection.

The infiltrating lymphoid cells were positive for CD3, CD5, CD2, and CD4 but negative for CD7, CD8, CD15, CD30, granzyme B, TIA-1, CD10, CD23 and CD103 (Fig. 2). The tumor cells have a betaF1-phenotype. A bone marrow biopsy was hypocellular and showed interstitial infiltration by small lymphocytes with a T-cell phenotype

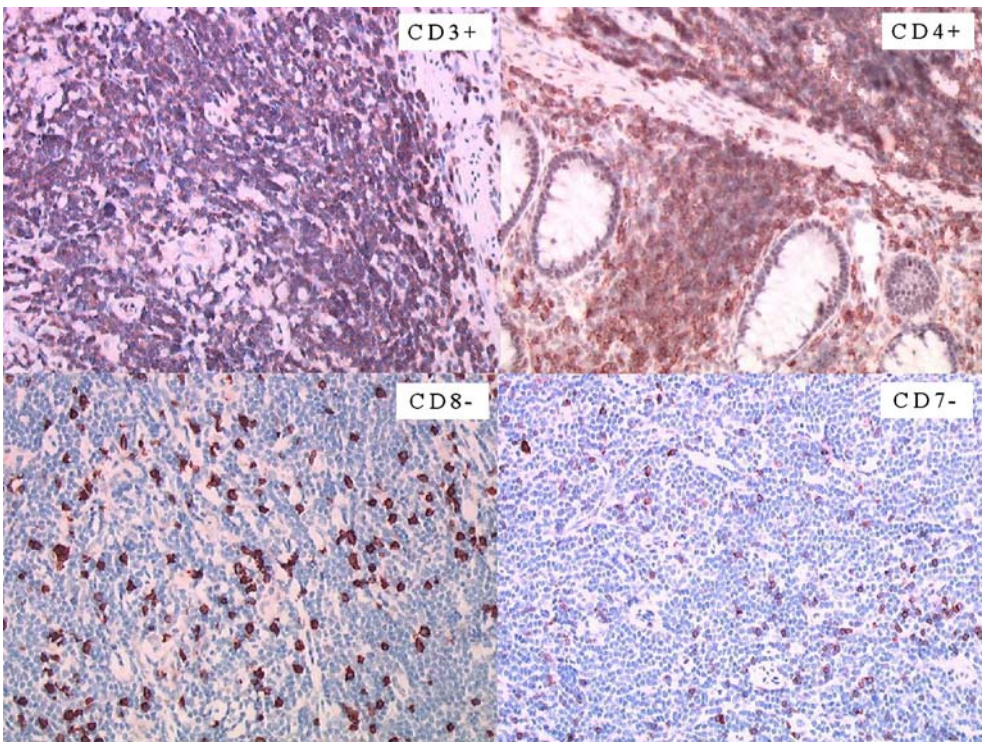

Fig. 2 Immunohistochemical analysis of the surgical ileal resection shows the neoplastic cells are positive for CD3, CD4 and negative for CD8 and CD7 (hematoxylin and dimethylaminoazobenzene, ×200)

similar to that seen in the ileal resection specimen. Molecular study by PCR analysis of TCR γ rearrangement showed a monoclonal pattern, both on the intestinal resection and mesenteric lymph node.

The patient was initially treated with prednisone, which improved his signs and symptoms. He then underwent systemic chemotherapy (ACVB-P regimen and anti-CD52 antibody therapy). Despite incomplete histological remission, the patient is symptom-free on steroid therapy alone, more than 3 years after diagnosis.

The clinical behaviour, the absence of a previous history of gluten-sensitive enteropathy and the immunophenotype of tumour cells distinguish this case from the WHO-defined distinctive enteropathy-type T-cell lymphoma. The current WHO classification of tumours of hematopoietic and lymphoid tissue also recognises two other major categories of extranodal T/NK cell tumours presenting in the intestine, which need to be included in the differential diagnosis: (1) extranodal NK/T cell lymphoma, nasal type and (2) hepatosplenic T-cell lymphoma. Our patient's low-grade T-cell lymphoma clearly differs from these two WHO-recognized distinctive entities.

Five cases with similar clinical and pathological features have been described in the English literature [1, 6]. In these five cases, the age ranged from 28 to 60 years (mean, 49.4 years). There were 4 men and 1 woman. These cases showed morphological homogeneity, with low-grade cytology. Immunophenotypic profiles were similar, with a constant expression of CD4 by tumour cells, whereas CD8 and CD103, which are expressed by normal IELs were negative. All patients had a slow relentless course and a prolonged survival compared to "classic" T-cell lymphomas. We had recently the opportunity to see one of these patients [1] who is still alive, more than 15 years after the diagnosis. Her clinical condition is good, although she presents intermittent skin lesions, mesenteric lymph nodes and non-specific lung opacities. The duodenal infiltrate is still present and unchanged on recent biopsies.

Our patient had hypereosinophilia. The relationship between hypereosinophilia and T-cell lymphoma is well established [2]. Like in three of the five patients reported in the literature, the neoplastic cells were admixed with numerous eosinophils and an extensive epithelioid cell granulomatous reaction. The granulomatous reaction may be due to aberrant cytokine production from T lymphocytes activated by the tumour or from the neoplastic cells themselves [3]. CD4+ lymphocytes are the most important source of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5, three cytokines active in promoting the development and differentiation of eosinophils in bone marrow.

This new case strengthens the hypothesis of a reproducible clinico-pathological entity, which could be individualized in the WHO classification as a second distinctive intestinal T-cell lymphoma category, as suggested by Isaacson [4]. The phenotype of this lymphoma seems to be in keeping with an origin from normal CD4+ lamina propria T cells.

It is noteworthy that this small intestinal CD4+ T-cell lymphoma shows similarities with the primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma in terms of behaviour, morphology and phenotype: this cutaneous lymphoma is defined by a predominance of small- to medium-sifszed CD4+ pleomorphic T cells and, in most cases, a favourable clinical course. Actually, this neoplasm is classified as "primary cutaneous peripheral T-cell lymphoma, unspecified" (WHO-EORTC classification of cutaneous lymphomas with primary cutaneous manifestations). However, recent studies have suggested that it can be separated out as a provisional entity from this broad group, called "primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma." A real distinctive category will probably be present in the forthcoming WHO classification.

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LETTER TO THE EDITOR

Mixed germ cell sex cord-stromal tumours of the testis

Michal Michal · Ondrej Hes · Petr Mukensnabl · Dmitry V. Kazakov

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Dear Editor,

We have recently published a series of seven cases of mixed germ cell sex cord-stromal tumours (MGSCT) of the testis and ovary comparing their morphological, immunohistochemical and molecular genetic findings [1]. MGSCT of the ovary and testes varied considerably in all these aspects. Germ cells in ovarian MGSCT stained positively with the antibodies to NSE, PLAP, and OCT4 with the exception of one case, which reacted positively for the c-kit protein antibody. The immunoprofile of the germ cells in ovarian MGSCT was identical to that of ovarian dysgerminomas and testicular classical seminomas. In addition, germ cells in one of the ovarian cases showed amplification of 12p, which is a typical feature of ovarian dysgerminomas and testicular classical seminomas [1].

Germ cells in testicular MGSCT were similar in morphology, immunoprofile and molecular genetic findings to those of spermatocytic seminomas. Immunohistochemically, they tested negative with all the above-enumerated antibodies and, in addition, they lacked on molecular biological analysis amplification of 12p [1]. However, there is not a single antibody positively distinguishing germ cells in spermatocytic seminoma from non-neoplastic testicular germ cells in contemporary pathology that we are aware of and that we could have used in our study. It may be one of the reasons why Drs. Ulbright and Young questioned the neoplastic nature of the germ cells in the testicular MGSCT [3, 4]. They reviewed nine testicular sex cord-stromal tumours containing germ cells, which closely mimicked MGSCT but differed from the latter, in that the germ cells were entrapped and non-neoplastic rather than neoplastic [3]. They thought that these tumours closely resembled MGSCT, but represented sex cord stromal tumours with entrapped germ cells, which they considered non-neoplastic. The evidence they presented to support their view was that the germ cells were distributed peripherally, were associated with entrapped seminiferous tubules or occurred in clusters with vaguely tubular shape consistent with preexisting seminiferous tubules [3]. Although some of these cases may have represented sex cord-stromal tumours with entrapped germ cells, in some of them, germ cells seemed not to have any relation to pre-existent seminiferous tubules and were scattered haphazardly forming a true predominant part of the lesion, whilst in other parts of the sex cord, the elements predominated. In one of those cases, inhibin stain showed an association of the germ cells with non-neoplastic Sertoli cells. The germ cells in Drs. Ulbright and Young's cases lacked large, vesicular nuclei with prominent nucleoli, closely resembling type A spermatogonia; the authors thus concluded that true testicular MGSCT most probably does not exist and these neoplasms represent sex cord-stromal tumours with entrapped, non-neoplastic germ cells [3]. A similar view was reiterated by the authors in a letter to the editor in relation to our paper [4].

In our paper, however, we have mentioned a very important morphological finding that proves the neoplastic nature of the germ cells in testicular MGSCT and militates against their reactive nature, namely, the presence of

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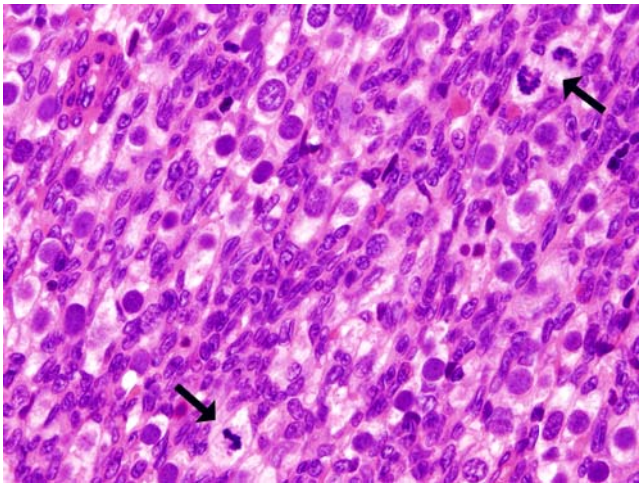

Fig. 1 Testicular MGSCT. Germ cells growing on the background of sex cord-stromal cells. There are three atypical mitoses in the germ cells at low magnification (*arrows*)

atypical, sometimes even bizarre mitotic figures in the germ cell component. We showed one atypical mitosis in Figure 4 of our original paper [1]; however, owing to the photographic reduction of the size, the details are not well apparent. Because this feature was very prominent in case 1 in our series [1] and from this case we were able to process the whole accessed surgical tumour tissues, we decided to cut through all blocks and review the slides again. The newly cut slides demonstrated places where we encountered several atypical mitoses within the germ cells (Fig. 1) (up to five atypical mitoses per one low-power field). When viewed on high magnification, these areas show clearly

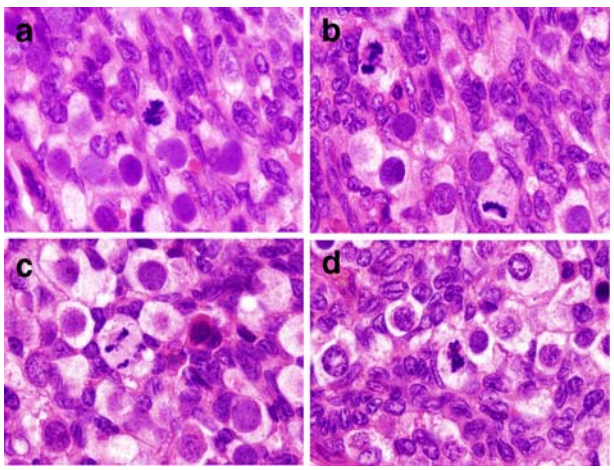

Fig. 2 **a**–**d** Testicular MGSCT. Atypical mitoses in germ cells at high magnification

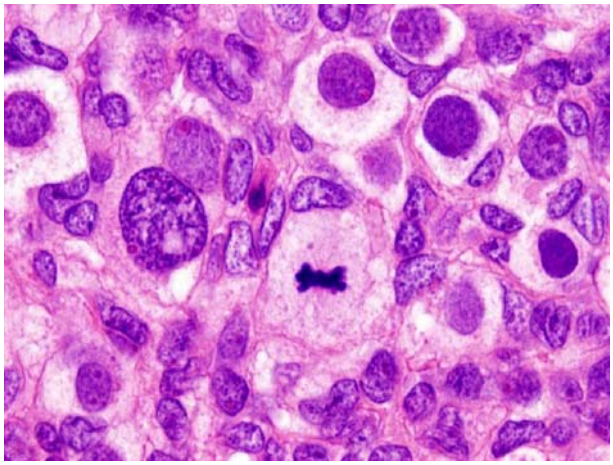

Fig. 3 Testicular MGSCT. Distinct variation in size of the germ cells

the atypical nature of the mitoses in the germ cells that leaves no doubt these germ cells are not only neoplastic, but malignant as well (Fig. 2). In addition, MIB1 antibody in these areas stained up to half of the germ cell component. We also found a distinct variation in size of the germ cells in this case, which would be quite unusual for the non-neoplastic germ cells (Fig. 3.). It is important to note that this morphological variation is quite similar to the germ cells of spermatocytic seminomas. In addition, we have not found a single focus in the entire neoplasm in which the germ cells resembled entrapped cells within testicular tubules or within a sex cord stromal tumour.

In our opinion, all the above-enumerated features point to the neoplastic, rather than reactive, nature of the germ cells in testicular MGSCT and to the existence of testicular MGSCT as an entity. Talerman and Roth recently expressed a similar opinion in their paper dealing with MGSCT [2].

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LETTER TO THE EDITOR

Presence of myeloid precursor cells in the endometrium of an AML patient: a diagnostic challenge!

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Dear Editor, myeloid precursor cells (myeloblasts) in the endometrium are very rare and occur in two conditions: myeloid sarcoma and extramedullary haematopoiesis. Both are very rare and are described in patients with an underlying haematological disorder. Diagnostic difficulties can present when myeloid precursor cells are found in an extramedullary location in an AML patient. We report the case of a female patient with acute myeloid leukaemia (AML) and endometrial extramedullary haematopoiesis.

A 49-year-old woman presents with progressive dyspnoea, leucocytosis, anaemia and thrombopenia. Differential white blood cell count shows 3% myeloblasts in the peripheral blood and 20% myeloblasts in the bone marrow. Examination of bone marrow smear and biopsy lead to the diagnosis of acute myeloid leukaemia, type FAB M4. No cytogenetic aberrations are found.

The patient is treated with chemotherapy and achieves complete remission. Four months later, the patient presents with metrorrhagia resistant to tranexaminic acid and progestins (lynestrenol).

An endometrium biopsy (Fig. 1) reveals arrested secretion due to prolonged intake of progestins, with foci of myeloblasts staining for myeloperoxidase and CD 68 (Fig. 2).

The differential diagnosis between extramedullary haematopoiesis and myeloid sarcoma is considered.

Myeloid sarcoma is an unusual extramedullary manifestation of acute myeloid leukaemia. It can precede or concur with AML, or can be the hallmark of relapse. As myeloid sarcoma can affect almost every organ, the clinical presentation is very diverse. Diagnosis depends on histology and immunohistochemistry. Myeloid sarcoma remains a diagnostic pitfall; up to 75% are initially misdiagnosed [3]. Uterine myeloid sarcoma has been described, although mainly in the cervix [1].

Endometrial extramedullary haematopoiesis is an extremely rare condition. Only a few cases are reported, most of them related to an underlying haematological disorder [4]. Two possible stimuli for endometrial haematopoiesis have been described: GM-CSF (granulocyte-macrophage colony-stimulating factor) and uteroferrin [2]. GM-CSF is secreted by fibroblasts and endothelial cells when appropriately stimulated by interleukin (IL)-1 or tumour necrosis factor (TNF)-α. Uteroferrin is secreted by endometrial cells in certain mammals during pregnancy and after administration of progestins. One case of endometrial extramedullary haematopoiesis associated with retained products of conception after termination of pregnancy is described [4]. This case could also be explained by the uteroferrin hypothesis.

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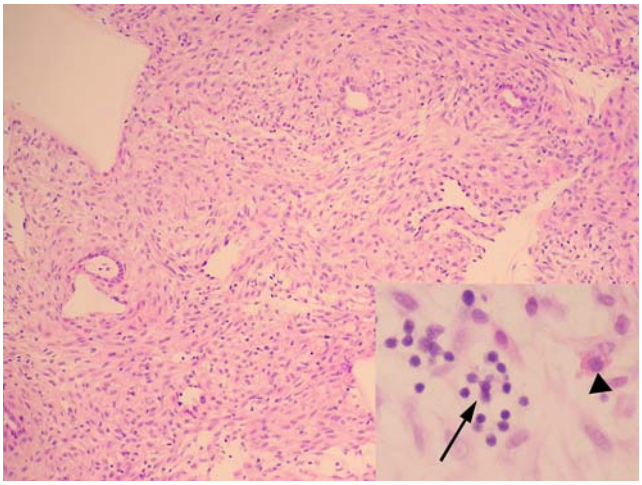

Fig. 1 *Background*: Low-power H&E of the endometrium. *Inset*: High-power H&E of the endometrium containing erythroid precursors (*arrow*) and myeloid precursors (*arrowhead*)

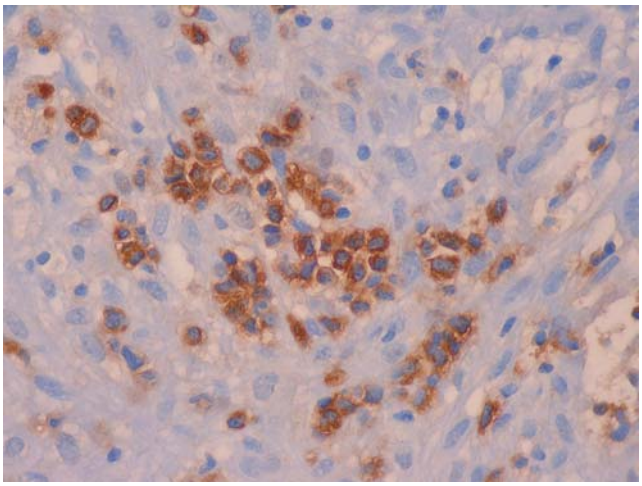

Fig. 2 Myeloperoxidase immunohistochemistry illustrating the myeloid precursors

In our case, the uteroferrin hypothesis seems plausible as our patient received long-time progestin therapy. The diagnostic dilemma in our case was solved with deeper tissue sections revealing the presence of erythroid cells, which were positive for glycophorin.

Female leukemia patients are often treated with progestins to prevent metrorrhagia, as is the case in our hospital. Progestin-induced endometrial extramedullary haematopoiesis could thus occur more often than has yet been proven. Clinicians often relate the uterine bleeding of leukemia patients to thrombopenia. As extramedullary hematopoiesis is not considered and an endometrial biopsy is not performed, little is known on this association.

However, bearing the uteroferrin hypothesis in mind, endometrial extramedullary haematopoiesis should be considered in female leukemia patients with uterine bleeding.

Raising the awareness of both pathologists and clinicians on this association should allow us a better insight into the incidence and the clinical impact of this matter.

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MEETING REPORT

The first Sino-German symposium on cancer research, Shanghai May 2007

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Keywords China · Germany · Sino-Germany Science Center

Introduction

This meeting report gives a short overview on the contributions of 17 leading scientists from China and Germany. The symposium was supported by the Sino-Germany Science Center Bejing, the Deutsche Forschungsgemeinschaft and the National Science Foundation, China. It intended to foster communication and collaboration of researchers from both countries.

The "Migrating Cancer Stem Cell"—Concept was discussed by Thomas Brabletz (Dept. of Surgery Univ. of Freiburg). Most colorectal adenocarcinomas (CRC) have mutations in the APC gene leading to overexpression of the Wnt-pathway effector beta-catenin. By acting as a transcriptional activator, nuclear beta-catenin is decisively involved in two fundamental processes in embryonic development: epithelial to mesenchymal transition (EMT) and generation of stem cells (e.g., in intestine development).

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We have previously shown that nuclear beta-catenin accumulates in tumor cells at the invasive front undergoing an EMT. The amount of these cells strongly correlates with clinical outcome and metastasis formation. In contrast, tumor cells in central tumor areas are differentiated and often show membranous, E-cadherin-bound beta-catenin. Strikingly, also, metastases show again a differentiated phenotype lacking nuclear beta-catenin, indicating a regulatory role of the tumor environment for malignant progression.

Based on the developmental functions of beta-catenin and our data, we propose that both primary tumors and metastases are derived from the pool of EMT-associated "migrating cancer stem cells" at the tumor host interface, which are defined by strong nuclear beta-catenin accumulation and expression of its target genes. This gives these cells a feature, which drives malignant tumor progression including metastasis: The unusual combination of abilities allowing simultaneously to migrate and behave as a cancer stem cell.

Molecular Targets in Cancer Therapy—New Possibilities and Old Limitations were reported by Reinhard Buettner (Inst. of Pathology, University of Bonn). Increasing knowledge on molecular pathogenesis of malignant tumors and activated signaling pathways has led to the concept of targeted therapies. Small molecularly designed molecules target specifically oncoproteins, and thereby modulate growth, apoptosis, or invasion of cancer cells. Currently, most experiences exit with membrane-coupled growth factor receptors and their intracellularly coupled signaling kinases.

In cancer cells, these tyrosine kinase-triggered signaling pathways are frequently altered and causally involved in malignant transformation. Indeed, some of the tyrosine

kinase-coupled receptors or their respective growth factors were originally cloned as retroviral oncogenes. Therefore, these critical oncogenic effector pathways represent attractive and often highly selective therapeutic targets. However, as experience with patients under long-term therapies with selective tyrosine kinase inhibitors accumulates, it becomes also evident that tumors develop escape strategies leading to resistance to therapy either via specific mutations or switches in intracellular signaling pathways.

We propose to discuss (1) current knowledge of the most frequently employed molecular targets and the strategies to design and develop specific inhibitors, (2) report first experiences with molecular diagnostics of resistance, and to (3) describe putative options for combinational therapies and second- or even third-line molecular therapies to address tumor-escape strategies in response to treatment. (4) Finally, we would also like to discuss implications on the health care system and reflect ethical considerations resulting from a science-based molecular medicine.

Reinhard Dengler (Dept. of Neurology Medical School) gave an overview on Modern Approaches in Functional Neurodiagnosis. A major goal of neurosurgery is to reduce the intervention-associated brain damage and functional deficits to a minimum. This holds especially true if brain tumors are localized in or close to important functional regions such as the eloquential or sensorimotor areas. Structural images may show the extension of the tumor and may allow for differentiation between tumor mass and brain edema, but do not provide precise information on the relation between the tumor and functional areas which may be entirely or partially involved or rather displaced by the space occupying lesion or may be even fully untouched.

In such situations, the neurosurgeon wishes a functionally oriented presurgical diagnosis to make the best strategy for the operation and to avoid as much unnecessary brain damage as possible. Modern functional imaging in conjunction with specific structural imaging is very valuable in solving this task. In addition to classical cranial computer tomography and magnetic resonance imaging (MRI) and its variations including Gadolinium enhancement, functional MRI, and PET studies using tracers such as F-18-Deoxyglucose or C-11-Methonin (or F-18-Tyrosin) can be used successfully. Matching of the images generated by these techniques provides information on tumor extension, metabolically active tumor portions vs inactive or necrotic portions, and anatomical relationship to functional areas. The power of these techniques can be further increased by modern neuronavigation which uses the computerized imaging data. This helps the surgeon to make optimal intraoperative decisions on how much tissue can or must be removed and to minimize postoperative functional deficits.

Manfred Dietel (Inst. of Pathology, Charitè, Berlin) discussed Molecular Pathology and Targeted Therapy. To read a patients' tissue as "deeply" as possible and to get information on morphological, genetic, proteomic, and epigenetic grounds will be the upcoming task of surgical pathologists to provide the clinicians with information relevant for individualized medicine. These data will provide the basis of clinical drug selection as well as development of new drugs.

Molecular diagnostic Following the WHO, malignant tumors are grouped on the basis of histogenesis, histological type, and differentiation. However, tumors of the same group may be heterogeneous. Although histopathology is only partly able to predict clinical behavior, it still plays the dominant role in selecting patients' management. To improve this situation, molecular approach will become part of the diagnostic process, hopefully adding that information which conventional morphology is not able to provide.

Predictive pathology Prediction of the clinical behavior of an individual tumor is a pushing challenge for pathology. Clinicians strongly demand for a reliable prediction regarding prognosis, metastatic potential, and/or response to therapy. Applying DNA arrays, several approaches have been performed successfully to improve predictive accuracy.

Development of new drugs In recent years, the efforts devoted to unraveling potential new therapeutic targets have lead to the development of new drugs. For example, Herceptin and Gleevec are the first designer drugs which are used with considerable success in the clinic. The basis of these developments was provided by tissue-based investigations performed by experimental pathologists.

Bernd Groner (Georg Speyer Haus, Inst. for Biomedical Research, Frankfurt) presented data on "Novel Approaches to Targeted Tumor Therapy". The inappropriate assembly of modular components plays a role in oncogenic transformation and other diseases and can be exploited for therapeutic purposes. These target structures, however, are conventionally considered as "not druggable". As protein–protein interactions are mediated by surface structures of folded proteins, peptides embedded in the context of a scaffold protein can serve as competitive inhibitors of protein recognition modules. Such peptide aptamers can target non-canonical drug binding sites and disrupt intracellular protein functions. Applications of this principle are dependent upon the binding specificities and affinities of the peptide aptamers to their target structures, recombinant expression, and purification of the peptide aptamer-scaffold protein and delivery of the purified protein into cells. We improved upon the scaffold structure and utilized the

human thioredoxin molecule. Removal of five internal cysteines allows the purification of a monomeric protein with high yield. Provision of this molecule with a protein transduction domain, consisting of nine arginines, results in a fusion protein, which is taken up efficiently by cultured cells, inhibits intracellular signaling functions and associated cellular phenotypes. A peptide aptamer which recognizes the intracellular part of the ErbB2 receptor was introduced into breast cancer cells. They reduced the extent of AKT kinase induction upon heregulin treatment of the cells and enhanced resistance towards chemotherapeutic agents in ErbB2 overexpressing cancer cells. A peptide aptamer directed against the dimerization domain of the transcription factor Stat3 prevented Stat3-mediated transactivation and induced growth inhibition and the induction of apoptosis.

Christian Hagemeier (Charité-Berlin, Children's Hospital, Lab for Molecular Biology, Berlin) spoke about Molecular Approaches to Understand and Tackle Acute Lymphoblastic Leukemias in Children. The probability of long-term survival after relapse from acute lymphoblastic leukemia (ALL) is predicted from well-established prognostic, mainly clinical, factors. However, the underlying biological determinants of these prognostic factors are poorly understood. In collaboration with the department of Pediatric Oncology that houses the BFM ALL-REZ study group, we have defined molecular prognostic markers for risk stratification, identified gene expression programs that characterize ALL pathogenesis in patients with poor outcome, and searched for genes that modulate therapeutic approaches. We found that early onset relapse of ALL, which is typically associated with a particularly low event-free survival rate, is distinguished from late disease recurrence by an increased proliferative capacity of leukemic blasts and a distinct expression of genes with functions in cell cycle control, particularly during the process of mitosis. In addition, by employing a genetic shRNA screen, we have identified sets of genes that modulate therapeutic approaches in cell culture-based models. The implications will be discussed.

Per Sonne Holm (Inst. of Experimental Oncology & Research, Technical University Munich) reported on YB-1 as a Target for the Development Oncolytic Adenovirus. It has been shown that YB-1 regulates the expression of a number of genes linked to cell proliferation and growth. In addition, YB-1 is a multifunctional protein involved in regulating translation, mRNA splicing, and DNA repair, indicating that YB-1 is involved in controlling cell survival. In recent years, several laboratories have demonstrated that YB-1 in response to genotoxic stress like chemotherapy translocates from the cytoplasm to the nucleus. Besides transcriptional activation of the human *MDR1* and *MRP1* genes, nuclear localization of the transcription factor YB-1 is associated with base excision and mismatch repair pathways through binding to several DNA repair proteins. YB-1 is found amongst others highly overexpressed in many malignancies, and current results suggest that YB-1 is important in conferring, e.g., drug resistance in many types of tumor cells. Oncolytic adenoviruses have attracted considerable attention for their use as an innovative class of promising cancer therapeutics. We have developed several oncolytic adenoviral vectors which replicate in the presence of YB-1 in tumor cells. The application of YB-1-dependent oncolytic vectors produces a strong antitumor effect in several carcinoma cell lines, and application in vivo in subcutaneous established tumors led to strong tumor growth inhibition, which could further be enhanced by radiation and chemotherapy. In conclusion, YB-1-dependent adenoviruses are highly effective against different malignancies and deserve further clinical testing.

Reinhold Schäfer (Inst. of Pathology Charité Berlin) explained Oncogenic Ras Signaling: Effector Pathways, Targets, Regulation and Interference. Hundreds of transcriptional targets are up-regulated or down-regulated on transition from the normal to the Ras-transformed state. A minority of targets is equally deregulated in different cell types, indicating that there exist a limited number of common Ras pathway targets. The set of up-regulated genes comprises factors involved in growth control, invasion, and metastasis, while many down-regulated genes encode proteins with anti-proliferative, anti-invasive, and anti-angiogenic properties. The involvement of most of the deregulated genes in triggering or executing various aspects of malignant transformation is unknown. Moreover, the global mechanisms controlling their expression are not yet understood. To unravel the regulatory mechanisms perturbing the transcriptional networks downstream of the Ras signaling cascade and to find out which of the targets are primarily involved in tumorigenesis, we are employing several experimental strategies: We have narrowed down the number of critical targets by correlating the effects of blocking individual effector pathways, using pharmacological inhibitors or RNA interference, with the transcriptional patterns and specific phenotypes. We have selected a few targets exhibiting significant differential expression in Ras-transformed vs normal cells for RNA interference experiments. To study the role of DNA methylation in target gene suppression, we subjected Ras-transformed cells to treatment with demethylating agents and determined the fraction of responding targets. As an example, we have shown that clusterin gene suppression is controlled by promoter methylation and by the MAPK-pathway, suggesting a dynamic relationship between Ras signaling and DNA methylation.

Unraveling the relationships between effector pathways and transcriptional networks will provide important clues for understanding the system biology of cancer, help to

identify novel biomarkers, and eventually, more specific nodes for therapeutic intervention.

Xuetao Cao (Inst. of Immunology & National Key Laboratory of Medical Immunology, SMMU, Shanghai) gave a report on Nuclear Entrapment of MEK1/2 by an Oncogenic Ras-Related GTP-Binding Protein Promotes Tumor Progression. Tumorigenesis is widely recognized as an intricate multistep process that involves malfunction in protooncogenes, tumor suppressor genes, and other key cellular genes implicated in cell proliferation, differentiation, survival, and genome integrity. Malfunction of protooncogenes and signaling components has been reported. Among them, the Ras/Raf/MEK [mitogen-activated protein kinase (MAPK)/ERK kinase]/ERK (extracellular signal-regulated kinase) signaling components have been identified to play important roles in tumorigenesis. MEK1/2 are cytoplasmic proteins containing nuclear export signal (NES) and play crucial roles in determining cell growth and differentiation through activation of extracellular signal-regulated kinase (ERK1/2).

RJLs are a family of Ras-related GTP-binding proteins that are characterized by the N-terminal GTP-binding domain (small GTPase domain) and the C-terminal J domain with unknown functions. The full-length human RJL (*hRJL*) cDNA was identified by us via large-scale sequencing of a human peripheral blood monocyte-derived dendritic cell cDNA library. Here, we report that hRJL, a nucleus-localized representative of RJL family, can mediate the accumulation of active MEK1/2 in the nucleus by masking the NES of MEK1/2, which in turn results in constitutive activation of ERK1/2 and malignant transformation. We show that hRJL is overexpressed in most of the tumor cells and tumor tissues and is oncogenic both in vitro and in vivo. Silencing of hRJL expression or disruption of hRJL-MEK1/2 interaction results in regression of tumor growth and metastasis. Our findings suggest that hRJL-mediated nuclear entrapment of MEK1/2 plays important roles in promotion of autonomous tumor progression, and hRJL is a novel oncogenic therapeutic target.

Guo-Qiang CHEN (Dept. of Pathophysiology, Shanghai Jiao Tong University School of Medicine) reported on Hypoxia and Leukemic Cell Differentiation, Evidence From In Vitro and In Vivo. He stated that cobalt chloride and desferrioxamine-mimicked hypoxia and moderate hypoxia can trigger acute myeloid leukemia (AML) cells to undergo differentiation, and intermittent hypoxia significantly prolongs the survival of the transplanted leukemic mice through inducing growth arrest and differentiation with inhibition of leukemic blast infiltration. Based on these in vitro and in vivo observations, we explore the role of hypoxia-inducible factor 1(HIF-1), a protein in leukemic cell differentiation. Our results show that the conditional HIF-1a induction triggers granulocytic differentiation of myeloid leukemic U937T transformants in which HIF-1a is tightly induced by tetracycline withdrawal. The suppression of HIF-1a expression, but not HIF-1b (an essential partner for transcription activity of HIF-1), by specific short hairpin RNAs (shRNAs) effectively inhibits hypoxia-induced differentiation of U937 cells. Further, the inhibition of hematopoietic differentiation-critical CCAAT/enhancer binding protein-alpha (C/EBPa) significantly eliminates HIF-1a-mediated myeloid leukemic cell differentiation. Furthermore, HIF-1a is also shown to interact physically with and to increase the transcriptional activity of C/EBPa and AML1, two critical factors for granulocytic differentiation. Collectively, this work provides several lines of direct evidence for the role of HIF-1a protein through its non-transcriptional activity in myeloid cell differentiation in which C/EBPa elicits a role as an effector downstream to HIF-1a. These discoveries would shed new insights for understanding mechanisms underlying leukemogenesis and designing the new therapeutic strategy for differentiation induction of AML.

Jing Cheng (Medical Systems Biology Research Center, Tsinghua University School of Medicine Haidian District, Beijing) explained Diagnostic Biochips: From Research to Consumer Market. Biochip-based platforms for research have developed rapidly in the past decade, which includes chips that can generate detailed biological information at four different levels—at the gene, protein, cell, and tissue levels. For example, at the gene level, we have SNP and mutation analysis microarrays, comparative genome hybridization microarrays, methylation detection microarray, and mRNA and miRNA expression profiling microarrays; at the protein level, we have established activated transcription factor profiling chips as well as ChIP-GLAS-based promoter chips; at the cellular level, we have chips for single cell ion flux monitoring and multichannel electrophysiology measurement of cell networks; and finally, for pathological examination, we can provide numerous different tissue microarrays, including normal and diseased tissues.

In recent years, demand for general consumption of these new classes of biochips has been keenly anticipated in consumer areas such as disease diagnostics, biosafety testing, etc. Furthermore, in this presentation, different types of consumer biochip products will be introduced, including DNA microarrays for the detection of the presence of SARS Corona virus RNA, for detection of Gram-positive bacteria and the associated drug resistance genes, for detection of mycobacterium and the drug resistance genes for TB, for detection of food-borne pathogen, and protein microarrays for the diagnosis of autoimmune diseases, and for the detection of veterinary drug residues such as antibiotics. This broad range of applications for biochips foreshadows future development of a wider range of sensitive testing products.

Jian-ren Gu (State Key Lab. of Oncogenes and Related Genes, Shanghai Cancer Institute, Jiao Tong University) discussed Conceptual Concerns on Cancer Biology. The major problems in cancer treatment are:

1. Cancer is not a cluster of cancer cells, but a well-organized tissue.
2. Cancer cells are heterogeneous. Among cancer cell population, the cancer stem cells may be crucial targets for treatment.
3. Dissemination may be an early event in most types of cancer, also a different event from metastasis. The mechanism to evoke the growth of disseminated cancer cells into a metastatic lesion is crucial in development of new therapeutic methods to the control of cancer.
4. Cancer could be a systems disease with local manifestation of abnormal cell growth developed on the basis of deregulation of host-cell homeostasis, including neural, humoral, and immune mechanism.

These conceptual considerations may provide some clues to explore the mechanism of carcinogenesis and cancer progression as well as the design of new therapeutic strategy against cancer.

Yajun Guo (International Joint Cancer Institute, SMMU, Shanghai, yjguo@smmu.edu.cn) explained the topic of "Novel Vaccine Development and Cancer Immunotherapy". We developed several novel bispecific antibodies, which consist of two normal binding sites for targeting antigens and a functioning portion of Flt3L. The constructed bispecific antibodies (SM5.1-Flt3L, Anti-her2-Flt3L, and anti-CD20-Flt3L) efficiently target hepatoma, breast, and lymphoma cancer cells both in vitro and in vivo, leading to accumulation of DCs, NK cells, and lymphocytes in local tumor tissues. Administration of these bispecific monoclonal antibodies can protect bone marrow injury caused by chemotherapeutic drugs and stimulates proliferation and maturation of lymphocytes, APCs, and NKs. Systemic administration of these bispecific antibodies (100 μg/each injection×3) significantly inhibited tumor growth and cured established tumors. These bispecific therapeutic proteins are effective in elicitation of long-lasting antitumor immunity. The tumor-specific immunity can be adoptively transferred into naïve animals successfully by transfusion of $CD3^{+}CD8^{+}$ T cells from the treated mice. The results suggest that fusion of Flt3L with therapeutic antibodies can create novel immunotherapeutic proteins, which are more effective in induction of antitumor immunity, and may provide a novel strategy for treatment of cancers.

Yongfeng Shang (Peking University) talked about Gene Regulation by Nuclear Receptors. Steroid receptor coactivators (SRCs) exert profound effects on animal development and physiology. These coactivators are nuclear proteins and transcription co-regulators that function to facilitate the transcription initiation mediated by nuclear receptors as well as by other well-known transcription factors. However, how these co-regulators are functionally regulated is poorly understood. During genome-wide screening for SRC-interacting proteins, we identified a novel ankyrin-repeat-containing protein, *S*RC-*i*nteracting *p*rotein (SIP), which interacts with SRC coactivators in the cytoplasm. We demonstrated that extracellular stimuli, such as the addition of estrogen, induced phosphorylation of SIP in its PEST (proline, glutamate, serine, and threonine-rich) domain by casein kinase II. The phosphorylation of SIP resulted in dissociation of SRC proteins from SIP in the cytoplasm and led to subsequent nuclear translocation of SRC proteins and gene coactivation. Both gain-of-function and loss-of-function experiments indicate that SIP functions to sequester SRC coactivators in the cytoplasm and buffer the availability of these coactivators, thus, providing a mechanism for the regulation of the transcription regulators.

Hongyang Wang (Inst. International Cooperation Laboratory on Signal Transduction Eastern Hepatobiliary Surgery Institute/Hospital, Shanghai SMMU) Negative Regulation of Hepatocellular Carcinoma Cell Growth by Signal Regulatory Protein. 1SIRPα1 is a member of the signal regulatory protein (SIRP) family that undergoes tyrosine phosphorylation and binds SHP-2 tyrosine phosphatase in response to various mitogens. The expression levels of SIRPα1 were decreased in human hepatocellular carcinoma (HCC) tissues, as compared with the matched normal tissues. Exogenous expression of wild-type SIRPα1, but not of a mutant SIRPα1 lacking the tyrosine phosphorylation sites, in SIRPα1 negative Huh7 human HCC cells resulted in suppression of tumor cell growth both in vitro and in vivo. Treatment of Huh7 transfectants with EGF or HGF induced tyrosine phosphorylation of SIRPα1 and its association with SHP-2, which were accompanied by reduced ERK1 activation. Expression of SIRPα1 significantly suppressed activation of NF-κB and also sensitized Huh7 cells to TNFα or cisplatin-induced cell death. In addition, SIRPα1-transfected Huh7 cells dis played reduced cell migration and cell spreading in a fashion that was dependent on SIRPα1/SHP-2 complex formation. In conclusion, these results suggest a negative regulatory effect of SIRPα1 on hepatocarcinogenesis through, at least in part, inhibition of ERK and NF-κB pathway. The heightened sensitivity of cells restoring SIRPα1 function could be exploited in the development of therapeutic regimens which may potentiate the antineoplastic effect of conventional cytokines or chemotherapeutic agents.

Qimin Zhan (Cancer Institute Chinese Academy of Medical Sciences) explained the role of Bacp, a BRCA1-Regulated Centrosomal Protein, in the Control of Genomic

Stability. The essence of successful mitosis in mammalian cells is the generation of two genetically identical daughter cells. The successful mitosis requires the assembly of a strictly bipolar mitotic apparatus that will ensure that chromosomes equally distribute to the daughter cells. This process is controlled by the centrosomes that are required for spindle formation and function. Centrosome stability is required for the successful mitosis in mammalian cells. Amplification of centrosome leads to chromosomal mis-segregation and generation of aneuploidy, which are closely associated with cell transformation and tumorigenesis. The machinery that controls centrosome stability involves multiple important cellular proteins, including p53, BRCA1, Gadd45, p21, and Cdk2/cyclin E. The precise coordination among those regulators maintains centrosome duplication and stability. However, there are currently limited insights into mechanism(s) for this critical biological event.

Breast cancer susceptibility gene BRCA1 is implicated in the control of mitotic machinery, although there is little insight into underlying mechanism(s). Here, we show that BRCA1 physically interacts and co-localizes with Bacp (*B*RCA1 *a*ssociated *c*entrosomal *p*rotein). Interestingly, Bacp centrosomal localization likely depends on normal cellular BRCA1 function, as cells containing BRCA1 mutations or silenced for endogenous BRCA1 reveal disrupted Bacp co-localization to centrosomes. Suppression of endogenous Bacp results in aberrant spindle formation, failure of chromosomal segregation and cytokinesis, and aneuploidy. Bacp is overexpressed in human breast and lung carcinomas, and its deregulation is in part associated with *BACP* amplification. Bacp exhibits strong oncogenic property and induces NIH3T3 fibroblast transformation. Importantly, Bacp transgenic mice mimic the phenotypes of disrupted BRCA1, including centrosome amplification and spontaneous tumorigenesis. Thus, Bacp may cooperatively act together with BRCA1 in mitotic machinery, and abnormalities of Bacp lead to genomic instability and tumorigenesis.

Jingde Zhu (Cancer Epigenetics and Gene Therapy Program, Shanghai Cancer Institute) Cancer Epigenetics (-omics) from DNA Methylation Perspectives in Liver Cancer as a Paradigm Cancer is a complex disease of broad genetic and epigenetic defects. The epigenetic circuit dictates the inheritable transcription profile and therefore the phenotype, better understanding of which would revolutionize the current anti-cancer practice. Epigenetic makeup comprises DNA methylation, histone modification, microRNAs, and chromatin remodeling, which act together to shape the chromatin structure of genome to meet the functional requirements. DNA methylation, an enzymatic process to add a methyl group at the fifth carbon of cytosine within the palindromic dinucleotide 5′ -CpG-3′ sequence is the best-studied pigenetic mechanism. Cancer-associated aberration of DNA methylation goes two opposite directions: global hypomethylation and local hypermethylation, that, in turn, dictate the transcription profile of both repetitive sequences and individual genes, and therefore, the phenotypic profiles unique to cancer states HCC present a major health threat to the people in China, owing to their high incidence and aggressive clinical nature. We are currently carrying out comprehensively profiling the disease-associated CpG methylation pattern at the multiple gene level (1) and at the global level (2) to gain the new mechanistic insights, establish a DNA methylation-based staging and classification system and find effective biomarkers for HCC. To demonstrate the great potential of DNA methylation in cancer detection, I will present the latest results in DNA methylation profiling in urine sediments for sensitive/specific detection of bladder cancer.

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EDITORIAL

Neuroendocrine tumors (2007): Oberndorfer's legacy

Günter Klöppel

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Who among us is not familiar with the difficulties that frequently occur in the diagnosis and therapy of neuroendocrine tumors of the gastrointestinal tract, the pancreas, and the lung? The main reason for the problems is that the tumors are quite heterogeneous; despite their relatively uniform histological appearance; their growth is slow but hard to influence, and because of their hormone activity, they often show special clinical features and biology. Moreover, they are so rare that our knowledge of them is relatively limited compared with other neoplasms.

Siegfried Oberndorfer was the first to give a special name to the neuroendocrine tumors. One hundred years ago, he coined the term "carcinoid" [1], and with this contribution, he set the path for the recognition and elucidation of the neuroendocrine tumors. This year's congress of the European Society for Pathology in Istanbul is an excellent occasion to celebrate the 100th anniversary of the term carcinoid and to remember Siegfried Oberndorfer, who worked in Munich, Germany and in his later years in Istanbul, Turkey (see Klöppel et al., this issue).

This supplement to *Virchows Archiv* attempts to provide up-to-date information on the most important neuroendocrine tumors. It contains contributions dealing with the pathology and biology of gastroenteropancreatic neuroendocrine tumors (GEP-NETs), the pathology and genetic background of hereditary GEP-NETs, the receptor status of these tumors, and their molecular profile. There is also a review of the second most frequent group of neuroendocrine tumors, the pulmonary neoplasms of neuroendocrine origin. Then follows a review of goblet cell carcinomas and mixed exocrine–endocrine neoplasms, and finally the rapidly advancing treatment modalities for GEP-NETs are discussed.

The authors of the various reviews published in this issue belong to the NET-WORKs group, which is a working group of the European Neuroendocrine Tumor Society (ENETS). This group recently provided guidelines for the staging and grading of GEP-NETs [2, 3], and these guidelines are also discussed in this issue.

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REVIEW AND PERSPECTIVE

Siegfried Oberndorfer: a tribute to his work and life between Munich, Kiel, Geneva, and Istanbul

Günter Klöppel · Katherine Dege · Wolfgang Remmele · Yersu Kapran · Sitki Tuzlali · Irvin M. Modlin

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Abstract In 1907 Siegfried Oberndorfer published his observations and interpretations on tumorlets ("Geschwulstchen") in the small intestine, which he called carcinoids ("karzinoide Tumoren"). This article pursues the questions why this discovery was so unique and what role it played in the later life of Siegfried Oberndorfer.

Keywords Oberndorfer · Carcinoid · Endocrine tumor

One hundred years ago, Siegfried Oberndorfer (Fig. 1) proposed the term "carcinoid", which he also used to call tumorlets ("Geschwülstchen") in the small intestine (Fig. 2). The name carcinoid was intended to express the distinctions between these tumors and true carcinomas, but also their similarity to carcinomas. Since then the carcinoids have been the topic of numerous studies, which revealed their fascinating nature as endocrine neoplasms. Siegfried Oberndorfer, whose contributions set the path for the recognition of the neuroendocrine tumors and stimulated the elucidation of this novel tumor type, lived in Germany until 1933, when he, being of Jewish origin, was forced by the Nazi regime to emigrate to Turkey. There, he remained as full professor and director of the Department of Pathology of the University of Istanbul until his death in 1944 (Fig. 3). The life of Siegfried Oberndorfer and the evolution of the concept of carcinoid tumors have been the focus of recent publications by one of us [7, 8] and were also extensively reviewed by Dr. Joachim Thomas Katz [2]. In this study, we focus on the questions why Oberndorfer became a pioneer in the field of carcinoid tumor biology, which of his original observations stood the test of time and how he followed the evolving concept of carcinoid tumors in his later years.

What was so special about Oberndorfer's observations?

Oberndorfer's article in which he described seven cases of carcinoid immediately received recognition, although in retrospect it is clear that he was not the first to describe carcinoids. Reports of similar tumors had been published before 1907 (cf. [7, 8]), in some of which the tumors can be clearly identified on the basis of the illustrations [3, 4, 14]. However, the authors of these case reports either did not give any interpretation of the described tumor [3] or regarded it as a primary carcinoma of the ileum [4, 14]. Oberndorfer's conclusion that the described tumors in the ileum form a special group of neoplasms because of their similarity and also dissimilarity to usual carcinomas and their special growth features were therefore novel. Moreover, and most importantly, he coined a term to unmistakably designate the new tumor group.

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Fig. 1 Siegfried Oberndorfer and his daughter Helena ("Leni") in Munich in 1911. At this time, when the nature of the carcinoids was heavily debated among the leading pathologists in Germany, Oberndorfer was appointed Professor at the Medical Faculty of the University of Munich at the age of 35

What qualified Siegfried Oberndorfer for his new observations?

The history of his life and particularly his so far unpublished autobiography characterize Siegfried Oberndorfer by four features: He had a general and deep interest in life and nature. He was diligent and highly disciplined. He had an analytical mind and he was an excellent writer.

He satisfied his interest in life and nature through excursions, tours and long journeys, and of course by studying medicine. He was not only a pathologist, but he worked temporarily as a general practitioner, as a ship's doctor, and also as a military doctor. He was interested in all kinds of diseases from both the clinical and the pathological view. The subjects he studied were therefore also manifold. Apart from the carcinoids, he contributed in numerous articles to the study of the pathogenesis of appendicitis, tuberculosis, and many different tumors. But it was not only this wide spanned interest; it was also his will to record what he observed and what he encountered. Thus, he wrote reports, articles, and descriptions of almost everything he experienced and studied during his life. Everything he saw and studied he also analyzed with regard to its nature, causes, and significance. In the last two years of his life, he wrote an autobiography, in which he not only gave a report of his life but also commented and reflected on what he had experienced and what he had done.

What helped him to make his decisive observations?

He started medical school in 1895 in Munich and spent one term in Kiel in northern Germany, which for a Bavarian was a

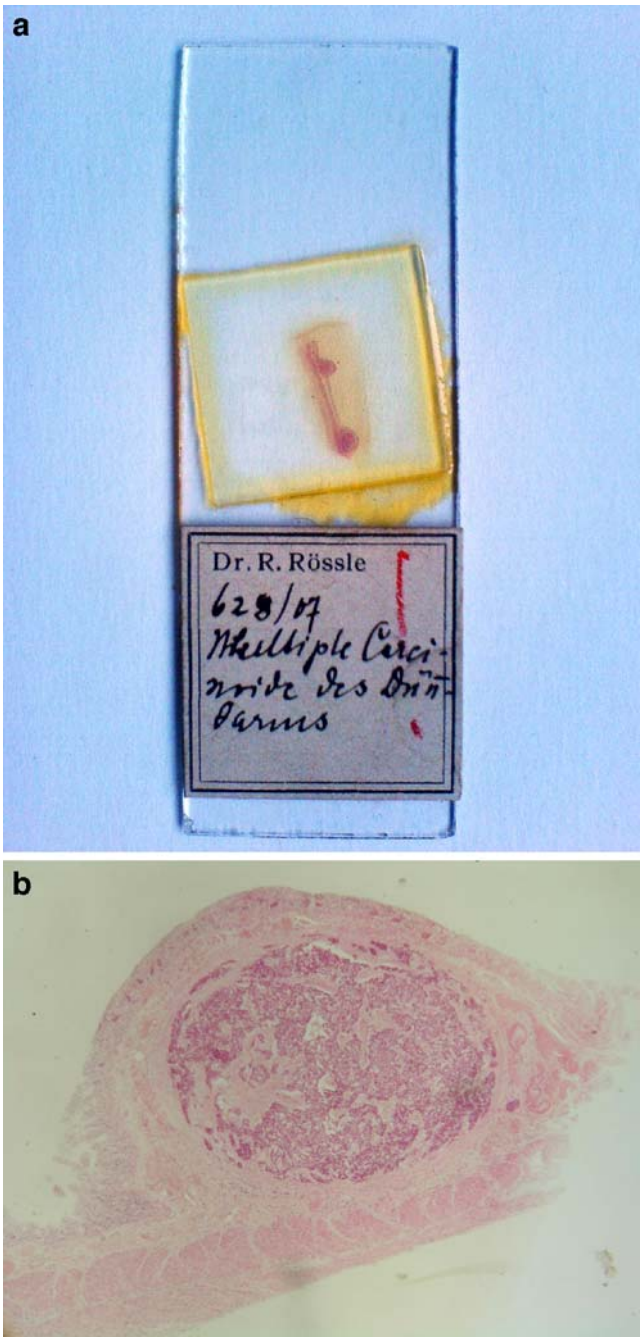


Fig. 2 a and **b** Photographs of original sections showing ileal carcinoids from the collection of Siegfried Oberndorfer, obviously provided in 1907 by Robert Rössle (1876–1956), at that time a colleague at the Department of Pathology, University of Munich

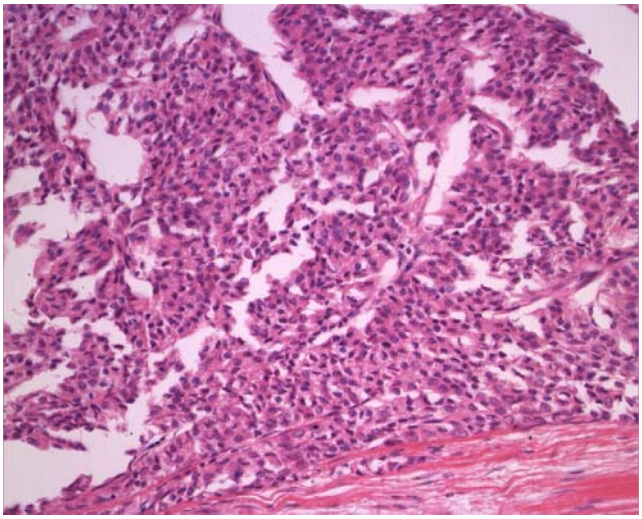


Fig. 3 Illustration of the thymoma that caused the death of Siegfried Oberndorfer in 1944 because of local extension in the mediastinum

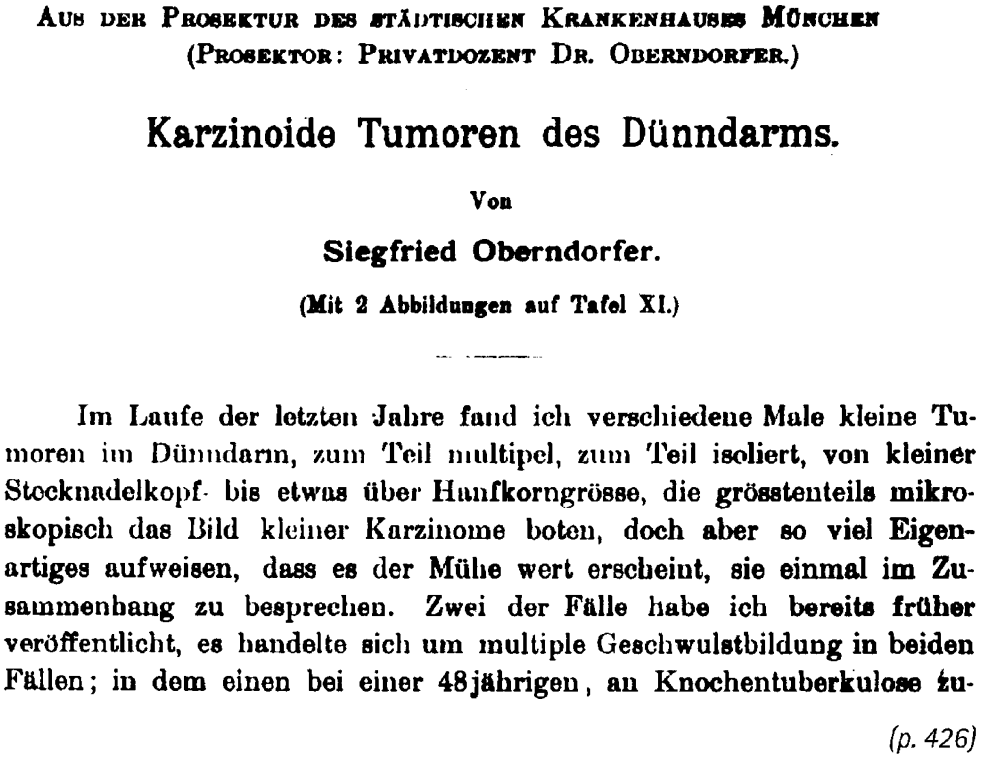
foreign country. Though only a student, the professor and head of the department of pathology in Kiel, Arnold Ludwig Heller (1840–1913), recognized his great interest in morbid anatomy and taught him his meticulous and "holoptic" autopsy technique which allowed the assessment of the organs in the context of their functional relationship to the surrounding organs. He quickly adopted Heller's technique and also his cleanliness, for which he himself became famous in later years when he was director of a pathology department. It is very likely that he profited very much in his professional and scientific life from his capacity to perform accurate postmortem examinations. At least his first observations of carcinoids, which he made in Geneva [9], are probably because of the fact that he was in the possession of a technique that allowed him to recognize even pea-sized tumorlets in the ileum, which could be easily overlooked.

Since he was never merely a morphologist who focused only on the appearance of lesions, he also considered the clinical history of the patients in whom he detected the carcinoids. As none of his patients had any symptoms related to the tumorlets in the ileum, he regarded these lesions as extremely slowly growing and apparently harmless in nature.

What were Oberndorfer's conclusions in 1907?

Oberndorfer observed the first two cases of carcinoids in Geneva (1900–1901) while working as an assistant to Professor Friedrich Wilhelm Zahn (1845–1904). He reported on these two tumors in the Beiträge zur pathologischen Anatomie und allgemeinen Pathologie in 1901. In Munich, where he started to work as assistant to Professor Otto Bollinger (1843–1909) in 1904, he added four other carcinoid cases and presented his results at the annual meeting of the Deutsche Gesellschaft für Pathologie in Dresden in 1907 [10] (Fig. 4). His lecture was heavily debated and its essence was published in December 1907 in the Frankfurter Zeitschrift für Pathologie. In this report, he summarized his observations and interpretations of the

Fig. 4 First page of Siegfried Oberndorfer's famous publication on carcinoids in the Frankfurter Zeitschrift für Pathologie (1907)



Aus der Prosektur des städtischen Krankenhauses München
(Prosektor: Privatdozent Dr. Oberndorfer.)

Karzinoide Tumoren des Dünndarms.

Von

Siegfried Oberndorfer.

(Mit 2 Abbildungen auf Tafel XI.)

Im Laufe der letzten Jahre fand ich verschiedene Male kleine Tumoren im Dünndarm, zum Teil multipel, zum Teil isoliert, von kleiner Stecknadelkopf- bis etwas über Hanfkorngrösse, die grösstenteils mikroskopisch das Bild kleiner Karzinome boten, doch aber so viel Eigenartiges aufweisen, dass es der Mühe wert erscheint, sie einmal im Zusammenhang zu besprechen. Zwei der Fälle habe ich bereits früher veröffentlicht, es handelte sich um multiple Geschwulstbildung in beiden Fällen; in dem einen bei einer 48jährigen, an Knochentuberkulose zu-

(p. 426)

lesions in five statements. The main characteristics of these tumorlets are:

1. They are usually small and often multiple.
2. Their cells form undifferentiated formations, at most with slight indications of glands.
3. They are well defined and show no tendency to penetrate infiltratively into the surroundings.
4. They do not metastasize.
5. They appear to grow extremely slowly, do not reach any great size, and are thus apparently harmless in nature.

The first three statements are still valid, except for the descriptive term "undifferentiated formations", which from today's vantage point does not reflect the differentiation of this particular tumor tissue. The last two statements, however, did not stand the test of time. As more and more carcinoids with lymph node and liver metastases were observed, Oberndorfer already admitted that some carcinoids may also be malignant. In his contribution to Henke and Lubarsch's textbook on special pathological anatomy and histology in 1929 [12], where he discussed his collection of 36 carcinoids of the ileum and appendix, he therefore distinguished carcinoids with benign behavior from "malignant carcinoids".

The carcinoids' endocrine nature was established by Masson [1, 5, 6], who suggested on the basis of his argentaffin staining results that the Kultchitzky cells in the crypts of Lieberkühn and the cells of the carcinoids have an endocrine function. Siegfried Oberndorfer himself abstained from speculating about the histogenesis of the carcinoids. Later, however, he discussed intensively, but also skeptically, the proposed neural-endocrine pathway that Masson proposed for the development of carcinoids in the appendix and their origin from the so-called enterochromaffin cells of the intestinal mucosa [11].

Why did he not return to the carcinoids after 1933?

Siegfried Oberndorfer was appointed full professor and director of the Institute of General and Experimental Pathology at the University of Istanbul shortly after he was forced to retire as director of the Department of Pathology at the München Schwabing Hospital and Associate Professor at the Medical Faculty of the University of Munich. Together with his colleague, Professor Philipp Schwartz (1894–1978) [13], who was also driven out of Germany because of his Jewish background and headed the Institute of Pathological Anatomy in Istanbul, he contributed very much to the development of pathology in Turkey in the fields of diagnostic pathology, cancer research, and training in pathology. Later (1938), he founded and headed the Turkish Institute of Cancer Research (Fig. 5). Conceivably, these

Fig. 5 Siegfried Oberndorfer in Istanbul in 1936

duties and his efforts to live in a foreign country and master its language consumed a great deal of his time. However, with his collaborators from Turkey (Drs. Uveis Maskar, Osman Saka, and Sati Eser) and from Germany (Dr. Peter Ladewig) he again produced approximately 100 contributions to many aspects of the pathology of neoplastic and nonneoplastic diseases and, finally, published textbooks in Turkish on general pathology (1937) and the histological diagnosis of some selected neoplasms (1941). An explanation for the fact that he never returned to the carcinoids may be that in his department he dealt only with surgical specimens, implying that at this time he had very little access to material to increase his experience with these tumors. But his interest in the carcinoids remained. Towards the end of his life he noted in his autobiography:

> "*Meine wesentlichen Arbeiten, so besonders die Wesentlichste, die fortleben wird, die Entdeckung der Bedeutung der "Carcinoide", habe ich nur in ganz kurzen Seiten veroeffentlicht; all das andere, was andere noch hinzugefuegt haben, habe ich zum grossen Teil auch gewusst, aber nicht verwertet. Zum Teil hinderte mich auch eine gewisse Scheu vor der Publizierung, der Verwertung der Arbeit in den grossen wissenschaftlichen Zeitungsschriften, so besonders in Virchows Archiv, eine gewisse Scheu vor dem Urteil der Autoritaeten, das ich retrospektiv nicht zu fuerchten gehabt haette. Aber "tempi passati"; im Ganzen war mein Leben doch nicht ganz sinnlos und ohne Ergebnisse, aber es haette wissenschaftlich noch glaenzender sein koennen*." (from Oberndorfer's unpublished autobiography, 1942–1943).

"My important works, especially the most significant work, which will survive, is the recognition of the significance of the carcinoids. It fills only a few pages. All the rest, which others added to it, I also knew, but made no use of it. In part, a certain hesitancy prevented me from publishing, from utilizing the work in the major scientific journals, particularly in Virchows Archiv, a certain dread of the verdict of the authorities that, retrospectively, I need not have feared. But "tempi passati", on the whole my life was not entirely futile and without results; but scientifically it could have been even more distinguished."

Acknowledgments We would like to thank Dr. Walter L. Castrillón-Oberndorfer, Siegfried Oberndorfer's grandson, for providing us with photographs and allowing us access to personal family records, in particular the unpublished autobiography. We are also grateful to Prof. K. Wurster, Munich, for providing us with information.

Conflict of interest statement We declare that we have no conflict of interest.

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REVIEW AND PERSPECTIVE

Site-specific biology and pathology of gastroenteropancreatic neuroendocrine tumors

Günter Klöppel · Guido Rindi · Martin Anlauf · Aurel Perren · Paul Komminoth

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Abstract The gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are composed of cells with a neuroendocrine phenotype. Well-differentiated tumors, well-differentiated carcinomas, poorly differentiated carcinomas, functioning tumors (with a hormonal syndrome), and nonfunctioning tumors are identified. To predict their clinical behavior, these neuroendocrine tumors are classified on the basis of their clinicopathological features, including size, local invasion, angioinvasion, proliferative activity, histological differentiation, and metastases, into neoplasms with benign, uncertain, low-grade malignant and high-grade malignant behavior. In addition, a tumor/nodes/metastases classification and a grading system are presented. In the light of these criteria, the various GEP-NET entities are reviewed.

Keywords Neuroendocrine tumors · Gut · Pancreas · Pathology · Classification · Biology · Prognosis

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Introduction

The neuroendocrine tumors (NETs) of the gastrointestinal tract and the pancreas express antigens that are also common in nerve elements. These antigens have been referred to as neuroendocrine markers, and their demonstration in normal cells and neoplasms has led to the terms neuroendocrine cell system and neuroendocrine neoplasms. Markers of the neuroendocrine phenotype such as synaptophysin, chromogranins A, B, and C [89], HISL-19, neuron-specific enolase (NSE), the proprotein convertases PC2 and PC3, the lymphoreticular epitope Leu-7, and the neural cell adhesion molecule (or CD56) reveal the neuroendocrine differentiation of gastroenteropancreatic NETs (GEP-NETs), independent of hormone production [88].

This review discusses the various GEP-NET entities that are observed in the foregut, midgut, and hindgut regions. The GEP-NETs associated with hereditary diseases and the molecular changes in GEP-NETs are dealt with in separate papers in this issue [5, 117].

Classification

In the gastrointestinal tract and pancreas, 15 neuroendocrine cell types producing different hormones but all expressing the general neuroendocrine marker synaptophysin can be distinguished [150]. They are the source of GEP-NETs. Traditionally, they are separated into benign or malignant neoplasms. In this paper, however, we follow the concept that all GEP-NETs are potentially malignant but differ in their metastasizing capacity (i.e., their biological behavior), depending on a number of features and criteria (see below).

The functional diversity of the neuroendocrine cell types and their nonrandom distribution in the gut and pancreas are probably the reason for the complexity of the tumors derived from them. Classifying these tumors is therefore extremely difficult. In 1963, Williams and Sandler [173] classified the GEP-NETs into foregut (stomach, duodenum, upper jejunum, and pancreas), midgut (lower jejunum, ileum, appendix, and cecum), and hindgut (colon and rectum) tumors, with considerable clinicopathological differences between the three groups. However, in the case of the foregut tumors, the usefulness of such a classification in practical diagnostic work is limited by its failure to characterize individual tumor entities with well-defined histological, hormonal, and/or clinicopathological profiles. The World Health Organization (WHO) 2000 classification, based on earlier work [28], therefore follows a different approach. In a first step, it distinguishes between pure endocrine tumors and mixed endocrine–exocrine tumors (for mixed tumors, see article by Volante et al. (in this issue and [167]). In a second step, a uniform scheme of classification is applied to all pure GEP-NETs, identifying three tumor categories, irrespective of their site of origin:

(1) Well-differentiated endocrine tumors with benign (a) or uncertain behavior (b) at the time of diagnosis
(2) Well-differentiated endocrine tumors with low grade malignant behavior
(3) Poorly differentiated endocrine carcinomas with high-grade malignant behavior

In a third step, the well-differentiated, slowly growing NETs and carcinomas of the gastrointestinal tract and the pancreas, those that are also called carcinoids [110] and islet cell tumors, respectively, and which comprise a number of well defined entities (e.g., insulinomas, gastrinomas, and others), are distinguished on the basis of their localization as well as their morphological and functional features. Poorly differentiated NETs that are composed of cells displaying high mitotic activity and few secretory granules form a separate group, as they are not difficult to recognize as invariably high-grade malignancies.

Undisputable criteria that predict the biological behavior of well-differentiated GEP-NETs are gross tumor infiltration of adjacent organs and/or metastases. Adverse prognostic factors include tumor size, angioinvasion, mitotic activity, and an elevated Ki-67/MiB-1 proliferative index (higher than 2%) [29, 58]. Tables 1 and 2, based on the WHO criteria, provide checklists that allow an appropriate classification of an individual GEP-NET.

As there is an increased demand for a standard in the stratification and treatment of patients with GEP-NETs, the European Neuroendocrine Tumour Society recently developed guidelines that were supplemented by a proposal for tumor/nodes/metastases (TNM) classification [127, 128]. Apart from theTNM staging proposal, a working formulation for the grading of GEP-NETs based on mitotic count and Ki-67 index has been suggested. Both the staging proposal and the grading system still need to be validated.

Epidemiology

Incidence data on GEP-NETs are difficult to obtain because of the rarity of these neoplasms, their long clinical courses, their incomplete recognition, and their classification, which was for a long time rather inaccurate. The Surveillance, Epidemiology, and End Results (SEER) data on carcinoids, which are usually cited when the incidence of GEP-NETs are discussed [99], are probably inexact for gastric and duodenal NETs, as the general availability of endoscopy

Table 1 Criteria for assessing the prognosis of neuroendocrine tumors of the gastrointestinal tract

Biological behavior	Metastases	Invasion of muscularis propria[a]	Histological differentiation	Tumor size (cm)	Angioinvasion	Ki-67 index (%)	Hormonal syndrome
Benign	−	−	Well differentiated	≤1[a]	−	<2	−[a]
Benign or low-grade malignant	−	−	Well differentiated	≤2	−/+	<2	−
Low-grade malignant	+	+[b]	Well differentiated	>2	+	>2	+
High-grade malignant	+	+	Poorly differentiated	Any	+	>20	−

[a] Exception: malignant duodenal gastrinomas are usually smaller than 1 cm and confined to the submucosa
[b] Exception: benign NETs of the appendix usually invade the muscularis propria

Table 2 Criteria for assessing the prognosis of neuroendocrine tumors of the pancreas

Biological behavior	Metastases	Invasion[a]	Histological differentiation	Tumor size (cm)	Angioinvasion	Ki-67 index (%)	Hormonal syndrome
Benign	–	–	Well differentiated	≤1	–	<2	–/+[b]
Benign or low-grade malignant	–	–	Well differentiated	>2	–/+	<2	–/+[c]
Low-grade malignant	+	+	Well differentiated	>3	+	>2	+[c]
High-grade malignant	+	+	Poorly differentiated	Any	+	>20	–

[a] Invasion of adjacent organs (e.g., duodenum, stomach)
[b] Insulinomas
[c] Insulinomas and other functioning tumours (e.g., glucagonomas)

has led to a considerable increase in the rates of these NETs that is not yet reflected in the SEER data. For these reasons, the following incidence figures for the NETs of the gut are interpreted with caution. The incidence of all NETs of the gut has been estimated according to a recent Swedish study to be 2.0/100,000 for men and 2.4/100,000 for women [59].

NETs of the esophagus are very rare and represent only 0.05% of all gastrointestinal NETs or approximately 1% of all esophageal cancers [34, 100]. Most patients are men, who are mainly in the sixth to seventh decade [87].

NETs of the stomach were thought to account for 2–4% of all gastrointestinal NETs [99, 100]. However, as the incidence of gastric NETs has not yet really been determined after the increased application of endoscopy, it is quite possible that gastric NETs may lead the list of the most frequent gastrointestinal NETs and account for 11–41% [75]. In Japan, they represent 30% of all gastrointestinal carcinoids.

Duodenal NETs account for approximately 2% of all gastrointestinal NETs in the old series [53, 100]. In more recent series, however, duodenal and jejunal NETs amounted to 22% of all gastrointestinal NETs [33]. The tumors occur slightly more frequently in men (male/female ratio=1.5:1) and are usually seen in the fifth and sixth decade [33].

Ileal NETs account for approximately 25% of all gastrointestinal NETs [33]. Men and women are affected equally. Their age ranges from the third to the tenth decade but has a peak in the sixth decade.

Appendiceal NETs account for approximately 20% of all NETs in the gastrointestinal tract [92, 100]. They are the most frequent tumors in the appendix. In contrast to all other NETs of the gastrointestinal tract, they most commonly present in the second to third decade and affect women more frequently than men [95, 110]. They may also occur in children [9].

While colon NETs are rare, NETs of the rectum account for 20% of the gastrointestinal NETs [32]. Their sex distribution is equal. An increase in incidence in colonic and rectal NETs has been observed in recent years [59, 85, 92, 99, 146]. In western countries, NETs of the colon are primarily diagnosed in male patients during the seventh decade of life and rectal lesions in the sixth decade [59]. A similar trend is observed in women, although at a relatively younger age, in the fifth decade in the colon and the sixth in the rectum [59]. Poorly differentiated neuroendocrine carcinomas develop predominantly in male patients [20].

The prevalence rate of PETs has been estimated at less than 1 in 100,000 [101]. Their incidence is not known, but it is probably substantially lower because of the generally rather favorable prognosis of PETs. For insulinomas alone, an incidence of approximately four tumors per 1 million patient-years has been calculated [141]. PETs appear at any age but occur preferentially between 30 and 60 years. There is no significant gender selection but a slight female preponderance of 55% to 45%.

PETs account for 1–2% of all pancreatic neoplasms [151]. Syndromic or functioning PETs (i.e., PETs that cause hormonal syndromes) make up 60%, with insulinomas being the most frequent type (up to 70%), followed by gastrinomas [22, 73, 76, 79, 135].

Insulinomas have been diagnosed in all age groups. The highest incidence is found between 40 and 60 years. Women seem to be slightly more frequently affected than men (ratio 6:4) [49, 51, 52, 84, 140, 141, 165].

Gastrinomas account for about 20% of endocrine pancreatic tumors [56, 151]. Zollinger–Ellison syndrome (ZES) is more common in men than in women, with a ratio of 3:2, and the mean age at diagnosis is 38 years [13, 27, 69, 126, 154].

Glucagonomas represent about 5% of all clinically relevant pancreatic endocrine tumors and 8% of functioning tumors [151]. The patients most often present between the ages of 40 and 70 years and women are slightly more often affected [136].

VIPomas constitute 3–8% of all pancreatic endocrine tumors, and women are more often (70%) affected than men.

Nonfunctioning PETs may occur at any age; however, they are rare in childhood [143]. Clinically relevant

nonfunctioning PETs are rare, with a prevalence of 0.2–2 per million inhabitants [58]. Small nonfunctioning PETs including microadenomas are much more frequent, with a reported prevalence in autopsy studies from 0.4 to 1.5% (largely depending upon the amount of pancreatic tissue examined) [54, 74]. There is no gender predilection [62].

Esophagus

Most NETs of the esophagus are poorly differentiated neuroendocrine carcinomas and mixed endocrine–exocrine carcinomas, usually of large size (from 4 to 10 cm in diameter) and located in the lower third of the esophagus [34]. They present as fungating or ulcerated masses deeply infiltrating into the esophageal wall and are associated with early spread to the regional lymph nodes or infiltration of adjacent organs. In contrast, most well-differentiated NETs/neuroendocrine carcinomas are less than 4 cm in diameter and present as polypoid lesions. Only few of them were found to be associated with lymph node metastases [61, 115].

Poorly differentiated neuroendocrine carcinomas of the esophagus are often of the large cell type and only positive for synaptophysin (Fig. 1). Well-differentiated NETs/carcinomas show a solid or trabecular–glandular growth pattern and are strongly immunoreactive for synaptophysin and chromogranin A, but mostly negative for the hormones expressed in the gastrointestinal tract. No established grading and staging scheme exists for these NETs.

The majority of well-differentiated esophageal NETs/carcinomas were found in association with Barrett's esophagus and adenocarcinomas. We saw two well-differentiated NETs of the esophagus. Both were incidental findings and associated with heterotopic oxyntic mucosa. They stained strongly positive for the vesicular monoamine transporter 2 (VMAT2) suggesting an origin from the enterochromaffin-like (ECL) cells within heterotopic oxyntic mucosa.

The initial symptoms of these tumors are unspecific because they are functionally silent. Inappropriate antidiuretic hormone syndrome, hypercalcemia, or ectopic production of vasoactive intestinal polypeptide (VIP) have been reported in only single poorly differentiated neuroendocrine carcinomas [34, 66, 169]. Well-differentiated NETs of the esophagus are rarely associated with lymph node metastases and thus have an excellent prognosis. Eleven patients suffering from primary esophageal well-differentiated NETs/neuroendocrine carcinomas for which follow-up was available were all alive and disease-free at 1–23 years after surgical excision (mean 5.5 years). The overall survival of patients with poorly differentiated carcinomas is usually less than 6 months [68, 83]. Mixed endocrine–exocrine carcinomas of the esophagus show a prognosis similar to that of adenocarcinomas, depending on their histological differentiation and tumor stage [36]. Neither associated diseases nor inherited syndromes have been reported for NETs/neuroendocrine carcinomas of the esophagus.

Stomach

There are three distinct types of NETs in the stomach [129], and if the poorly differentiated NET is added, four types of NETs can be distinguished [35, 75]. Type 1 comprises approximately 70–80% of all cases, followed by types 3, 2, and 4 [129]. Recently, a proposal for a TNM staging classification and a grading system for tumors of the stomach was published (Tables 3, 4, and 5).

Type 1 NETs of the stomach present as multiple small tumors (0.3–1 cm; Fig. 2a). Histologically, they are well differentiated (proliferation rate <2%), show a solid pattern, and are composed of intensely synaptophysin and

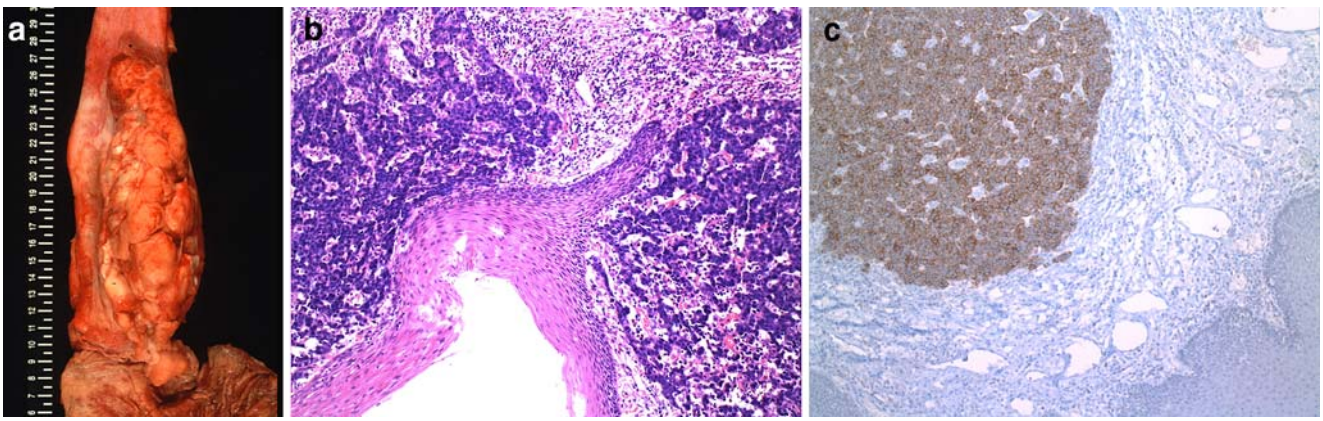

Fig. 1 Poorly differentiated neuroendocrine carcinoma of the esophagus (**a**) with subepithelial infiltration by large atypical (**b**) and synaptophysin-positive cells (**c**)

Table 3 Proposal for a pTNM classification for neuroendocrine tumors of the stomach [127]

Abbreviation	Characteristics
T—primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	In situ tumor/dysplasia (<0.5 cm)
T1	Tumor invades lamina propria or submucosa and ≤1 cm
T2	Tumor invades muscularis propria or subserosa or >1 cm
T3	Tumor penetrates serosa
T4	Tumor invades adjacent structures
	For any T add (m) for multiple tumors
N—regional lymph nodes	
NX	Regional lymph node status cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M—distant metastases	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1	Distant metastasis

chromogranin A positive ECL cells. These cells, which produce histamin, are specifically recognized by the marker VMAT2 [45, 130] (Fig. 2b). In addition to the tumors, there is always ECL cell hyperplasia in the oxyntic mucosa. The tumors are not associated with any hormonal syndrome but are always found on a background of chronic atrophic gastritis of the oxyntic mucosa of the autoimmune type. This disease occurs mainly in women, aged between 50 and 60 (70–80% of the patients) and leads to the disappearance of the specific glands of the oxyntic mucosa harboring the parietal cells. The consequences of the loss of parietal cells are the insufficient production of intrinsic factor, which triggers pernicious anemia via the decreased resorption of vitamin B12, and deficient production of gastric acid, which stimulates the antral G cells to persistent hypersecretion of gastrin. It is thought that hypergastrinemia promotes the growth of the ECL cells of the oxyntic mucosa so that diffusion to micronodular ECL cell hyperplasia develops and, after a latent period of many years, multiple ECL tumors [14]. The observation that the tumors can also occur in only partially atrophic gastritis and the detection of growth factors such as tumor growth

Table 4 Proposal for disease staging for neuroendocrine tumors of the stomach

Stage	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIa	T2	N0	M0
Stage IIb	T3	N0	M0
Stage IIIa	T4	N0	M0
Stage IIIb	Any T	N1	M0
Stage IV	Any T	any N	M1

Table 5 Proposal for a grading system for neuroendocrine tumors [127]

Grade	Mitotic count (10HPF)[a]	Ki-67 index (%)[b]
G1	<2	≤2
G2	2–20	3–20
G3	>20	>20

[a] Ten HPF: high power field=2 mm^2, at least 40 fields evaluated in areas at highest mitotic density
[b] MiB1 antibody
Percent of 2,000 cells in areas of highest nuclear labeling

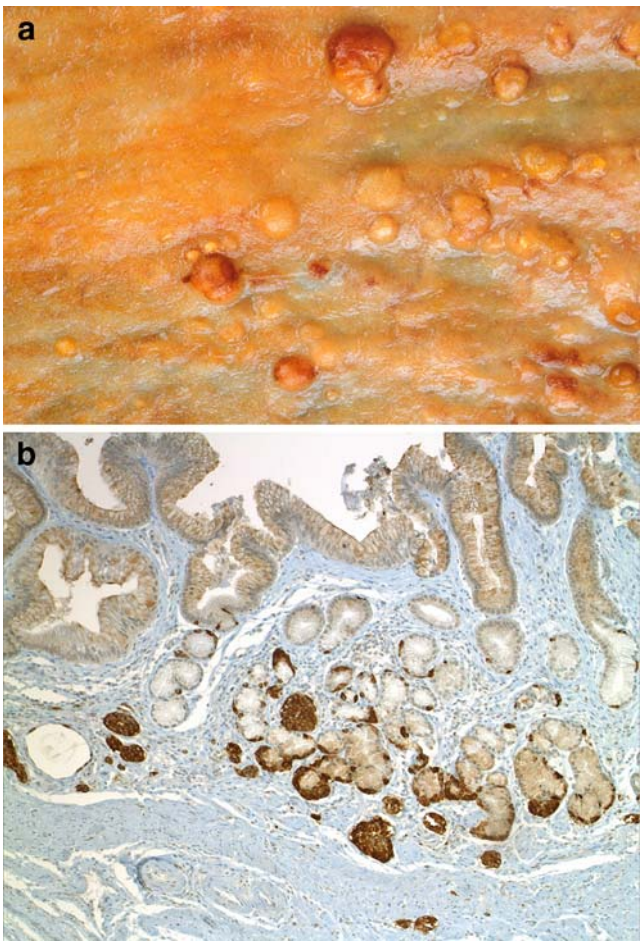

Fig. 2 Well-differentiated neuroendocrine tumors of the stomach associated with chronic atrophic gastritis of the oxyntic mucosa (type 1 gastric NET): **a** multiple small polypoid tumors in the corpus region of the stomach. **b** VMAT2-positive ECL cell hyperplasia in the oxyntic mucosa with microtumors

factor alpha, basic fibroblast growth factor, and B cell leukemia/lymphoma 2 are indications that hypergastrinemia alone probably does not cause these tumors to develop [14, 17]. The prognosis of these tumors is good because they are usually so small that they can be removed endoscopically. Regional lymph node metastases seem to occur only in those very rare cases in which the tumors are larger than 2 cm in size and infiltrate the muscularis propria [124].

Type 2 gastric NETs occur in association with multiple endocrine neoplasia type 1 (MEN1), a hereditary, autosomal dominant disorder, in the course of which a ZES has developed. The genetic changes associated with MEN1 are probably needed for tumor development [42] because so far no such tumors have been found in patients with ZES but without MEN1. As in type 1 NETs, multiple ECL cell NETs are found in the oxyntic mucosa, which, however, is not atrophic but hypertrophic. Men and women with a mean age of 50 years are equally affected [35]. Lymph node metastases may develop in rare cases in which the tumors exceed a size of 1–2 cm [149].

Type 3 gastric NETs are sporadic (unassociated with MEN1) solitary tumors that develop unrelated to chronic atrophic gastritis and occur throughout the stomach (Fig. 3). In one third of the cases, the tumor is already larger than 2 cm at the time of diagnosis. Histologically, they are well differentiated, show a trabecular to solid pattern, and often have a proliferation rate exceeding 2–5%. They consist in most cases of ECL cells. Tumors with EC (serotonin) cells or gastrin cells, by contrast, are extremely rare [75]. They show a predilection for the male sex and occur at a mean age of 55 years [35]. If the tumor is larger than 2 cm, has invaded the muscular layer, and/or shows angioinvasion, metastases are very likely to be present [35]. In 71% of such cases that were surgically removed, lymph node metastases were found [35]. In rare cases, type 3 tumors may be associated with a so-called atypical carcinoid syndrome, characterized by cutaneous flushing in the absence of diarrhea, usually coupled with liver metastases and production of histamine and 5-hydroxytryptophan [35].

Poorly differentiated neuroendocrine carcinomas of the stomach ("type 4 gastric NETs") are rare. Histologically, they often show no special arrangement of the cells, which are medium sized, with a chromatin-rich nucleus and sparse cytoplasm. Immunohistochemically, they are positive for synaptophysin and to some extent also for chromogranin A. Hormones cannot be demonstrated. These tumors are more common in men than in women, aged between 60 to 70 years. They present as a large ulcerated lump with symptoms similar to those of adenocarcinomas, including gastric hemorrhage and obstruction. Hormonal symptoms are absent, and there is no relationship to chronic atrophic gastritis but, in exceptional cases, to MEN1 [16]. At the time of diagnosis, most of the tumors are already in an advanced stage (tumor diameter more than 4 cm) and show extensive metastasis. Their prognosis is poor, with three quarters of the patients dying within 1 year of diagnosis because of extensive metastatic disease [16]. In addition, poorly differentiated large cell neuroendocrine carcinomas or mixed endocrine–exocrine carcinomas (endocrine differentiation in greater than 30% of the tumor) may occur but are extremely rare. Mixed exocrine–endocrine carcinomas should generally be classified as adenocarcinomas [35].

Recently, multiple, up to 1.3 cm large ECL cell tumors were found in a background of ECL cell hyperplasia and parietal cell hyperplasia in patients with hypergastrinemia but without ZES [1, 112]. It was suggested that the development of these NETs is associated with an intrinsic acid secretion abnormality of the parietal cells.

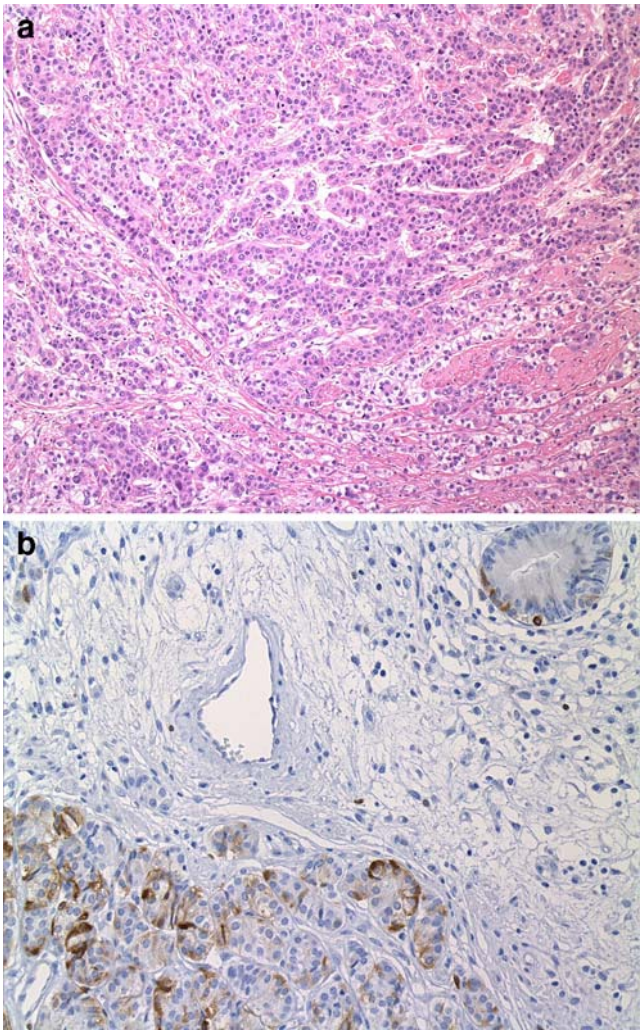

Fig. 3 Well-differentiated neuroendocrine carcinoma of the stomach (**a**) unrelated to chronic atrophic gastritis (type 3 gastric NET). VMAT2-positive ECL cell tumor (**b**)

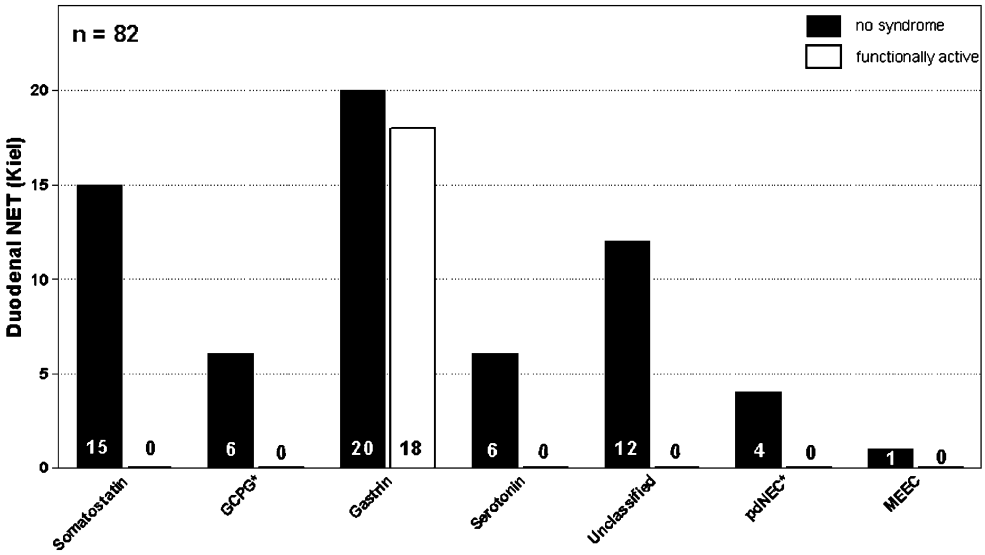

Fig. 4 Relative ratios of neuroendocrine tumors of the duodenum defined by their hormone expression. Neuroendocrine tumor archives of the Department of Pathology, University of Kiel, 1970 and 2006

Duodenum and upper jejunum

On the basis of their clinical, morphological, hormonal, and genetic features, several types have to be distinguished: gastrin-producing NETs with ZES (i.e., gastrinomas), gastrin-producing NETs without ZES, somatostatin-producing tumors with or without neurofibromatosis type 1 (NF1), serotonin- or calcitonin-producing NETs, poorly differentiated neuroendocrine carcinomas, and gangliocytic paragangliomas [14, 25, 28, 152]. Their relative frequency is shown in Fig. 4. Tables 6 and 7 show the recently proposed TNM classification for these NETs.

Gastrin-producing and gastrin-secreting NETs causing a ZES are called gastrinomas. Twenty-five to 33% occur in the setting of an MEN1 syndrome. Sporadic and MEN1-associated duodenal gastrinomas usually arise in the first part of the duodenum [43, 109, 156, 163]. They usually form small (<2 cm in diameter) polypoid lesions within the submucosa with an intact or focally ulcerated overlying mucosa (Fig. 5). In a large series of 96 duodenal NETs, the mean size was 0.8 cm for gastrinomas [31, 156]. The gastrinomas in MEN1 are multiple and tiny (sometimes less than 1 mm in diameter) [7]. Both the sporadic and MEN1 gastrinomas are well differentiated, showing a trabecular–pseudoglandular pattern. They all stain for gastrin. Caudal-related homeobox 2 (CDX2) is positive in half of the cases. MEN1-associated gastrinomas are associated with focally accentuated gastrin and somatostatin cell hyperplasia in the mucosa and/or Brunner's glands, while sporadic gastrinomas lack such lesions [6, 7]. Despite their small size, metastases to regional lymph nodes are already found in 60 to 80% of the cases at the time of diagnosis [162, 170]. These metastases may be much larger than the primary tumor and may erroneously be considered pancreatic tumors, especially if they are located at the upper margin of the head of the pancreas [43]. This peculiarity is probably the reason why in early reports primary lymph node gastrinomas were diagnosed and many more pancreatic gastrinomas were recorded than today. Apart from lymph node metastases, duodenal gastrinomas may metas-

Table 6 Proposal for a pTNM classification for neuroendocrine tumors of the duodenum/ampulla/proximal jejunum

Abbreviation	Characteristics
T—primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumors
T1	Tumor invades lamina propria oder submucosa and size ≤1 cm
T2	Tumor invades muscularis propria or >1 cm
T3	Tumor invades pancreas or retroperitoneum
T4	Tumor invades peritoneum oder other organs
	For any T: add (m) for multiple tumors
N—regional lymph nodes	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Regional lymph node metastases
M—distant metastases	
Mx	Distant metastases cannot be assessed
M0	No distant metastases
M1	Distant metastases

Table 7 Proposal for disease staging for neuroendocrine tumors of the duodenum/ampulla/proximal jejunum

Stage	T	N	M
Stage I	T1	N0	M0
Stage IIa	T2	N0	M0
Stage IIb	T3	N0	M0
Stage IIIa	T4	N0	M0
Stage IIIb	Any T	N1	M0
Stage IV	Any T	Any N	M1

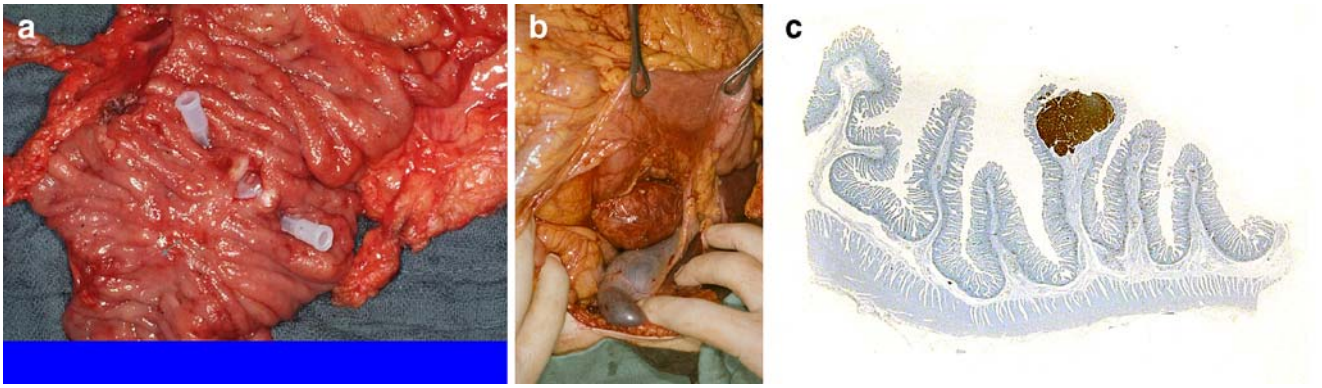

Fig. 5 Well-differentiated neuroendocrine carcinoma of the duodenum producing gastrin and associated with a Zollinger–Ellison syndrome. **a** Duodenal mucosa showing a small submucosal tumor. **b** Lymph node metastasis at the upper margin of the pancreas. **c** Duodenal mucosa with a gastrin-positive NET

tasize to the liver but only in a small percentage of cases (approximately 10%) and only many years after the manifestation of the disease. Thus, 10-year survival rates of 84% have been reported in patients with duodenal gastrinomas [107, 162, 170]. Fast-growing poorly differentiated and metastasizing duodenal gastrinomas are extremely rare.

The ZES is characterized by elevated fasting gastrin serum levels, a positive gastrin secretin stimulation test, and clinical symptoms such as recurrent peptic ulcer disease, gastroesophageal reflux disease, and occasional diarrhea [8, 176]. Between 60 and 75% of patients with sporadic ZES are found to have a duodenal gastrinoma, while the remaining patients are found to have a pancreatic gastrinoma. In MEN1-associated ZES, most, if not all, patients have their gastrinomas in the duodenum [4, 8, 118, 119].

Somatostatin-producing tumors account for approximately 20% of all duodenal NETs (Table 2). Their preferential localization is in the region of the papilla of Vater, and their mean size is 2.3 cm [31, 41, 156]. Histologically, these tumors often have a glandular pattern, frequently with psammoma bodies in the lumina. About 50% of the somatostatin-producing tumors lack chromogranin A immunoreactivity but stain brightly for synaptophysin and somatostatin. If the tumors invade the muscularis propria, metastasis to the paraduodenal lymph nodes has probably occurred [41]. They are not associated with a hormonal syndrome, particularly not with a somatostatinoma syndrome, but may be found in association with NF1 [33, 156]. There are no morphological features to distinguish between NF1- and non-NF1-associated duodenal NETs.

Gangliocytic paragangliomas are rare (Table 2). They are mainly found in the second (periampullary) portion of the duodenum, and their size ranges from 1.5 to 7 cm [31, 125, 156]. The tumors are composed of three different mature cell types: (1) epithelial/endocrine cells, which are arranged in ribbons, solid nests, or pseudoglandular structures, (2) S100-positive neural spindle cells, which usually represent the major component, and (3) scattered or aggregated ganglionic cells. Despite their infiltrative growth, metastasis to the regional lymph nodes is rarely observed [24, 67, 137].

Well-differentiated duodenal NETs producing gastrin but not associated with a ZES have been increasingly recog-

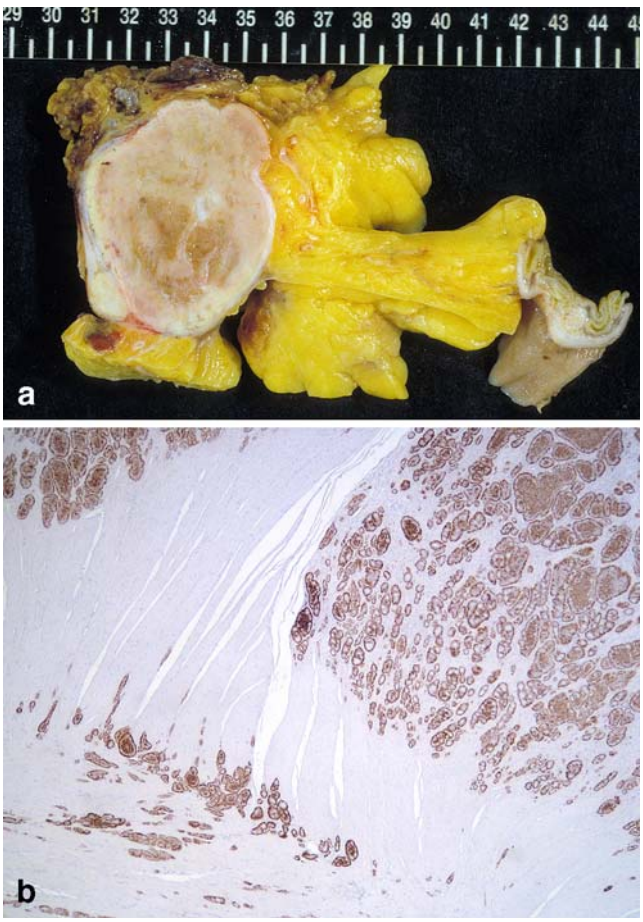

Fig. 6 Small neuroendocrine carcinoma of the ileum with large lymph node metastasis in the mesenterium (**a**). The tumor infiltrates the muscular layer and produces serotonin (**b**)

nized during recent years and are so far not well characterized. They are mostly found in the duodenal bulb as small polypous tumors localized in the mucosa and submucosa. Histologically and immunohistochemically, they are indistinguishable from gastrinomas. Recently, a series of these NETs were reported in patients who were on proton pump blockers and had *Helicobacter pylori* [97]. These tumors were found to be associated with gastrin cell hyperplasia in the adjacent mucosa. Clinically, they are often incidental findings during endoscopy or cause symptoms by virtue of local infiltration such as obstructive jaundice, pancreatitis, hemorrhage, and intestinal obstruction [33]. The prognosis of this group of nonfunctioning tumors is much more favorable than ZES-associated gastrinomas or ampullary somatostatinomas [33]. Metastases are not to be expected until the tumor extends beyond the submucosa.

In rare cases, there are well-differentiated duodenal NETs producing serotonin or calcitonin [31, 156]. Their biology is not well characterized but seems to follow the criteria listed in Tables 6 and 7.

Poorly differentiated neuroendocrine carcinomas of the duodenum occur primarily in the region of the papilla of Vater. They present as ulcerated or protuberant tumors ranging in size between 2 and 3 cm [33]. Histologically, they show a solid arrangement of medium-sized or large cells staining for synaptophysin but only rarely for chromogranin A. A small number of tumor cells may also stain for somatostatin. Clinically, most patients develop jaundice and/or hemorrhage. Hormonally, they are inactive. At the time of diagnosis, advanced metastasis to the regional lymph nodes and the liver has usually occurred [104].

Table 8 Proposal for a TNM classification for neuroendocrine tumors of the lower jejunum and ileum

Abbreviation	Characteristics
T—primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor invades mucosa or submucosa and size ≤1 cm
T2	Tumor invades muscularis propria or size >1 cm
T3	Tumor invades subserosa
T4	Tumor invades peritoneum/other organs
	For any T add (m) for multiple tumors
N—regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M—distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1	Distant metastasis

Neuroendocrine tumors of the distal jejunum, ileum, and Meckel's diverticulum

The well-differentiated NETs (carcinoids) of the lower jejunum and the ileum present as small sessile nodules with a diameter ranging between 0.5 and 3 cm but usually between 1 and 2 cm. Most of them occur in the terminal ileum, close to the ileocecal valve, and in 26–30% of the cases, they are multiple [26, 116, 175]. Tumors that are larger than 1 cm almost invariably show infiltration of the muscular layer and the adjacent subserosal fatty tissue. On the cut surface, they are yellow and small (Fig. 6a). Histologically, they are characterized by an insular growth pattern with solid to cribriform tumor structures showing pallisading of the peripheral cell layer. In the glandular cavities, there are sometimes PAS-positive material. The tumor structures are embedded in a sclerotic paucicellular stroma that may lead to kinking of the foregut and subsequently to bowel obstruction. The uniform tumor cells have faintly eosinophilic cytoplasm and a very low mitotic rate, and immunohistochemically, they show the phenotype of serotonin-producing enterochromaffin (EC) cells (Fig. 6b). Between the tumor cells, there may be single S100-positive cells [26]. In addition, carcinoembryonic antigen is found in approximately two thirds of the tumors. The tumor cells may also express neurotransmitters and are positive for CDX2 [81]. There is no endocrine cell hyperplasia in the mucosa adjacent to or distant from the tumors.

If the tumors are smaller than 1 cm in diameter, lymph node metastases are rare, not exceeding 5% of the cases. If the tumors are larger than 2 cm, lymph node metastases are present in 85% of the cases, and very often, there are also liver metastases [158]. The lymph node metastases lie in the mesenterial tissue and are often larger than the primary.

Table 9 Proposal for disease staging for neuroendocrine tumors of the lower jejunum and ileum

Stage	T	N	M
Stage I	T1	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	any T	N1	M0
Stage IV	any T	any N	M1

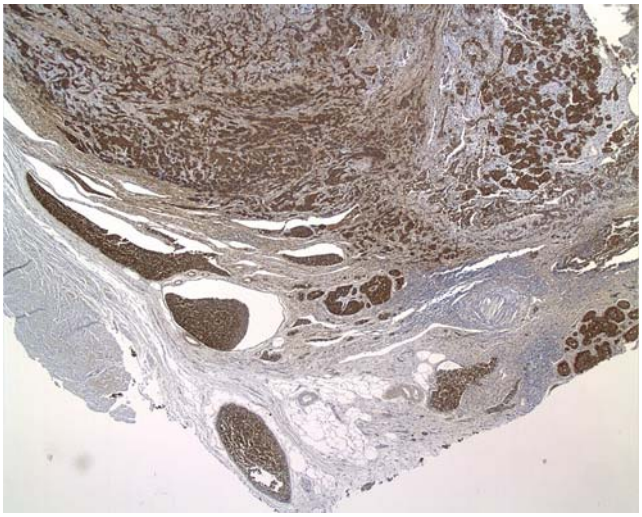

Fig. 7 Well-differentiated serotonin-producing neuroendocrine carcinoma of the appendix with infiltration of the mesoappendixand angioinvasion

Tables 8 and 9 show the newly developed TNM staging system for the tumors [128].

Clinically, the tumors may be discovered during exploration of the gut in search of a primary tumor that gave rise to liver metastases, or the patients have local (bowel obstruction, subileus) and/or systemic symptoms. The systemic symptoms are due to the hormonal effects of serotonin and are called carcinoid syndrome. This is characterized by flush, diarrhea, and carcinoid heart disease mostly causing right-sided heart failure because of tricuspid regurgitation. The carcinoid syndrome is usually seen in patients with liver metastases (95%). However, in some cases, liver metastases can occur without the syndrome. Carcinoid heart disease is a rather late event in the carcinoid syndrome and is responsible for a large number of deaths in these patients. Overall 5-year survival rates range from 50 to 60%, decreasing to 35% if liver metastases are present [158]. There is no association with any known hereditary disease, but approximately one third of the patients have other, mostly intestinal, carcinomas, either synchronous or metachronous [26, 175].

Meckel's diverticulum is a rare site of well-differentiated NETs. These tumors are usually small (<1.7 cm) and when found incidentally have not yet metastasized [26]. However, if symptomatic, metastases are likely to be found [98].

Table 10 Proposal for a TNM classification for neuroendocrine tumors of the appendix

Abbreviation	Characteristics
T—primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor ≤1 cm invading submucosa and muscularis propria
T2	Tumor ≤ 2 cm invading submucosa, muscularis propria and/or minimally (up to 3 mm) subserosa/mesoappendix
T3	Tumor >2 cm and/or extensive (more than 3 mm) invasion of subserosa/mesoappendix
T4	Tumor invades peritoneum/other organs
N—regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M—distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1	Distant metastasis

Neuroendocrine tumors of the appendix

The well-differentiated NETs/neuroendocrine carcinomas of the appendix (appendiceal carcinoids) usually occur in the tip of the organ, are 1–2 cm in size, and infiltrate the appendix wall. Histologically, they are characterized by a solid islet-like pattern (i.e., type A pattern according to Soga and Tazawa [147]. Rarely, they show trabeculae and ribbons (Soga's type B). Tumor cells are uniform, with low atypia and rare mitoses, if any. The tumors with a solid pattern are composed of serotonin-producing EC cells. In addition, there are sustentacular cells that are positive for S-100 [150].

A size greater than 1 cm, a location at the base of the appendix, extensive involvement of the mesoappendix, and angioinvasion are important negative prognostic factors (Fig. 7) and are potentially associated with metastases [95]. The risk of lymph node metastases in tumors measuring 1 to 2 cm is 1% and increases to 30% in tumors measuring more than 2 cm [158]. Mesoappendix invasion is a debated variable [91, 134]. Series with sufficiently long follow-up, including children with a median age of 12 years, revealed that no patient treated by appendectomy died of appendiceal NETs with a diameter below 2 cm [114, 158]. A poorly differentiated neuroendocrine carcinoma, as part of a mixed

Table 11 Proposal for disease staging for neuroendocrine tumors of the appendix

Stage	T	N	M
Stage I	T1	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	any T	N1	M0
Stage IV	any T	any N	M1

exocrine–endocrine carcinoma, has only been reported once so far [133]. Tables 10 and 11 show the newly developed TNM stage system and the grading of the tumors [128].

Most tumors are usually detected because of symptoms of acute appendicitis. A carcinoid syndrome has not been reported in association with a metastasized well-differentiated appendiceal NET. The carcinoid syndrome is exceedingly rare [102].

Neuroendocrine tumors of the colon and rectum

Well-differentiated NETs (carcinoids) are more frequent in the rectum than the colon, whereas poorly differentiated neuroendocrine carcinomas are more common in the colon. The well-differentiated rectal tumors mostly appear during endoscopy as small (<1 cm) movable submucosal tumors. The poorly differentiated neuroendocrine carcinomas of the colon are usually of large size (>2 cm). An average diameter of 4.9 cm was reported [10], and 55 of 129 cases reported by Soga were between 5 and 10 cm in size [145]. The well-differentiated NETs of the colon are mostly small and occur in the cecal region [10, 132, 145] or throughout the colon in cases of ulcerative colitis [94]. Recently, "microcarcinoids" (0.5 to 1.5 mm in size) have been described in polypous colonic adenomas [122].

Histologically, the well-differentiated NETs (carcinoids) of the rectum are characterized by a trabecular pattern (Soga's type B) [150]. The well-differentiated NETs of the colon, in contrast, show the solid pattern that is also found in the tumors of the small intestine (Soga's type A) or a mixed pattern (Soga's type E) [145, 150]. Immunohistochemically, the well-differentiated NETs of the rectum are positive for synaptophysin, glucagon, glicentin, and/or pancreatic polypeptide and are usually negative for chromogranin. In addition to synaptophysin and chromogranin A, the well-differentiated NETs of the cecal region stain for serotonin. In minor tumor cell populations, positivity for motilin, neurotensin, substance P, enkephalins, and other hormones has been described [150]. In addition, prostatic acidic phosphatase was reported positive in the majority of the well-differentiated colorectal NETs, making this a potentially useful marker of lower hindgut origin [46]. Expression of the transcription factor CDX2, although specific for colorectal adenocarcinomas [123], proved to be restricted to the rare colorectal serotonin-positive NETs [81].

Histologically, poorly differentiated neuroendocrine carcinomas are characterized by solid structures, sometimes with "organoid" appearance, extensive "geographical chart" necrosis, small or large cell cytology and high mitotic counts, and Ki67 index [23, 38, 152]. Mucin stain (periodic acid Schiff [PAS] and Alcian/PAS) may be useful to identify occasional, although not uncommon, minor foci of adenocarcinoma or squamous cell carcinoma [20, 21]. Immunocytochemically, there is diffuse immunostaining for synaptophysin and CD56 as well as NSE, while chromogranin A may be absent or only present in individual cells. c-kit expression is found in approximately 20% of the cases and is not associated with an activating mutation in exon 11 of the c-kit gene [3].

Prognostically, well-differentiated NETs of the rectum but also of the colon are likely to have metastasized to the regional lymph nodes and elsewhere if they are larger than 2 cm and have invaded the muscularis propria. Rectal well differentiated NETs less than 1 cm in size have a very low risk of metastasis, while those between 1 and 2 cm may show metastasis to the regional lymph nodes in up to 5% of the cases. If the tumors are poorly differentiated, there is a high rate of metastasis at the time of diagnosis [20, 21]. A recent proposal for a TNM classification and grading system may be of value in the stratification and management of patients with colon and rectal NETs (Tables 12 and 13) [128]. NETs of the colon and rectum rarely cause hormonal symptoms. Some cases of classical carcinoid syndrome were reported in patients with metastatic lesions of cecal origin [144, 145, 150]. An association with inflammatory bowel diseases has been reported in both Crohn's and ulcerative colitis, suggesting that chronic inflammation is a potentially triggering condition for the development of NETs [63, 103, 150]. Synchronous or metachronous colorectal carcinomas are frequently seen [144, 145]. This is particularly the case in poorly differentiated neuroendo-

Table 12 Proposal for a TNM classification for neuroendocrine tumors of the colon and rectum

Abbreviation	Characteristics
T—primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor invades mucosa or submucosa
	T1a size <1 cm
	T1b size 1–2 cm
T2	Tumor invades muscularis propria or size >2 cm
T3	Tumor invades subserosa/pericolic/perirectal fatty tissue
T4	Tumor directly invades other organs/structures and/or perforates visceral peritoneum
	For any T add (m) for multiple tumors
N—regional lymph nodes	
NX	Regional lymph node status cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M—distant metastases (subspecification as in small bowel)	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1	Distant metastasis

Table 13 Proposal for disease staging for neuroendocrine tumors of the colon and rectum

Stage	T	N	M
Stage IA	T1a	N0	M0
Stage IB	T1b	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	any T	N1	M0
Stage IV	any T	any N	M1

crine carcinomas [21, 23, 47]. Therefore, a common histogenesis for neuroendocrine and nonneuroendocrine aggressive cancers of the colon has been discussed [164, 168].

Neuroendocrine tumors of the presacral region

NETs involving the presacral region between the rectum and the sacrum are rare but well documented [64, 161]. They affect adults of both sexes and are frequently associated with tail gut cysts. Histologically, most of them are well-differentiated NETs resembling rectal carcinoids. Metastases may occur.

Pancreas

Pancreatic NETs (PETs) usually present as solitary well-demarcated neoplasms but lack a well defined capsule (Fig. 8). Their size ranges between 1 and 5 cm. Multiple tumors are rare and should always raise the suspicion of MEN1 or VHL (for details on hereditary NETs, see Anlauf et al. this issue). The majority of the tumors are well differentiated, showing a trabecular or solid pattern [77]. Poorly differentiated carcinomas, which are rare, show a diffuse infiltrative growth pattern.

The biological behavior, i.e., the metastatic potential, may be predicted by a number of criteria and indicators that are summarized in Table 2. Recent studies provided evidence that this multiparameter approach that is applied in the 2004 WHO classification of PETs is a reliable tool for stratifying patients into risk groups and that, in addition, the immunohistochemical marker CK19 may refine the prognostic power of the WHO classification [29, 58, 139]. Furthermore, a proposal for a TNM classification and a grading system has recently been published (Tables 14 and 15) [127].

PETs include several entities that are divided on the basis of their symptomatology into functioning neoplasms (i.e., with hormonal syndromes such as insulinomas, gastrinomas, glucagonomas, or VIPomas) and nonfunctioning neoplasms (i.e., without hormonal syndromes). The majority of the functioning and nonfunctioning PETs fall into the category of well-differentiated carcinomas, with the exception of insulinomas.

Insulinoma These tumors are located in the pancreas or are directly attached to it and present as solitary red brown, mostly soft, well-demarcated tumors with a size between 0.5 and 2 cm. Histologically, they show either solid or trabecular, gland-like tumor growth [56, 79]. The tumor cells are often bland, and cells with large, pleomorphic nuclei are rare. If they occur, they are not predictive of malignancy. A special finding in insulinomas is the deposition of amyloid that can be immunostained for amylin [172]. Immunohistochemically, they stain for insulin and proinsulin. In addition there may be cells

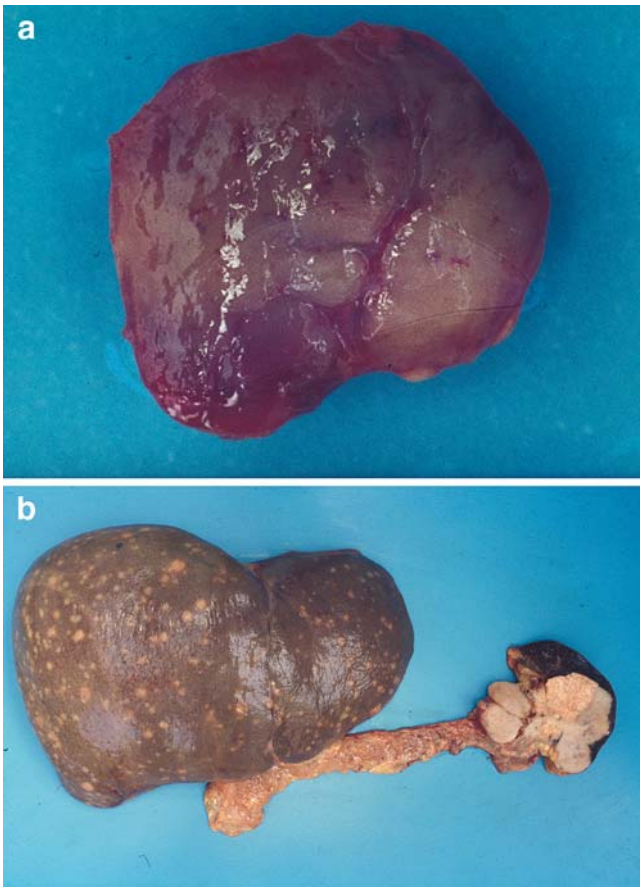

Fig. 8 Pancreatic neuroendocrine tumors: **a** tumor with a diameter less than 2 cm (insulinoma without metastases). **b** Large tumor (>2 cm) in the tail of the pancreas with multiple liver metastases (malignant insulinoma)

Table 14 Proposal for a pTNM classification and disease staging for endocrine tumors of the pancreas

Abbreviation	Characteristics
T—primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Limited to the pancreas and size <2 cm
T2	Limited to the pancreas and size 2–4 cm
T3	Limited to the pancreas and size >4 cm or invading duodenum or bile duct
T4	Invading the wall of adjacent large vessels (celiac axis or superior mesenteric artery), stomach, spleen, colon, adrenal gland
	For any T add (m) for multiple tumors
N—regional lymph nodes	
NX	Regional lymph node status not assessed
N0	Absence of lymph node metastasis
N1	Presence of regional lymph node metastasis
M—distant metastases	
MX	Distant metastasis not assessed
M0	Absence of distant metastases
M1	Distant metastasis

expressing glucagon, somatostatin, pancreatic polypeptide or other hormones [72, 79].

The vast majority of insulinomas are benign at the time of diagnosis [151]. This may be due in part to their early detection, as they already become symptomatic at a small size [78, 148] (Fig. 8a). Approximately 8.4% of insulinomas are malignant [49, 51, 52, 84, 140, 148, 157, 165] (Fig. 8b). Malignant insulinomas occur in an older age group and are rare in children [39, 159].

Tumors producing a hypoglycemic syndrome are usually larger than 1 cm; microadenomas (below 0.5 cm in diameter) are functionally silent. Approximately 4–7% of patients with insulinomas suffer from MEN1 [141]. Rare examples of insulinomas have also been described in patients suffering from NF1 [48]. Between 12 and 17% of VHL patients develop endocrine pancreatic tumors, which may show focal insulin immunoreactivity [90], but most of these tumors are clinically nonfunctioning.

Gastrinoma In recent years, it has become obvious that pancreatic gastrinomas are less common than duodenal gastrinomas (see "Duodenum and upper jejenum"). They are associated with the sporadic form of ZES and only very rarely with the MEN1-associated form of ZES [118, 119]. Their preferential localization seems to be in the pancreatic head, where they present as solitary tumors that usually have a diameter of 2 cm or more [43, 153]. The anatomical area comprising the head of the pancreas, the superior and ascending portion of the duodenum, and the relevant lymph nodes has been called the "gastrinoma triangle," as it harbors the vast majority of these tumors [65, 70, 105, 106, 153]. Histologically, they predominantly show a mixed trabecular and solid pattern with some pseudoglandular structures. Immunohistochemically, they are positive for gastrin but may also show some glucagon, somatostatin, or pancreatic polypeptide cells [15, 119]. The risk of liver metastases increases with tumor size, and they occur with a frequency of 30% [151]. Metastases to other organs are rare [155]. In general, the progression of gastrinomas is relatively slow with a combined 5-year survival rate of 65% amd a 10-year survival rate of 51% [70]. Even with metastatic disease, a 10-year survival of 46% (lymph node metastases) and 40% (liver metastases) has been reported [108]. Patients with complete tumor resection have 5- and 10-year survival rates of 90–100%, respectively.

Glucagonoma These are NETs that produce glucagon and are associated a syndrome consisting of skin rash (necrolytic migratory erythema), mild glucose intolerance, anemia, weight loss, depression, diarrhea, and a tendency to develop deep-vein thrombosis [58]. They are usually large, solitary tumors with a diameter between 3 and 7 cm commonly occurring in the tail of the pancreas [136, 151]. Extrapancreatic glucagonomas are extremely rare [131]. On their cut surface, they show a brown red to pink color and a soft consistency. Histologically, they display a mixed trabecular and solid pattern, and immunohistochemically, they stain (often weakly) for glucagon or proglucagon-derived peptides (glicentin, glucagon-like peptides 1 and 2) [18, 55, 136]. In addition, numerous pancreatic polypeptide cells can often be identified.

Approximately 60–70% of glucagonomas are already metastatic at the time of diagnosis [60, 121, 136]. Malignant glucagonomas tend to grow slowly, and patients may survive for many years.

Table 15 Proposal for a pTNM classification and disease staging for endocrine tumors of the pancreas

Stage	T	N	M
Stage I	T1	N0	M0
Stage IIa	T2	N0	M0
Stage IIb	T3	N0	M0
Stage IIIa	T4	N0	M0
Stage IIIb	any T	N1	M0
Stage IV	any T	any N	M1

VIPoma This PET is associated with the Verner–Morrison syndrome or WDHA syndrome (watery diarrhea, hypokalemia, hypochlorhydria, alkalosis), characterized by diarrhea (up to 20 l a day), hypokalemia because of potassium loss in the stool, achlorhydria, glucose intolerance, and anemia. These symptoms are caused by inappropriate secretion of VIP and peptide histidine methionine (PHM). The tumors are located in the pancreas, where they are preferentially seen in the tail. They are large and solitary [30]. Histologically, they show a solid or trabecular growth pattern. Immunohistochemically, they stain for VIP and PHM and often in addition for pancreatic polypeptide and other hormones [2, 12, 111].

Most VIPomas have led to metastases in the regional lymph nodes and the liver at the time of diagnosis [93]. The 5-year survival rate is about 59% for patients with metastases and 94% for those without metastases [58].

Somatostatin-producing PETs (so-called somatostatinomas) In 1979, the somatostatinoma syndrome was described in patients presenting with symptoms of diabetes mellitus, cholecystolithiasis, steatorrhea, indigestion, hypochlorhydria, and occasionally anemia in association with a somatostatin-producing and somatostatin-secreting PET [80, 82, 120, 142, 166]. The symptoms were attributed to the inhibitory effects of somatostatin on the function of various cell systems. However, since then, no further convincing reports on the somatostatinoma syndrome have appeared in the literature, although somatostatin-producing NETs (SOM-NETs) have been identified not only in the pancreas but also at other sites, particularly the duodenum [41, 160]. This casts doubt on the existence of a somatostatinoma syndrome and raises the question whether the described symptoms were unspecific manifestations of large malignant PETs [166] that happened to produce somatostatin. The last view is supported by our results in a series of 386 PETs, collected between 1972 and 2006, which contains ten well-differentiated somatostatin-producing PETs, none of which were associated with the so-called somatostatinoma syndrome [50].

SOM-NETs of the pancreas are rare and in approximately 50% of the cases malignant. While most tumors show a trabecular pattern, some display a paraganglioma-like architecture with occasional psammomatous calcifications. The somatostatin-producing PETs in our series were outnumbered by the SOM-NETs found in the duodenum (see "Duodenum and upper jejenum") [31, 156].

Other functioning PETs Apart from insulinomas, gastrinomas, glucagonomas, and VIPomas there may be adrenocorticotropic hormone-producing tumors causing Cushings's syndrome [37, 57, 96], tumors producing growth hormone-releasing hormone (GHRH) causing acromegaly [11, 19, 40, 138], calcitonin-producing tumors causing diarrhea [44, 71], and serotonin-producing tumors causing a carcinoid syndrome [113, 171]. Many of these neoplasms are solitary and large and have metastasized to the liver and lymph nodes when detected. Histologically, they do not differ from the more common functioning tumors. Exceptions are some GHRH-producing tumors, which show a distinct paraganglion-like microglandular pattern and spindle-shaped cells [138]. The hormone causing the syndrome can usually be detected by immunohistochemistry. These tumors tend to be large, and at diagnosis, many patients have already liver metastases. The prognosis is therefore usually poor [58].

Nonfunctioning PETs These PETs are either incidental findings or become clinically apparent because of size, invasion of adjacent organs, or the occurrence of metastases. In rare cases, they present as pancreatitis. They are observed more frequently than previously, although this probably does not reflect an actual increase in frequency but rather improved diagnostic methods [139]. Historically, most of these tumors were large when detected and frequently malignant [73]. More recently, however, smaller nonfunctioning tumors are increasingly detected by modern imaging techniques [139]. Large nonfunctioning PETs are reported to occur most frequently in the head of the pancreas, possibly because they are most likely to produce symptoms such as cholestasis in this location. In general, the presenting symptoms are most often unspecific and may consist of nausea, vomiting, or diarrhea.

Histologically, they may show various types of patterns: a trabecular, solid, pseudoglandular, or gyriform pattern. Immunohistochemically, these tumors stain for synaptophysin and chromogranin A and show a wide range of positivity for hormones including glucagon, somatostatin, and pancreatic polypeptide. Some of these tumors are associated with elevated hormone levels in the blood (e.g., glucagon, somatostatin, or pancreatic polypeptide), reflecting the hormonal immunoreactivity in the tumor. Because the patients lack any hormonal syndrome, they should not be called glucagonomas, somatostatinomas, or Ppomas. A special feature of glucagon-producing but hormonally silent NETs are grossly cystic changes [86, 174].

A minority of nonfunctioning NETs of the pancreas are histologically poorly differentiated, showing a diffuse infiltrative growth pattern and composed of small- to medium-sized cells, occasionally also large cells, that have a high mitotic rate and proliferative activity of more than 20%. Their neuroendocrine differentiation is demonstrated by diffuse staining for synaptophysin and, rarely, for

chromogranin A. Hormones are usually not detected.They may stain for p53.

Prognostically, well-differentiated nonfunctioning NETs of the pancreas have a 5-year survival rate of approximately 65% and a 10-year survival rate of 45%. Follow-up in patients with PETs having a diameter of less than 2 cm revealed that they are often cured by surgery [139].

Tumors smaller than 0.5 cm are designated microadenomas and are usually not visible macroscopically. Most of these tumors are detected incidentally or at autopsy and are present throughout the pancreas. Histologically, they show a trabecular pattern, and immunohistochemically, they stain for glucagon.

Conflicts of interest statement We declare that we have no conflict of interest.

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REVIEW AND PERSPECTIVE

Hereditary neuroendocrine tumors of the gastroenteropancreatic system

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Abstract Approximately 5–10% of neuroendocrine tumors (NETs) of the gastroenteropancreatic system (GEP) have a hereditary background. The known inherited syndromes include multiple endocrine neoplasia type 1, neurofibromatosis type 1, von Hippel–Lindau disease, and the tuberous sclerosis complex. This review discusses for each of these syndromes the: (1) involved genes and specific types of mutations, (2) disease prevalence and penetrance, (3) affected neuroendocrine tissues and related clinical syndromes, (4) special morphological features of NETs and their putative precursor lesions. In addition, GEP-NETs clustering in individual families or associated with other malignancies without known genetic background are discussed.

Keywords Neuroendocrine tumors · Pancreas · Gut · Hereditary syndromes · Multiple endocrine neoplasia type 1 · Neurofibromatosis · Tuberous sclerosis complex · von Hippel–Lindau disease · Gastrinoma · Insulinoma

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Introduction

Hereditary cancer susceptibility is more frequent and variable in tumors of endocrine organs than in any other category of human neoplasms. Often, a variety of endocrine and nonendocrine tissues are involved, resulting in complex clinical syndromes.

In the digestive tract, approximately 5–10% of neuroendocrine tumors (NETs) have a hereditary background. Inherited tumor syndromes include multiple endocrine neoplasia type 1 (MEN1), neurofibromatosis type 1 (NF1), von Hippel–Lindau disease (VHL), and the tuberous sclerosis complex (TSC). In this article, we focus for each of these syndromes on: (1) the involved genes and specific types of mutations, (2) the disease prevalence and penetrance, (3) the affected neuroendocrine tissues and the related clinical syndromes, and (4) the morphological features of the respective NETs and their putative precursor lesions. In addition, suspected hereditary backgrounds [e.g., association with other malignancies, familial clustering of gastroenteropancreatic system (GEP)-NETs] are discussed.

Multiple endocrine neoplasia type 1

MEN1 is an autosomal-dominant disorder characterized by multifocal endocrine tumors affecting the anterior pituitary, parathyroids, stomach, duodenum, pancreas, adrenal cortex, thymus, and lungs. In addition, various uncommon tumoral lesions may occur in the skin, central nervous system, and soft tissues [12, 57].

The *MEN1* gene is localized on chromosome 11q13 and consists of 10 exons spanning approximately 9 kb of genomic sequence and encoding a 68-kDa protein of 610 amino acids, named menin [12, 15, 57] (Table 1). Menin is

Table 1 Genetic and clinicopathological features of MEN1 and NF1

	MEN1	NF1
Function	Tumor suppressor gene	Tumor suppressor gene
Chromosomal location	11q13	17q11.2
Gene structure	10 exons (~9 kb)	>50 exons (~300 kb)
Protein	Menin (610 amino acids)	Neurofibromin (2,818 amino acids)
Mode of inheritance	Autosomal-dominant (10% de novo)	Autosomal-dominant
Prevalence	~1:20,000–1:40,000	~1:2,000–1:5,000
Penetrance	>95% (at age 50)	~100% (in childhood)
Diagnosis	According to WHO clinical criteria (genetic testing of family members recommended)	According to WHO clinical criteria (genetic testing not recommended)
Intestinal tract		
NETs	Multiple duodenal gastrinomas	Duodenal NETs (somatostatin)
Penetrance	20–60%	~1%
Functional activity	Zollinger–Ellison syndrome	No
Malignancy	Early metastases	~20% metastases
Pancreas		
NETs	Macrotumors and microadenomatosis	Somatostatin/insulin-producing NETs or functionally active insulinomas (case reports)
Penetrance	30–70% (>90% microadenomas)	
Functional activity	Nonfunctioning>insulinoma>PETs with ectopic hormone production	
Malignancy	<10% metastases	<20% metastases
Other GEP tumors	ECL cell tumors (associated with ZES)	GIST (often multiple)
	Esophageal leiomyomas (rare)	Neurofibroma
Tumors or endocrine hyperfunction outside the GEP	Primary hyperparathyroidism, anterior pituitary adenoma, adrenocortical tumor, thymic and bronchial NET, cutaneous lipoma and angiofibroma	*Cafe au lait* macules, neurofibroma, plexiform neurofibroma, MPNST, pheochromocytoma, optic/brain stem gliomas, bone lesions, renal artery stenosis, congenital glaucoma

NETs Neuroendocrine tumors, *GEP* gastroenteropancreatic system, *MEN1* multiple endocrine neoplasia type 1, *WdNEC* well-differentiated neuroendocrine carcinoma (defined by presence of lymph node metastases or infiltrative growth in the outer smooth muscle layers), *ECL* enterochromaffin-like, *PETs* pancreatic endocrine tumors, *ZES* Zollinger–Ellison syndrome, *NF1* neurofibromatosis type 1, *GIST* gastrointestinal stroma tumor, *MPNST* malignant peripheral nerve sheath tumor

a cell cycle-regulated nuclear protein. Menin is assumed to play an important role in pathways controlling cell growth and differentiation during embryogenesis and postnatal life. To date, menin has been shown to interact with numerous proteins involved in regulation of transcription, DNA replication, mitosis, apoptosis, genome integrity, growth factor signaling pathways, and extracellular matrix organization [12, 57]. However, it remains still unresolved why *MEN1* mutations affect primarily endocrine tissues.

The prevalence of the MEN1 syndrome has been estimated to be between 1:20,000 and 1:40,000. In approximately 10% of patients, MEN1 germline mutations arise de novo without any family history [8, 12, 15] (Table 1). The *MEN1* germline mutations are found spread over the entire exonic and intronic sequences and are not clustered in hotspots. Approximately 60% are truncating mutations, either frameshift (~40%) or nonsense (~20%) mutations; 20% are missense mutations; 10% are in-frame deletions or insertions, and about 10% are intronic and splice-site mutations. Large germline deletions encompassing the whole *MEN1* locus have also been detected [12, 14, 15]. A stringent genotype/phenotype relation correlation could not be demonstrated. Among patients meeting the clinical criteria of a MEN1 syndrome, approximately 10% have no identifiable mutations [43]. Most MEN1-associated tumors show somatic loss of the wild-type allele (loss of heterozygosity; LOH) on chromosome 11q13, consistent with the role of *MEN1* as a tumor suppressor gene [6, 26, 54, 66].

Twenty to 60% of MEN1 patients suffer from a Zollinger–Ellison syndrome (ZES), characterized by elevated fasting gastrin serum levels, a positive secretin stimulation test, and clinical symptoms such as recurrent peptic ulcer disease, gastroesophageal reflux disease, and occasionally, diarrhea [44, 87]. The gastrinomas observed in MEN1 are almost exclusively localized in the duodenum (Table 1). They are multiple and are associated with multifocal gastrin cell hyperplasia and tiny gastrin-producing microtumors. Despite their small size of 0.3 to 5 mm, the gastrinomas tend to metastasize to regional lymph nodes [3, 4, 67]. In addition, the duodenum harbors multiple tiny somatostatin cell neoplasms and multifocal somatostatin cell hyperplasia [6] (Fig. 1). Recently, molecular studies have

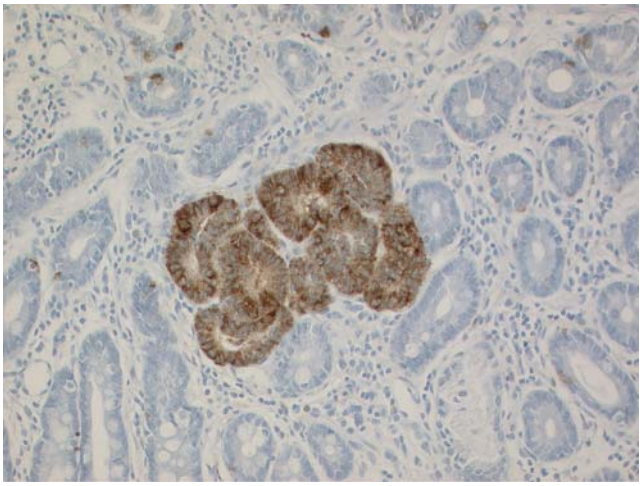

Fig. 1 Linear hyperplasia of somatostatin cells in the duodenal mucosa of a MEN1 patient with ZES

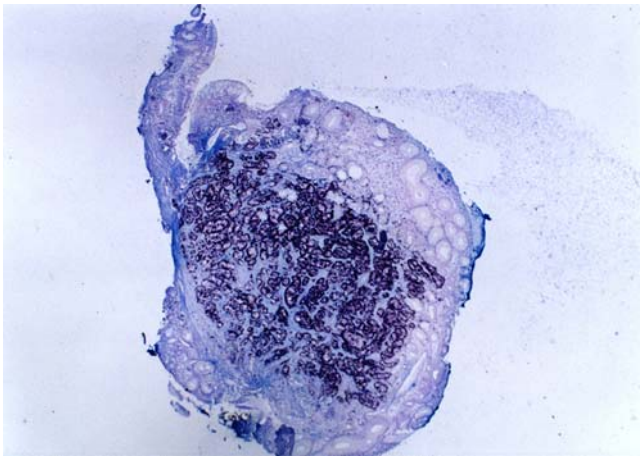

Fig. 3 Polypectomy specimen showing a small benign ECL cell tumor in a MEN1 patient with ZES (immunostaining for the vesicular monoamine transporter 2)

allowed the separation from hyperplastic and neoplastic lesions. LOH of the *MEN1* locus was found in approximately 50% of MEN1-associated duodenal NETs [6]. Allelic loss was detected in tumors as small as 300 μm (gastrin) and 400 μm (somatostatin) in diameter, which therefore represent true neoplasms. In contrast, hyperplastic gastrin and somatostatin cells lacked LOH on chromosome 11q13. These findings suggested that although the hyperplastic cells were hyperproliferative and carried the *MEN1* germline mutation, they had not yet assumed the neoplastic genotype characterized by the loss of the *MEN1* wild-type allele.

In addition to duodenal NETs, MEN1 patients with ZES may show multiple gastric NETs of the enterochromaffin-like (ECL) cells (Fig. 2). These tumors occur in a hypertrophic oxyntic mucosa and are associated with an ECL cell hyperplasia, which can be visualized using antisera against the ECL cell-specific marker vesicular monoamine transporter 2 [25] (Fig. 3). They are probably induced by both the MEN1 germline mutation and the trophic effect of hypergastrinemia. Metastases of these ECL cell tumors are rare, and tumor-related deaths are the exception [9, 10, 12].

Pancreatic NETs lead to symptoms in 30–75% of MEN1 patients [12] and up to 10% of all pancreatic NETs may be associated with a MEN1 syndrome [57]. In a recent study on surgical specimens of the pancreas from MEN1 patients in more than 90% of the cases, numerous microadenomas (i.e., up to 5 mm in diameter) were found [5], a condition that has been called pancreatic microadenomatosis. Although this finding is a hallmark for MEN1, it is not MEN1 specific, as recently, pancreatic microadenomatosis characterized by multiple glucagon-producing or multiple insulin-producing microadenomas was described in several patients who had no evidence of MEN1 or any other known hereditary syndrome [5].

In MEN1 patients, the pancreatic microadenomas are typically multihormonal and are often associated with one or more macrotumors (diameter >5 mm; Fig. 4) [12, 42,

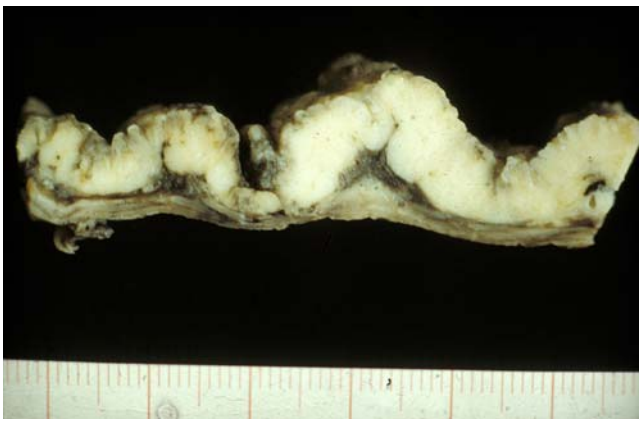

Fig. 2 Cut surface of a gastric specimen from a MEN1 patient with ZES, showing multiple small tumors in the mucosa and submucosa

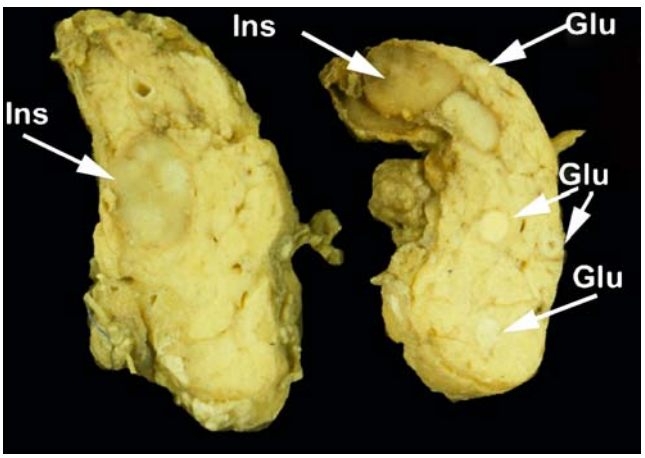

Fig. 4 Pancreatic specimen from a MEN1 patient showing two insulin-positive (*Ins*) macrotumors and four glucagon-positive (*Glu*) microadenomas

77]. The microadenomas in MEN1 are characterized, irrespective of their size (i.e., less than 200 μm), by the following features: (1) frequent expression of glucagon and/or pancreatic polypeptide, (2) trabecular growth pattern, and (3) a distinct stromal component [5, 42, 77]. Their neoplastic nature was demonstrated by LOH of the MEN1 gene [66, 82]. The MEN1-associated macrotumors (≥5 mm) also frequently express glucagon and pancreatic polypeptide or, rarely, somatostatin. In all these cases, the patients do not have a hormonal syndrome. However, if one of the macrotumors produces insulin, the patient presents with a hyperinsulinemic hypoglycemia syndrome. This is seen in approximately 10–25% of the cases. Only exceptionally, there are macrotumors that express hormones such as gastrin, vasoactive intestinal polypeptide, or growth hormone releasing hormone, which then may give rise to the respective hormonal syndromes. Regarding MEN1 patients with ZES, this implies that, with all likelihood, the source of hypergastrinemia is not a pancreatic gastrinoma but multiple duodenal gastrinomas.

Recently, forerunners of microadenomas were identified in the MEN1 pancreas, which have been called monohormononal islet-like endocrine cell clusters [66]. In addition, single irregularly shaped and enlarged islets with an increased number of glucagon cells were found. However, in contrast to the monohormononal islet-like endocrine cell clusters, the cells of the glucagon-cell-rich islets show retention of heterozygosity of the *MEN1* gene and are therefore still nonneoplastic in nature.

In summary, the MEN1 syndrome is characterized by multiple endocrine duodenal and pancreatic NETs expressing several peptide hormones, but preferentially either gastrin or somatostatin (duodenum) or glucagon or pancreatic polypeptide (pancreas). The duodenal NETs are associated with multifocal gastrin and somatostatin cell hyperplasia that can be considered precursor lesions. In the pancreas, the islets with hyperplastic glucagon cells are probably the precursors from which microadenomas evolve. Similar precursor changes have so far not been observed in the usually solitary nonhereditary NETs [6].

Neurofibromatosis type 1

NF1 (i.e., von Recklinghausen disease) shows an autosomal-dominant inheritance and a high penetrance: In almost all patients, there is sufficient evidence of the disorder to allow diagnosis in childhood [27]. The condition is characterized by neurofibromas, *Café au lait* patches of the skin, and bone dysplasia. Neurofibromas occur widely throughout the body, but affect mainly the skin. Other tumors are optic nerve and brain stem gliomas, pheochromocytomas, and malignant nerve sheath tumors [27] (Table 1). Gastroenteropancreatic NETs are rare and occur in 1% of the NF1 patients. They typically arise in the duodenum [23].

The *NF1* gene is localized on chromosome 17q11.2 and consists of more than 50 exons spanning approximately 300 kb of genomic sequence and encoding a 327-kDa protein of 2,818 amino acids, called neurofibromin [81]. Neurofibromin acts as a negative regulator of the ras-related G-proteins by increasing Ras GTPase activity, and thereby, functions at multiple levels: transcription, cell proliferation, cytoskeletal microtubule assembly. Mice that are homozygous for an NF1 mutation fail to develop the normal structure of heart and various neuroectodermal tissues and die in utero [27, 86]. Neurofibromin has been referred to as a tumor suppressor, as malignant peripheral nerve sheath tumors in NF1 patients display LOH of the NF1 gene [27].

Mutation screening is costly and not recommended clinically because of the size of the gene (50 exons) and the fact that the clinical manifestations are so characteristic. Mutations have been identified throughout the *NF1* gene; most mutations are protein-truncating mutations, consisting of nonsense, frameshift, and splice-site mutations. Five to 10% of patients have large deletions, often involving the whole gene, that are easily detectable by fluorescence in situ hybridization [36]. Large deletions of the NF1 gene have been correlated with a greater neurofibroma burden, as well as dysmorphic features, greater mental retardation, and a higher risk of developing malignant peripheral nerve sheath tumors [27]. Except for large deletions, genotype–phenotype correlations have not been described.

Our knowledge about the incidence, histopathology, biology, and functional activity of NF1-associated intestinal NETs is based on reports of single cases or small series of patients and reviews [22, 23, 75, 76]. Most of the GEP-NETs arise in the ampullary region of the duodenum and show glandular structures containing PAS-positive psammoma bodies. Immunohistochemically, they consistently express somatostatin and synaptophysin, but rarely chromogranin A. An association with the so-called somatostatinoma syndrome [i.e., markedly elevated somatostatin levels in the plasma and/or tumor, diabetes mellitus of recent onset, hypochlorhydria, gallbladder disease (cholelithiasis), diarrhea and steatorrhea, anemia, and weight loss] [24] has yet not been described. The clinical symptoms are caused by the site of the tumors, i.e., the ampulla, and lead to obstructive jaundice, intestinal obstruction, and/or bleeding. In our series of 82 duodenal NETs, we identified 15 somatostatin-producing NETs (SOM-NETs), 3 of them associated with the NF1 syndrome (Fig. 5). None of these tumors was associated with a somatostatinoma syndrome. Therefore, the high rate of NF1-associated SOM-NETs reviewed by Soga and Yakuwa [74] could not be confirmed in our series. Precursor lesions of NF1-associated SOM-NETs have not been identified. NF1-associated SOM-NETs

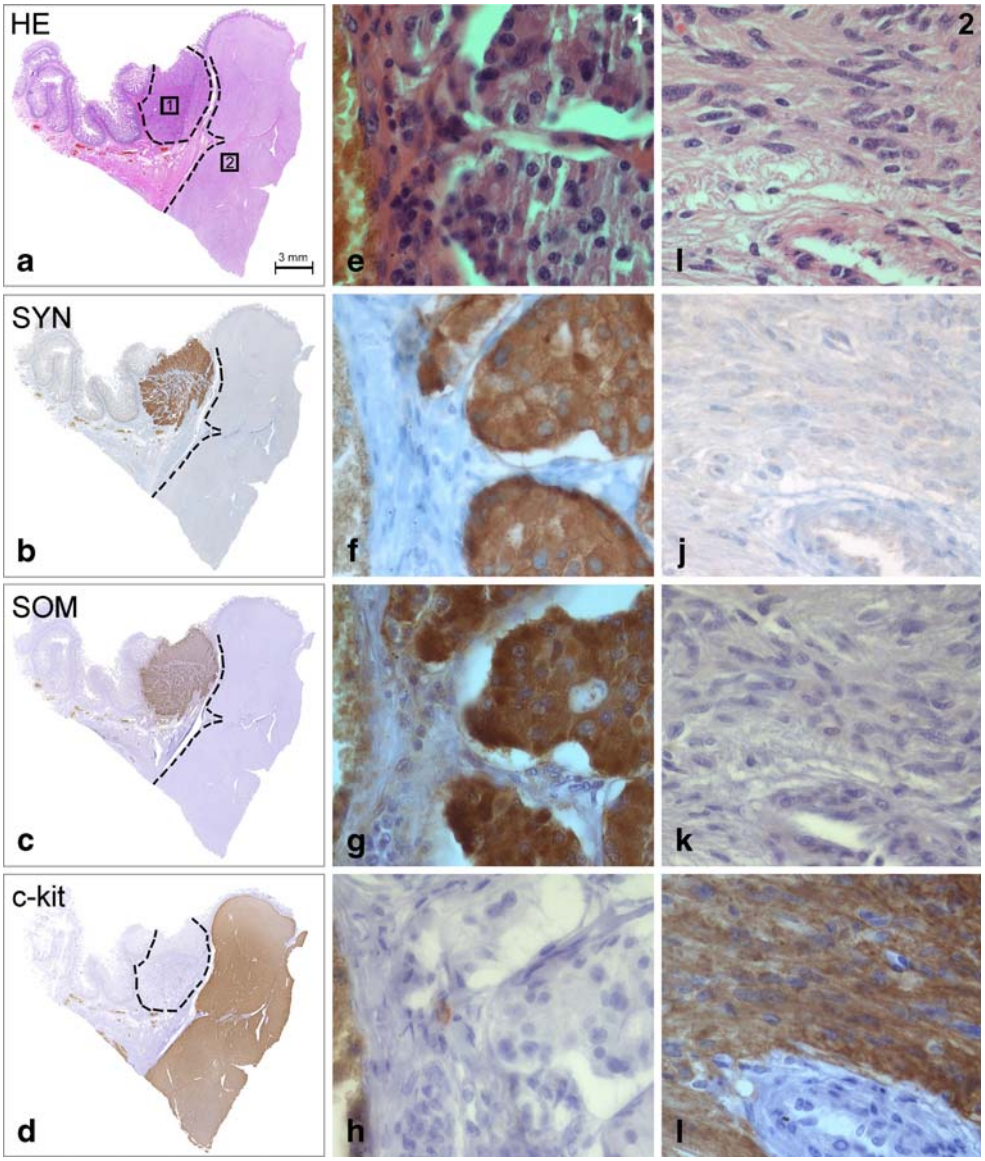

Fig. 5 Simultaneous occurrences of SOM-NET and GIST in a patient with the NF1 syndrome. Whole section scan (**a**–**d**) and high magnification of the SOM-NET (**e**–**h**) showing strong immunoreactivity for synaptophysin (*SYN*, **f**) and somatostatin (*SOM*, **g**) but not for c-kit (**h**). High magnification of the GIST (**i**–**l**) showing absence of synaptophysin (**j**) and somatostatin (**k**) but strong expression of the c-kit antigen (**l**)

may be associated with gastrointestinal stroma tumors (Fig. 5). In addition, gangliocytic paragangliomas and ampullary adenocarcinomas have been reported; however, it remains unclear whether these tumors are coincidental or due to the NF1 germline mutations.

Metastases have been described in 27% of SOM-NETs, mainly to the lymph nodes. They appear to be less aggressive than their sporadically occurring duodenal and pancreatic counterparts [35]. A tumor size of more than 2 cm and infiltration of the outer smooth muscle layers increase the risk of metastases ([76] and personal observations). In addition, a few cases of pancreatic NETs have been described in patients with the NF1 syndrome. These were somatostatin-producing NETs or insulinomas [29, 73, 74]. In our series of 541 non-MEN1-associated pancreatic NETs, we identified 19 somatostatin-producing NETs, none of them associated with NF1, and 162 insulinomas, one of them in a patient with NF1. In this patient, the tumor was proven to lack expression of the wild-type NF1 allele, providing strong evidence that there is indeed a close relationship between NF1 and the development of this insulinoma [65].

von Hippel–Lindau syndrome

VHL is a dominantly inherited familial cancer syndrome caused by germline mutations in the *VHL* tumor suppressor gene. VHL disease shows marked phenotypic variability and age-dependent penetrance [55, 84] (Table 2).

The VHL gene is localized on chromosome 3p25 and consists of three exons encoding two VHL transcripts. The major transcript (isoform I) represents all three exons, whereas exon 2 is absent from isoform II. They encode two

Table 2 Genetic and clinicopathological features of VHL and TSC

	VHL	TSC
Function	Tumor suppressor gene	Tumor suppressor gene
Chromosomal location	3p25	TSC1 9q34 TSC2 16p13.3
Gene structure	3 exons	TSC1 23 exons TSC2 42 exons
Protein	pVHL30 (~28–30 kDa) pVHL19 (~18–19 kDa)	TSC1 Hamartin (~140 kDa) TSC2 Tuberin (~200 kDa)
Mode of inheritance	Autosomal-dominant (~20% de novo)	Autosomal-dominant (~two thirds de novo)
Prevalence	~1:36,000	~1:10,000
Penetrance	~50% (at age 50) >95% (at age 60)	~100%
Diagnosis	According to WHO clinical criteria (genetic testing recommended)	According to clinical and radiological criteria
Intestinal tract		
NETs	Not described	Not described
Penetrance		
Functional activity		
Malignancy		
Pancreas		
NETs	Clear cell type	Insulin->somatostatin-producing
Penetrance	5–17%	<1%
Functional activity	Nonfunctioning	Insulinoma
Malignancy	10–20% metastases	n.d.
Other GEP tumors	Pancreatic microcystic adenoma or benign serous cysts	Hamartomatous rectal poyps
Tumors/lesions or endocrine hyperfunction outside the GEP	Pheochromocytoma, parasympathetic paraganglioma, renal cell carcinoma (clear cell), CNS hemangioblastoma, retinal angioma, papillary cystadenoma epididymis, papillary cystadenoma mesosalpinx, endolymphatic sac tumor, capillary hemangioblastoma and cysts at various locations, hyperparathyroidism	Hamartomatous tubers in cerebral cortex and subependymal nodules, giant cell astrocytomas, ocular retinal astrocytic hamartoma, cardiac rhabdomyoma, angiomyolipomas (kidney/liver), hypopigmented skin macules, shagreen patches, ungual and gingival fibromas, multiple renal cysts, pulmonary lymphangioleiomyomatosis, bone cysts

NETs Neuroendocrine tumors, *GEP* gastroenteropancreatic system, *VHL* von Hippel–Lindau syndrome, *CNS* central nervous system, *TBC* tuberous sclerosis complex, *n.d.* not enough data

gene products: a full-length 213-amino-acid protein (pVHL30; ~28–30 kDa) and a shorter protein (pVHL19; ~18–19 kDa). The *VHL* gene product has multiple functions, the best characterized of which is the role of pVHL in regulating proteolytic degradation of the subunits of the HIF transcription factors. In addition, there is strong evidence that pVHL targets other proteins for polyubiquination [18, 48, 53, 55]. VHL-associated tumors such as renal clear cell carcinoma, hemangioblastoma, and pheochromocytoma are highly vascular and overexpress a wide range of hypoxia-inducible mRNAs, including vascular endothelial growth factor (VEGF) and VEGF receptor. A variety of VHL mutations have been described. Large genomic deletions account for up to 40% of all mutations, and the rest are divided approximately equally between intragenic missense mutations and protein-truncating mutations (nonsense, frameshift insertions and deletions, splice-site mutations). Germline VHL mutations have been characterized in >500 patients and have provided a wealth of data for genotype–phenotype correlations. VHL mutations may cause (1) VHL diseases, (2) isolated familial pheochromocytoma (VHL disease type 2C), and (3) autosomal recessively inherited polycythemia due to homozygous missense mutations [17, 19, 55, 69, 84].

Although pancreatic involvement in VHL is very common (50–77%), the majority of lesions are cysts. These are rarely of clinical significance, and impairment of pancreatic function is uncommon. Hemangioblastoma occurs rarely in the pancreas of VHL patients [34, 55, 62].

The prevalence of pancreatic NETs in VHL patients has been reported at frequencies of 5–17%. VHL-associated NETs are usually confined to the pancreas and show a solid, trabecular, and/or glandular architecture. In approximately 30–50% of VHL patients, the tumors are multiple. Most of them reveal clear-cell cytology. Immunohistochemically, they are positive for general neuroendocrine

markers (chromogranin A and synaptophysin). A minor subset of tumor cells may be immunoreactive for pancreatic polypeptide, glucagon, somatostatin, or insulin. Clinically, however, almost all of the tumors are functionally inactive and are usually detected by routine radiological assessment of the abdomen in VHL patients [1, 51, 52, 54, 55]. Almost all VHL-related pancreatic NETs reveal LOH of the VHL gene locus on chromosome 3p25, supporting the concept that the VHL gene product acts as a tumor suppressor [54]. Although VHL-related tumors are often multifocal, precursor lesions were not identified in a systematic analysis of 14 patients [54]. VHL-related pancreatic NETs grow slowly. As with other NETs, the risk of malignancy seems to be directly proportional to the diameter of the tumor. In the largest reported series of 30 patients, the median size of the tumor in patients with no metastases was 2 cm (n=25) compared to 5 cm for those with metastases (n=5) [51].

Tuberous sclerosis complex

Tuberous sclerosis is an autosomal-dominant genetic disorder with a prevalence of 1:10,000 and a disease penetrance of approximately 100%. TSC is a multisystem disorder exhibiting a wide range of manifestations characterized by hamartomatous lesions in the brain, skin, eyes, heart, lungs, and kidneys (Table 2). Epilepsy, mental retardation, and autism are often present. A few cases of pancreatic NETs have been described in patients with TSC. TSC is caused by inactivating mutations in either the *TSC1* gene at 9q34 or the *TSC2* gene at 16p13.3 encoding the proteins hamartin (~140 kDa) and tuberin (~200 kDa), which form a complex that affects cell growth, differentiation, and proliferation [70, 71, 78, 85] (Table 2). The diagnosis of TSC is usually based on the clinical and radiological findings. Two thirds of the cases result from new dominant mutations. Clinical manifestations may vary, but there is no well-documented report on nonpenetrance.

The TSC1/TSC2 dimer mediates a key step in the phosphoinositide 3-kinase signaling pathway. Thereby, the TSC1/TSC2 complex is involved in the regulation of the activity of mTOR, a master controller of protein translation, integrating information on growth stimuli, cellular energy levels, nutrient availability, hypoxia, and cell growth [47, 49, 68, 79].

Mutations of the TSC1 and TSC2 genes were detected throughout the genes, with some clustering but no striking hotspots, and include inactivating nonsense, frameshift, and splice-site mutations. For TSC2, additionally in-frame deletions and large deletions involving the adjacent PKD1 gene have been reported. Somatic mosaicism occurs in a minority of patients with TSC and seems to be associated with a milder phenotype. Several studies showed that sporadic TSC2 mutations tend to be associated with a more severe phenotype than sporadic mutations of the TSC1 gene [20, 39, 46, 50]. Among patients meeting the clinical criteria of TSC, 15–20% have no identifiable mutations. These persons generally have milder clinical disease [20, 72]. Somatic mutations often involving large deletions spanning the gene (LOH) account for the majority of tumors occurring in TSC, following Knudsen's two-hit paradigm for tumor suppressor genes [30, 31, 85].

Pancreatic NETs have rarely been described in adults with TSC [21, 28, 33, 38, 41, 80]. In a case report analyzing a 6-year-old child with a de novo mutation of the TSC2 gene, who suffered from an endocrinologically silent malignant pancreatic NET, 16p13 LOH and absence of tuberin protein expression from the tumor were demonstrated [28]. These findings provide evidence for a role of tuberin in the pathogenesis of pancreatic NETs. Based on the small series of reported cases, pancreatic TSC-associated pancreatic NETs are insulinomas or endocrinologically silent. Some of them are malignant. Interestingly, in a postmortem series of nine TSC patients, one 21-year-old woman was found to have an incidental nonfunctioning pancreatic NET in association with multiple endocrine adenomas of the pituitary gland, adrenal, and parathyroid glands [38].

GEP-NETs with suspected hereditary background

Data regarding an association of GEP-NETs with other malignancies are limited. In the older literature, several studies indicated that patients with intestinal NETs are at increased risk for developing another primary malignancy [11, 45, 61]. Chen et al. [16] postulated that intestinal adenocarcinomas and intestinal NETs might have common endogenous or environmental risk factors. Kothari and Mangla [45] found that 36% of patients with ileal NETs (carcinoids) had an associated malignancy. However, these results could not be confirmed by two large population studies that included 1,029 and 245 patients from Denmark and the USA, respectively, and failed to confirm a general excess cancer risk in patients with GEP-NETs [7, 83].

Case reports described ileal and rectal NETs in first-degree relatives without any evidence of a known inherited disease [2, 56, 60, 64]. These observations were also confirmed in a large study including 245 patients with intestinal NETs in which 3.7% of patients had at least one first-degree relative with the same malignancy [7]. This rate was found to be much higher than age-adjusted incidence rates for intestinal endocrine tumors (p<0.00001 small bowel; p=0.008 large intestine). The findings of this study were confirmed by a nationwide epidemiological study from Sweden including 1,933 offspring and 4,713 parents with endocrine tumors at various sites [37]. Similar findings

were reported in four families suffering from lung NETs [63]. None of these studies identified environmental risk factors, which suggests a genetic background for the familial clustering of intestinal NETs that remains to be analyzed in detail.

Further clustering of intestinal NETs was very well established in patients with inflammatory bowel disease [13, 32, 58]. In addition, some case reports described GEP-NETs in patients suffering from familial adenomatous polyposis [40, 59]. However, for both conditions, it remains to be clarified whether and how a hereditary predisposition exists.

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Conflict of interest statement We declare that we have no conflict of interest.

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REVIEW AND PERSPECTIVE

Molecular profiles of gastroenteropancreatic endocrine tumors

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Abstract Neuroendocrine tumors of the gastroenteropancreatic system are defined by their endocrine phenotype and share many histopathological and clinical features. However, the fact that the hormone production of tumors depends on their site of origin, that the tumors differ in their biology, and that the association with familial syndromes is nonrandom suggests heterogeneity. It is therefore conceivable that the gastroenteropancreatic neuroendocrine tumors also differ in their molecular profile. This review summarizes and discusses the available data in this field.

Keywords Gastrointestinal · Pancreatic · Endocrine tumor · Genetic · Expression array · Comparative genomic hybridization · LOH · Mutation analysis

Introduction

Well-differentiated gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are defined by their capacity to express neuroendocrine markers and to produce and/or secrete peptide hormones, biogenic amines, and tachykinins.

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Pancreatic endocrine tumors (PETs) may arise in association with a multiple endocrine neoplasia type 1 (MEN1), von Hippel–Lindau (VHL), or rarely neurofibromatosis type 1 (NF1) or tuberous sclerosis (TSC) syndrome, and the genes responsible for these familial diseases have been described. However, about 90% of PETs and the vast majority of gastrointestinal endocrine tumors are sporadic, and still little is known about the molecular basis of GEP-NETs.

Activation of oncogenes is rare in sporadic PETs [21, 23, 29]. Activating mutations of β-catenin were described in 37% of gastrointestinal NETs [14] but were not detected in exon 3 in a series of 15 PETs (own unpublished data). Mutations of *TP53*, *K-ras*, *p16*, *DPC4*, or *DCC*, which are found frequently in gastrointestinal adenocarcinomas, were not identified in GEP-NETs [22, 28–30, 33, 41]. CGH and loss of heterozygosity (LOH) data point toward the involvement of other chromosomal regions. In this article, we review the genetic alterations that have been identified in GEP-NETs, using ribonucleic acid expression arrays, comparative genomic hybridization (CGH), LOH, and gene mutation analysis.

Pancreatic endocrine tumors

Genome-wide analysis: RNA expression arrays

With the development of high-throughput techniques, analysis of the expression of thousands of genes in a given tumor has become possible. The use of RNA expression arrays can further our understanding of tumor classification. It can also provide signatures (gene lists classifying tumors into distinct groups) that may have prognostic implications. While this technique has led to new (sub)classifications in other tumors (lymphoma, breast cancer), only limited

results have been published for GEP-NETs. For these tumors, class comparison studies rather than class prediction or class discovery studies have been performed. Class comparison studies analyze the gene expression in groups of specimens defined by other methods such as histopathological, immunohistochemical, or clinical features. This analysis identifies genes that are differentially expressed between the different tumor types.

As biological behavior is difficult to predict in PETs, the largest published RNA expression array study on PETs compared metastasized with nonmetastasized PETs [10]. By comparing 12 localized to 12 metastasized PETs, the signature of 51 genes that are downregulated in malignant PETs and 72 that are upregulated in malignant PETs emerged. These genes were involved in pathways related to (1) angiogenesis and remodeling, (2) signal transduction through tyrosine kinases, (3) calcium-dependant cell signaling, and (4) response to therapeutic drugs.

A different approach was taken in the study by Maitra et al. [31]. In this study, transcripts from eight well-differentiated PETs were compared with transcripts of three enriched pancreatic islet cell samples with 80–90% purity. Their aim was to identify potential therapeutic targets and novel tumor markers in PETs. None of the patients had an associated endocrine hypersecretion syndrome; therefore, all eight tumors were nonfunctioning tumors. One of the tumors revealed somatostatin expression. Two tumors had lymph node metastases and measured 2 cm or less in diameter. The authors described 66 transcripts with threefold overexpression in PETs and 119 underexpressed transcripts. The underexpressed genes included cell cycle checkpoint proteins (p21, MICT) and genes involving deoxyribonucleic acid (DNA) damage repair and genomic stability (*O*-methylguanine DNA-methyltransferase and GADD 45). The number of differentially expressed genes was rather low in this study. One reason might be the applied high threshold of "threefold change" and the other, the selection of tumor types. The group of nonfunctioning tumors might be a potpourri of PETs expressing hormones at low levels. Furthermore, the study included many large tumors that may have accumulated extensive genetic changes, at least as expected from CGH studies. While the aim of the study to detect therapeutic markers is not yet accomplished, these gene lists will provide useful candidate genes for further detailed analyses.

In a second study, this group extended their analysis to the differential expression of genes in well-differentiated metastatic and nonmetastatic PETs [18]. The expression profiles of seven metastatic PETs were compared with the profiles of five nonmetastatic PETs. Sixty-five transcripts were overexpressed, and 57 transcripts were underexpressed in metastatic PETs compared to nonmetastatic ones. In this attempt to find a progression model from nonmetastatic to metastatic PETs, the gain of function of genes involved in growth regulation, cholesterol homeostasis, osmotic regulation, and hypoxia-inducible factors were described. Underexpression of genes involved in cell cycle regulation, developmental regulation, and DNA damage repair was described. Theses two studies showed a continuum of upregulation of IGFBP3 from normal islets to nonmetastasizing PETs and to metastasizing PETs. Malignant tumors showed an overall pathway activation of the insulin-like growth factor-signaling cascade, but the underlying mechanisms still have to be elucidated.

The rate of differentially expressed genes described was dramatically higher in the study by Carpuso et al. [7]. Comparing RNA extracts of 13 nonfunctioning PET (NF-PET) samples (eight primaries and five metastases) to extracts of four isolated islet preparations, they found 990 differentially expressed individual genes. Only 20 of the reported 667 upregulated genes were also described in the aforementioned similar studies (fibronectin [31], elongation of very long fatty acid-like 2 [6], coagulation factor V [18], and mitogen activated protein kinase 3 [17]). The authors described a striking similarity between primary malignant PETs and their metastases concerning their genetic profile: Metastases clustered with their corresponding primary tumors in most instances. This is evidence that the metastatic potential is acquired in an early stage of tumor transformation because cells of the primary tumor already carry the metastatic signatures.

Shalev et al. [42] compared isolated islets with and without glucose challenge. Fourteen genes were upregulated by glucose in all three independent experiments, and only six genes were downregulated. The authors were able to show that upregulation of transforming growth factor β (TGFβ) signaling was induced by glucose. As TGFβ is also known to be involved in differentiation, this raises the intriguing possibility that there might be crosstalk between glucose metabolism, development, and the immune system in human pancreatic islets.

The research on PETs using expression array techniques has only recently started, and it is anticipated that significant results will be published in the years to come. Future studies comprising larger numbers of well-characterized tumors are needed, and class prediction studies rather than simple class confirmation studies will allow the identification of subsets of endocrine tumors that are caused by different genetic changes and that might therefore respond to targeted therapies.

Genome-wide analysis: CGH studies

Four conventional CGH studies included a total of 102 PETs. These studies indicate that chromosomal losses occur slightly more frequently than gains, whereas amplifications

are uncommon [45–47, 58]. Genetic alterations seem to accumulate during tumor progression: The total number of genomic changes per tumor appears to be associated with both the tumor volume and the stage of the disease [46] Thus, large and/or malignant tumors and especially metastases harbor a larger number of genetic alterations than small and clinically benign neoplasms [46, 58] These findings point toward a tumor suppressor pathway and chromosomal instability as important mechanisms associated with malignancy in PET. These data have recently been confirmed for insulinomas in a large array CGH analysis studying 30 insulinomas. At least 20 chromosomal alterations and six telomeric losses were the best predictors of malignancy [26]. The alterations described are not randomly distributed on chromosomes but are particularly common in distinct chromosomal regions. Gains are common on 4pq (17% of the tumors), 5q (25%), 7pq (41%), 9q (28%), 12q (23%), 14q (32%), 17pq (31%), and 20q (27%), whereas genomic losses frequently occur on 1p (21%), 3p (19%), 6q (28%), 10pq (14%), 11q (30%), Y (31%), and X (31%) [45–47, 58]. Furthermore, genomic alterations in some chromosomal regions appear to be associated with the stage of the disease and, additionally, with particular tumor types. For example, losses of chromosome 1 and 11q as well as gains of 9q appear to be early events in the development of PETs because they are already present in many small tumors (<2 cm) [46]. Some defects, e.g., losses of 3p, 6pq, and 10pq and gains of 5q, 12q, 18q, and 20q, were shown to be associated with malignant behavior [2, 3, 46], whereas gains of chromosomes 4 and 7 in the presence of losses of 21q prevailed in metastases [58].

Insulinomas exhibit a lower number of genomic alterations than other PETs [45–47, 58]. Insulinomas often have chromosomal aberrations consisting of a gain of 9q32 and loss of 22q13.1, which may occur in the same tumor and appear to be early genetic events in these tumors [25]. Losses of 3p and 6q associated with malignancy are particularly rare in insulinomas [2, 3]. Malignant insulinomas, in contrast, harbor a large number of chromosomal alterations similar to that seen in other types of malignant PET [26].

In pancreatic gastrinomas, only limited chromosomal imbalances are encountered: Losses at 3p and 18q21 occur in approximately 33 and 22% of cases, respectively [46, 47, 58]. It is interesting to know that 18q losses are also common in gastrointestinal NETs, indicating that NETs and pancreatic gastrinomas might be related. NF-PETs in general harbor higher numbers of chromosomal gains and losses than functioning tumors. These genetic aberrations occur in chromosomal loci frequently involved in malignant tumors [58]. Gains of chromosome 4 and losses of 6q appear to be early events because they are already detectable in 40 and 50% of tumors with a diameter of less than 2 cm [46].

Loss of heterozygosity and mutation analysis

Analyses of LOH using polymerase chain reaction microsatellite markers identify the same chromosomal regions as those described by CGH. The rate of alterations detected by microsatellite analysis is, however, approximately twice that of the allelic losses detected by CGH. In the regions 3p23, 6q22, 9p, 11q13, 18q21, and 22q12.1, the differences are even more pronounced, indicating that small deletions that are not detectable by CGH may be involved [2, 3, 16, 19, 20, 32, 33, 37, 41, 59]. These regions displaying losses might harbor tumor suppressor genes involved in the initiation and progression of PETs. However, only a few candidate genes have been thoroughly investigated, and many genes remain to be tested.

The most important candidate gene is the *MEN1* gene. We know that almost all PETs in MEN1 patients, including microadenomas show allelic loss of the wild-type allele [19, 34, 51]. However, somatic mutations of the *MEN1* gene located on 11q13 are detectable in only 21% (33 of 155) of sporadic PETs [11, 16, 32, 43, 53, 59]. When PETs are stratified according to the hormonal syndrome they cause, the rate of *MEN1* mutations varies considerably between the subgroups. Thus, *MEN1* mutations are only detected in 7.7% (5 of 65) of insulinomas and 8% (2 of 26) of NF-PETs. In contrast, gastrinomas, glucagonomas, and VIPomas show a higher mutation rate of 37, 67, and 44%, respectively [11, 15, 16, 32, 43, 53, 56, 59].

In contrast to the low rate of 21% *MEN1* mutations, 68% of all PETs exhibit losses at 11q13 and/or of more distal parts of the long arm of chromosome 11, indicating that there might be a haploinsufficiency of the *MEN1* gene or that the other allele might be inactivated by epigenetic mechanisms. CpG island methylation in the *MEN1* promoter, however, was not identified [8]. Another explanation for this high rate of LOH is that further, yet unknown tumor suppressor genes in this region might be involved [11, 12, 16, 32, 43, 53, 59]. An important role of the *SDHD* gene (one of the candidate genes on 11q23) in the pathogenesis of PETs and other endocrine tumors has recently been excluded [35].

Another familial syndrome associated with PETs is VHL. Intriguingly, somatic *VHL* gene mutations have been detected in only 1 of 22 sporadic PETs, despite the high LOH rate on 3p25, indicating that the gene is less frequently involved than expected [9, 32]. NF1 and TSC patients may also develop PETs. However, the role of the *NF1* and *TSC1/2* genes in sporadic tumors has not been examined so far.

Several other tumor suppressor genes or oncogenes have been examined in PETs. These genes include *p16*, *PTEN*, *k-RAS*, and *p53*, which are only occasionally mutated and therefore seem to be of minor importance [5, 26, 33, 36, 41, 54]. *RET*, *hZAC*, *BRAF*, and *SMAD3* have been excluded as candidate genes [28, 38, 44, 49]. Furthermore, we recently were unable to detect any somatic mutation of the *HRPT2* gene on 1q25 in 25 examined PETs (unpublished observations).

The role of *DPC4/Smad4* in sporadic PETs also seems to be of minor importance. The high mutation rates described by one group [4] could not be confirmed by subsequent studies on a larger number of tumors by several other groups [30, 33, 37]. The classical tumor suppressor genes *PTEN*, *p53*, and *DPC4* are only rarely mutated, mostly in malignant tumors, and alterations of these genes therefore most likely represent late events in PET progression.

Gastrointestinal endocrine tumors

Genome-wide analysis

Knowledge about the genetic background of sporadic gastrointestinal NETs is even sparser than that of PETs. No results of RNA expression array analyses have been reported so far. Only three studies using CGH [50, 57] or 131 microsatellite LOH markers [30] examined genome-wide allelic imbalances in gastrointestinal NETs. The average number (2.9) of genomic changes was lower in gastrointestinal NETs than in PETs, and there was no clear correlation between the number of aberrations and tumor stage [57]. Furthermore, the number of different chromosomes involved was low, genetic alterations apparently being concentrated on chromosome 18. The study by Zhao et al. [57] described losses of the entire chromosome 18 or of its long arm in 38% of gastrointestinal NETs, whereas others reported these losses to occur in 50 [50] or even in 88% of tumors [30]. The high percentage reported by Lollgen et al. [30] is based on microsatellite LOH analysis, in which small deletions are detected with a higher sensitivity than obtained by CGH. The loss of chromosome 18 in gastrointestinal NETs is strong evidence that important candidate tumor suppressor genes are located on this chromosome. Most of these genes are most likely located on the long arm of chromosome 18.

In the three aforementioned studies [30, 50, 57], significant genetic differences between bronchial NETs, gastrointestinal NETs, and PETs were described, suggesting a different genetic background for these tumor groups. For example, losses of chromosome 18 are rare in bronchial tumors and PETs, whereas losses of chromosome 11 are common [57]. Furthermore, losses of 9p, which are detectable in 50% of gastrointestinal NETs, are rare in PETs, which in contrast more frequently exhibit gains of 17q and losses of 11q.37.

LOH and mutation analysis

The candidate genes *DPC4*, *DCC*, and *Smad2* were investigated because of the frequent allelic loss on 18q (88% of gastrointestinal NETs) [30] but were found to be unaltered in a small series of tumors. In the study by Lollgen et al. [30], all eight examined gastrointestinal NETs contained a wild-type C-terminal *DPC4* sequence encoded by exon 8–11 and conserved DPC4 protein expression as demonstrated by immunohistochemistry [30]. In our study on 21 gastrointestinal NETs, *DPC4* and *DCC* mutations were not detected using single-strand conformation polymorphism analysis (unpublished observations). Therefore, other possible tumor suppressor genes located on chromosome 18 remain to be investigated.

Patients suffering from MEN1 often develop NETs; most of which are localized in the duodenum and the stomach. Allelic loss of the corresponding chromosomal arm 11q has been detected in these types of endocrine tumors associated with the MEN1 syndrome. LOH of 11q is also consistently found in a subset of sporadic endocrine tumors of various localizations, although the observed LOH frequency varies. Although it is very low in the ileum (14% of tumors), it amounts to 25% in the ascending colon, to 30% in the pancreas and stomach, to approximately 50% in NETs of the descending colon and rectum, and to 60% in the duodenum [13, 15, 16, 24, 30, 56].

Enterochromaffin-like (ECL)-cell gastric NETs associated with chronic atrophic gastritis (type I gastric NETs) arise in association with ECL-cell hyperplasia and are often multiple [40]. LOH of 11q was present in only one of six such tumors examined. In the setting of the MEN1 syndrome, *MEN1* LOH, however, occurred in 15 of 20 type II gastric NETs arising in patients with a Zollinger–Ellison syndrome because of duodenal gastrinomas [13]. Two examined sporadic gastric NETs (type III gastric NETs) exhibited allelic loss of 11q microsatellite markers [16].

With the exception of sporadic gastrinomas, only few gastrointestinal NETs have been examined for *MEN1* gene mutations. The *MEN1* mutation rate in sporadic gastrinomas was high (31%). This ratio was comparable to that found in PETs [15]. This is not surprising, as duodenal gastrinomas and PETs are both classical MEN1-associated tumors. Somatic *MEN1* mutations have also been detected in a small subset of NETs of the ileum (one of four tumors examined) and colon, indicating that these mutations are not restricted to foregut NETs but may also occur rarely in midgut and hindgut tumors [16].

The tumor suppressor genes *p16* and *TP53* have also been investigated in NETs. Like in PETs, no mutations of the *p16* tumor suppressor gene have been identified. However, a 5′ CpG island of the p16 gene promoter was methylated in 52% of gastrinomas [41] and in other types of gastrointestinal NET [8]. Furthermore, methylation of *p16* was significantly more frequent in NETs than in PETs and thus represents an additional molecular difference between the two tumor groups [8]. It is interesting to know that a recent study also described higher rates of promoter methylation of the *APC*, *MEN1*, *HIC1*, and *RASSF1a* genes in gastrointestinal NETs [1]. This is good evidence that CpG island methylator phenotype (CIMP) is frequent in gastrointestinal NETs. Microsatellite instability does not occur in PETs or gastrointestinal NETs, and the *hMLH1* gene is not methylated [1, 27]. The high rate of *RASSF1a* promoter methylation might explain the frequent expression of extracellular signaling-related kinase (ERK) 1/2, an important downstream point of convergence in the ras-RAF-mitogen-activated protein ERK pathway [49].

Accumulation of the *TP53* protein was detected by immunohistochemistry in only one duodenal gastrinoma with an identified 17p loss and thus seems to be a rare event in NETs [50]. However, in 25% of goblet cell carcinoids of the appendix, *TP53* mutations were reported to occur [39]. The most frequently reported mutated gene in gastrointestinal NETs is β-*catenin*. Mutations in exon 3 of this gene protecting the corresponding protein from phosphorylation and degradation have been reported in 38% (27 of 72) of NETs [14].This leads to cytoplasmic and nuclear accumulation of β-*catenin* (demonstrated by immunohistochemistry) in 79% of these tumors, indicating other, yet unknown, mechanisms of accumulation of this protein. Only one of these 72 tumors (1.4%) displayed a mutation of the APC gene. However, the rate of gastrointestinal NETs with nuclear translocation of β-*catenin* was only 30% in a later study, and exon 3 mutations were absent [48]. The reported overexpression of cyclin D1 and c-*myc* [52] may be a downstream effect of the alterations of the Wnt signaling pathway. Other examined oncogenes appear to be of minor importance. Hardly any tumors show amplifications of the *HER2* gene [50], and in contrast to gastrointestinal adenocarcinomas, no *K-Ras* mutations were detected in gastrointestinal NETs [55] (Table 1).

Conclusion and perspective

The available molecular data indicate that the tumorigenesis of well-differentiated GEP-NETs differs from that of their nonendocrine counterparts. This is supported by results of CGH, LOH, and mutation analyses that point to different genetic pathways leading to the formation of GEP-NETs. In addition, subgroups of GEP-NETs appear to have special genetic pathways. PETs (especially NF-PETs, glucagonomas, and VIPomas) show a higher average number of chromosomal aberrations and allelic losses than gastrointestinal NETs. RNA expression analysis describes deregulation of cell cycle checkpoint proteins as well as genes

Table 1 Genetic changes frequently found in GEP-NETs

	Gene(s)	Involvement		
		Frequent	Rare	Absent
Genes characterizing endocrine tumor syndromes	*men1*	PET, D	G, I	
	vhl		PET	
	nf-1			
	tsc-1/2			
	hrpt-2			PET
	SDHx			PET, gasNET
Wnt signaling pathway	*b-catenin*	gasNET		PET
	APC		gasNET	
TGFbeta signaling pathway	*TGFbR2*			PET, gasNET
	Smad4		PET	
	Smad3			PET
Common tumor suppressor genes/oncogenes	*DCC*			PET, gasNET
	p53		PET, gasNET	
	PTEN		PET	
	K-Ras		PET, gasNET	
Mechanisms of tumorigenesis	CIMP pathway	gasNET	PET	
	Chromosomal instability	PET	gasNET	
	MSI		PET, gasNET	

GEP-NET Gastroenteropancreatic neuroendocrine tumor, *PET* pancreatic endocrine tumor, *D* duodenal endocrine tumor, *I* ileal endocrine tumor, *gasNET* gastrointestinal neuroendocrine tumor, *CIMP* CpG island methylator phenotype, *MSI* microsatellite instability

involved in DNA damage repair and genomic stability. It will be an important task to find the underlying cause of chromosomal instability (that is particularly observed in malignant PETs and less pronounced in benign insulinomas and duodenal gastrinomas) because this might identify new therapeutic targets. Furthermore, some chromosomal loci are preferentially altered in NETs: Gastrointestinal NETs frequently show 18q losses, while PETs commonly exhibit 11q losses involving the *MEN1* gene. Because PETs, in particular insulinomas and NF-PETs, only rarely harbor somatic *MEN1* mutations and there is no known tumor suppressor gene on 18q, the 11q and 18q losses may suggest that potential new tumor suppressor genes are located in both chromosomal areas. *MEN1* gene deletions are very rare in ileal and appendiceal NET, as are somatic *MEN1* mutations. Additional differences between PETs and gastrointestinal NETs concern the tumor suppressor gene p16 and β-*catenin* as well as the CIMP pathway (Table 1, bottom). p16 is more frequently methylated and β-*catenin* more often mutated in gastrointestinal NETs than in PETs, and gastrointestinal NETs more often exhibit alterations associated with the CIMP pathway, whereas advanced PETs frequently reveal chromosomal instability. Taken together, these data may explain why GEP-NETs, depending on their site of origin and phenotype, differ in their biological behavior and, in addition, stress the importance of classifying endocrine tumors according to their localization and hormone production.

Current results combined with those obtained by new technologies, such as DNA and RNA expression and tissue arrays, will probably result in a more clear-cut picture of the molecular background of the oncogenesis and progression of GEP-NETs. These findings, in turn, might subsequently lead to precisely tailored diagnostic and therapeutic strategies in the near future.

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Conflict of interest statement We declare that we have no conflict of interest.

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REVIEW AND PERSPECTIVE

Peptide receptor expression in GEP-NET

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Abstract Numerous peptide receptors have recently been reported to be expressed or overexpressed in various human cancers. For instance, somatostatin receptors are particularly frequently expressed in gastroenteropancreatic neuroendocrine tumors (GEP-NET), including both primaries and metastases. The density is often high, and the distribution is usually homogenous. While various somatostatin receptor subtypes can be expressed in these tumors, the sst_2 is clearly predominant. These receptors represent the molecular basis for a number of clinical applications, including symptomatic therapy with octreotide in hormone-secreting GEP-NET, in vivo diagnostic with radiolabeled diethylene triamine pentaacetic acid octreotide (Octreoscan) to evaluate the extend of the disease, and ^{90}Y- or ^{177}Lu-[^{90}Y-DOTA]-D-Phe^{1}-Tyr^{3} octreotide radiotherapy. GEP-NET can, however, express peptide receptors other than somatostatin receptor: Insulinomas have more glucagon-like peptide 1 receptors than somatostatin receptors; gastrinomas express very high levels of secretin receptors. GEP-NET may also express cholecystokinin 2, bombesin, neuropeptide Y, or vasoactive intestinal peptide receptors. Often, several of these peptide receptors are expressed simultaneously in GEP-NET, providing a molecular basis for in vivo multi-receptor targeting of those tumors.

Keywords Peptide receptors

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Introduction

In the past decade, there has been increasing evidence for peptide receptor expression on various human cancers [15]. This observation has permitted to develop in vivo peptide receptor targeting of these tumors, for diagnostic and/or therapeutic purposes [12, 13, 15]. The best evidence has been provided for somatostatin receptors expressed in neuroendocrine tumors, which can currently be targeted with ^{111}In diethylene triamine pentaacetic acid octreotide for their in vivo localization or with ^{90}Yttrium- or ^{177}Lutetium-[^{90}Y-DOTA]-D-Phe^{1}-Tyr^{3} (DOTATOC) for targeted radiotherapy [12]. Somatostatin receptor scintigraphy has been shown to be the diagnostic tool of first choice for a subgroup of gut neuroendocrine tumors, as it was superior to all other conventional imaging methods [6], and radiotherapy with ^{90}Y-DOTATOC appears extremely promising in tumors expressing somatostatin receptors, with more than 25% remissions and about 50% disease stabilization [10, 14, 23, 25]. More recently, other peptide receptors have emerged as being overexpressed in selected tumors [15] and appear to have a promising in vivo targeting potential. These are bombesin receptors of the BB_2 subtype, better known as gastrin-releasing peptide (GRP) receptors, which are overexpressed in prostate and breast cancers [15] and can be visualized in vivo in these tumors [22, 24]. Furthermore, cholecystokinin (CCK) 2 receptors expressed in medullary thyroid carcinomas [15] can be selectively targeted in vivo [1, 11], and more recently, neurotensin receptors were visualized in exocrine pancreatic carcinomas [2].

Extensive information on in vitro peptide receptor expression including incidence, density, and subtype characteristics in tumors is required before a novel peptide receptor is chosen as target for in vivo clinical investigations in tumor patients. The aim of the present article is to

review the in vitro evidence for the expression of several regulatory peptide receptors in cancer tissues with morphological methods, including receptor autoradiography and immunohistochemistry. It will focus on human gastroenteropancreatic neuroendocrine tumors (GEP-NET) and somatostatin receptors but will also expand to other peptide receptors such as vasoactive intestinal peptide (VIP), CCK, bombesin, neuropeptide Y (NPY), neurotensin (NT), corticotropin-releasing factor (CRF), and glucagon-like peptide (GLP-1) receptors.

Somatostatin receptors in GEP-NET

GEP-NET are generally known as a group of tumors expressing frequently somatostatin receptors. Table 1 summarizes the main features that are explained in more detail further in the text. There are several possibilities to detect somatostatin receptors morphologically in tissue sections [15]. One possibility is in situ hybridization, which, however, identifies the messenger ribonucleic acid and not the protein. Another possibility is autoradiography that identifies the receptor binding site (protein) that can be pharmacologically characterized and quantitatively assessed. It is a highly sensitive and specific method. Recently, it has been improved to identify the five somatostatin receptor subtypes by using subtype-selective analogs [18]. One drawback is, however, that in vitro autoradiography does not have a very high resolution. A method analyzing the protein with a higher resolution is receptor immunohistochemistry, which, while dependent on a high quality antibody, can precisely identify membrane-bound receptors in formalin-fixed tissues [16].

It is not sufficient to know whether a tumor is somatostatin receptor positive or negative; it is also necessary to assess its density, its distribution, and its somatostatin receptor subtype profile. GEP-NET can have a wide variability of somatostatin receptor density among individuals, ranging from low density, as found in lymphomas, to high density, as seen in meningiomas, medulloblastomas, or growth hormone adenomas. In the majority of the cases, GEP-NET belong preferably to the group of tumors with a high density range of receptors. Both pancreatic NET (including gastrinomas, glucagomas, vipomas) and gut NET (foregut, midgut, and hind gut tumors) can express somatostatin receptors in 80–100% of the cases. Insulinomas have a lower incidence (50–70%). In general, the somatostatin receptors are expressed homogeneously in these tumors, a characteristic that represents an advantage in view of an optimal in vivo targeting of as much tumor cells as possible during peptide receptor radiotherapy. Worth mentioning is also the differentiation-dependent expression of somatostatin receptors. Well-differentiated tumors express usually somatostatin receptors, while undifferentiated GEP-NET may not [17]. Such observations are relevant in regard to therapeutic options [9]. The existence of five sst subtypes has made the evaluation of somatostatin receptors in tumors quite complex. There is consensus, based on various methodologies, that GEP-NET can often express more than one sst subtype; moreover, sst_2 is usually the most prominent, followed by sst_1 and sst_5, while sst_3 is less frequent and sst_4 almost absent [15, 18]. Because excellent sst_2-selective antibodies are now available, the sst_2 receptors can conveniently be assessed by immunohistochemistry on formalin-fixed tissues [7, 16, 21]; if frozen tissue is available, receptor autoradiography using subtype-selective analogs is the method of choice to identify the five sst subtypes.

In clinical practice, in vivo Octreoscan scintigraphy has become the method of choice to evaluate the somatostatin receptor status in the great majority of GEP-NET patients. It is a sensitive, specific, and noninvasive method giving receptor information for the patient's whole body. However, next to in vivo scintigraphy, the in vitro somatostatin receptor evaluation of tumors remains in specific cases an important additional current diagnostic option. It may simply be used as a complementary and confirmatory method to Octreoscan, providing information on sst subtypes, tissue localization, and on receptor homogeneity. It may replace Octreoscan if Octreoscan is not available or uneasy to interpret. The somatostatin receptor status can be established in vitro, either immunohistochemically in the formalin-fixed resected tumor using appropriate antibodies [7, 16, 21] or, if frozen tumor tissue samples have been secured, by somatostatin receptor autoradiography [17].

Table 1 Somatostatin receptor expression in GEP-NET

Main features	Characteristics
Receptor incidence	80–100% (Most pancreatic and gut NET) 50–70% (Insulinomas)
Receptor density	predominantly high
Receptor distribution	predominantly homogeneous
Receptor expression	differentiated tumor>undifferentiated tumor
Receptor subtype	$sst_2 >> sst_1 = sst_5 > sst_3 >> sst_4$
Receptor localization	usually membrane bound (sst_2)

Other peptide receptors in GEP-NET

Although somatostatin receptors have been found to be extremely useful targets for the diagnosis and therapy for a majority of GEP-NET, it is worth summarizing the data available for other regulatory peptide receptors in this type of tumors, as they may potentially play an equally important role. Indeed, several peptide receptors are

Table 2 Selection of peptide receptors expressed in GEP-NET (see text for detail)

Somatostatin receptors
GLP-1 receptors
Secretin receptors
Cholecystokinin receptors
VIP receptors
Bombesin receptors
CRF receptors
NPY receptors

expressed in selected GEP-NET in much higher incidence and/or density than somatostatin receptors [18].

GLP-1 receptors

This is the case for GLP-1 receptors in insulinomas, which are expressed in virtually all cases in extremely high concentrations. We have identified several examples of insulinomas with a high density of GLP-1 receptors but no sst_2 receptors [18]. Furthermore, gastrinomas express GLP-1 receptors with a very high incidence but a lower density than insulinomas. One third of carcinoids have a low to moderate GLP-1 receptor density, as well as half of the four tested glucagonomas [18].

CCK receptors

CCK receptors are also widely expressed in GEP-NET. Virtually all insulinomas express CCK_2 receptors, often in high amounts, but no CCK_1 receptors [18]. More than half of the ileal carcinomas express CCK_2 receptors, and one third expresses CCK_1 receptors [18]. In several cases, both receptor subtypes are expressed concomitantly. Half of the gastrinomas express CCK_1 but not CCK_2 receptors.

VIP receptors

Virtually all ileal carcinomas and insulinomas and a large proportion gastrinomas and glucagonomas express $VPAC_1$, while $VPAC_2$ is absent [18].

Bombesin receptors

While bombesin receptors are rarely detected in insulinomas, gastrinomas express frequently the GRP receptor subtypes (BB_2), while ileal carcinoids preferentially express the neuromedin B receptor subtype (BB_1) [18].

NPY receptors

NPY receptors have been identified in two thirds of ileal carcinoids and one fourth of pancreatic carcinoids, in a relatively low density however (Körner and Reubi, unpublished data).

Secretin receptors

Secretin receptors are expressed in extremely high amounts in the great majority of gastrinomas cases [8]. These wild-type secretin receptors are likely to be responsible for the positive secretin provocation test in Zollinger–Ellison syndrome [5]. However, these receptors can be silenced if, exceptionally, they are expressed concomitantly with a misspliced variant with exon 3 deletion [4].

CRF receptors

Only one study has investigated CRF receptors in GEP tumors, where only endocrine pancreatic tumors were analyzed: 6 of 15 insulinomas express CRF_2 receptors, while glucagomas and gastrinomas rarely express CRF_1 or CRF_2 receptors [20].

NT receptors

GEP-NET have not been found to express significant amounts of NT receptors [19].

Multiple receptors

It is now well documented that a large proportion of GEP-NET can express concomitantly several peptide receptors that could be used as targets simultaneously. Insulinomas often express CCK_2, GLP-1, sst_2, and $VPAC_1$ receptors simultaneously. Gastrinomas are characterized not only by a very high incidence and density of sst_2 and secretin receptors but also a high incidence of GLP-1 receptors and a marked expression of GRP receptors [18]. The coexpression of multiple receptors in human GEP-NET may be biologically relevant [18]. Indeed, many of the involved peptides, e.g., GRP, CCK, VIP, SS, somatostatin secretin, are known to have growth stimulatory or inhibitory properties [15]. Many of these peptides will affect tumor growth, depending on the individual receptor profile of the tumor. Peptide receptor coexpression may also have clinical implications. The concomitant application of multiple radioligands may be extremely attractive to improve the efficacy of peptide targeting in tumors; it will selectively increase the accumulation of radioactivity in the tumors, an advantage not only for diagnostic but especially for radiotherapeutic purposes. Specifically, GLP-1 and CCK_2 receptors may be highly efficient targets in all insulinomas, and the use of a mixture of sst_2, secretin, GLP-1, and GRP radioligands could offer optimal targeting of gastrinomas. As some of the receptors are nonhomoge-

neously expressed by tumors, such as CCK_1 and CCK_2 in ileal carcinoids [18], a combination of the corresponding receptor-selective radiopeptides may further improve the targeting efficacy during radiotherapy by destroying more than one receptor-expressing tumor area. Furthermore, a cocktail of different peptides may possibly reduce the risk of a loss of efficacy during peptide radiotherapy that may be due to tumor dedifferentiation with a resulting loss of some but not all peptide receptors. Finally, an advantage of using a cocktail of radioligands is the possibility to label each of them with different isotopes namely, with β-emitters of different ranges to obtain an optimal radiotherapy for large and small tumoral lesions [3]. Whenever possible, before the concomitant use of several radiopeptide ligands in vivo, it would be worth determining the individual peptide receptor affinity profile of the tumor under consideration, by in vitro receptor determination using the previously described methodology in a surgically resected biopsy sample.

Conflict of interest statement We declare that we have no conflict of interest.

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REVIEW AND PERSPECTIVE

Neuro-endocrine tumours of the lung. A review of relevant pathological and molecular data

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Abstract Neuroendocrine (NE) tumours of the lung include pure and mixed forms. In the former group, a continuum of lesions is recognised ranging from benign typical carcinoids to atypical carcinoids (having a low-grade behaviour, although often associated with regional and distant metastases), to the highly aggressive poorly differentiated carcinomas of the small and large cell types. In the mixed tumour group, the NE component is extensively represented in association with any of the non-small cell carcinoma subtypes (so-called combined carcinomas), or the NE component is restricted to a cell population scattered among adenocarcinoma cells (or more rarely within squamous or large cell carcinomas). The molecular profile of NE tumours has been widely investigated to identify features helpful for the diagnosis, prognosis and even therapy for this special lung tumour category. Specific chromosomal alterations, oncogene mutations and cell cycle molecule disregulation has been documented in NE tumours of the lung, as well as the expression of specific receptors or enzymes implicated in the response to biotherapies or to chemotherapeutic agents. The "molecular classification" of NE tumours should be integrated to morphology, for a better definition of the different histological types and a more appropriate selection of the therapeutic strategy.

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Introduction

Neuroendocrine tumours (NET) of the lung share most morphological and clinical features observed in neuroendocrine (NE; carcinoid) tumours of other organs, e.g. pancreas and intestine. Similarly, pulmonary NETs do not constitute a single, uniform entity but build up a spectrum of NE differentiated lesions associated to specific pathological features on the one side and to a variable clinical behaviour on the other. Typical carcinoids (TC) and atypical carcinoids (AC), as well as poorly differentiated NE carcinomas of the large and small cell types were identified.

Appropriate diagnostic and classification criteria, of high clinical and prognostic value, have been established for NETs of the lung, although a precise "functional" classification of these tumours, in terms of hormonal production, is still lacking. As a consequence, only "common" NE markers are of diagnostic significance in the definition and identification of pulmonary NETs. Among such markers, chromogranins/secretogranins are of major interest. Their detection by specific anti-bodies or by molecular procedures (to reveal the specific mRNA) is not only of diagnostic importance, but it allows drawing of information on cell metabolism and on the storage or release of neurosecretory granules as well. Chromogranin A (CgA), the most widely and intensely expressed member of the family, is stored in high amounts in carcinoids and to a variable extent in poorly differentiated NE carcinomas.

This review will briefly summarise the current diagnostic criteria of each single type of pulmonary NET (both pure and combined forms) with special reference to difficult issues for the differential diagnosis, and will finally

consider the phenotypic and molecular data having a pathological and/or clinical relevance.

NE cells in normal lung and in non-neoplastic conditions

Normal NE cells (also called Kulchitsky cells) of the bronchial tree occur solitarily or in small aggregates, so-called neuroepithelial bodies, within the ciliated epithelium [17]. Lung NE cells produce a variety of peptides such as serotonin, bombesin/gastric releasing peptide, calcitonin and the recently identified ghrelin [60], while in hyperplastic or neoplastic conditions, adrenocorticotropic hormone (ACTH), vasoactive intestinal peptide or somatostatin productions have been reported [9].

A role of NE cells in lung development, growth and repair has been suggested based on their increased expression levels in infants with pulmonary dysplasia, cystic fibrosis or prolonged assisted ventilation [52].

NE cell hyperplasia is a microscopic finding only that displays three different patterns including: (1) increased number of scattered NE cells, (2) linear proliferations along the bronchial mucosa and (3) nodular hyperplasia in the mucosa. Most cases are idiopathic ("diffuse idiopathic pulmonary NE cell hyperplasia," associated with obliterative bronchiolar fibrosis in the absence of known causes of interstitial or airway fibrosis or inflammation) [28], while others may be incidentally found in chronic bronchial inflammation [42], Langerhans cell hystiocytosis [2] or bronchopulmonary dysplasia [16].

At the extreme of this spectrum, the term "tumourlet" defines NE cell proliferations that extend beyond the basal membrane of bronchi and bronchioles, having a size less than 5 mm and a dense fibrous stroma surrounding cell clusters.

NE tumours

The WHO classification of NETs of the lung has combined architectural patterns (e.g. organoid growth vs small cell diffuse growth) with other parameters, with the mitotic index and the presence of necrosis as the two most relevant, for the purpose of recognising the four different categories proposed in the spectrum of pure NETs of the lung (Table 1).

Typical carcinoid

TC represents less than 1% of lung tumours. As opposed to other NE lung tumours, it is not related to smoking and develops in adults (mean age of 55 years) with an equal male/female distribution. The most common location is in the large airways, presenting as a polypoid mass, which protrudes in the bronchial lumen. Several histo-morphologic patterns can be recognised, most often in combination. Tumour cells are polygonal, with abundant, finely granular and eosinophilic cytoplasm. Nuclei show clumped open chromatin and small dark nucleoli. The growth pattern is most often trabecular with cords or ribbons of tumour cells dispersed in a delicate fibrovascular stroma. More rarely, spindle cell, acinar or glandular patterns, with occasional oncocytic and clear cell changes, can be recognised.

Necrosis is always absent and mitosis are less than 2×10 high power fields (HPF): both features are essential to differentiate TCs from other NE lung tumours of higher grade (see also Table 1).

Spindle cell variety of TC should be distinguished from nerve sheath proliferations and haemangiopericytomas, defined by S-100 protein and vascular markers immunoreactivity, respectively, in contrast to the presence of NE markers in TCs. Carcinoids having glandular formation may be hardly distinguished from well-differentiated adenocarcinomas, especially when mucin secretion is observed. Clear cell changes may resemble clear cell (sugar) tumours, which are invariably positive for CD34 and HMB45 and lack NE markers.

Atypical carcinoid

AC represents nearly 10% of NE lung tumours, even if different series are difficult to compare because, in some of them, large cell NE carcinomas (LCNEC) are included in

Table 1 Differential pathological findings in NE lung tumors

	Typical carcinoid	Atypical carcinoid	Large cell NE carcinoma	Small cell carcinoma
Organoid pattern	Characteristic	Characteristic	Present, less extensive	Absent
Cell size	Large	Large	Large	Small
Cytoplasm	Abundant	Abundant	Abundant	Scanty
Nuclear pleomorphism	Usually absent	Occasionally present	Present	Present
Prominent nucleoli	No	No	Yes	No
Mitoses (x10 HPF)	<2	2–10	>10 (mean 70)	Mean 70
Necrosis	Absent	Usually focal	Extensive	Extensive
Approximate 5-year survival	100%	70%	25%	<10%

this same group. ACs are more often associated with cigarette smoking, they have a male pre-dominance and are more frequently peripherally located, compared to TCs. Conversely, they share the same morphologic, cytologic and architectural features as TCs (Fig. 1), and the correct diagnosis of the two forms may be difficult, especially in small biopsies. As already mentioned, the main differential criteria are the presence of necrosis and a higher mitotic count (2–10 × 10 HPF) in ACs. Necrosis has a peculiar focal, punctate, sharply demarcated appearance. Focal nuclear pleomorphism may also occur. It has to be considered that up to 20% of ACs may lack NE markers. Peripherally located tumours more frequently show spindle cell appearance and smaller cell size. These cases have to be separated from small cell carcinomas, which usually have a higher mitotic count, a dense and uniform nuclear chromatin, and extensive necrosis. The distinction between ACs and LCNECs is even more difficult and will be discussed in the next paragraph.

Large cell NE carcinoma

LCNEC was recognised in 1991 as a separate entity [54] based on the observation that a group of highly aggressive NE lung carcinomas exists, apart from the well-known small cell (oat cell) carcinoma. Because these tumours maintain at least in part the organoid and trabecular growth patterns (typical of carcinoid tumours) and are made of large cells, the term LCNEC was proposed. NE differentiated carcinomas with extensive necrosis, high mitotic index and marked propensity to invasion and distant spread were grouped in this novel category and found to have a clinical behaviour not different from that of small cell carcinoma [5, 54, 55] (Fig. 2). Unfortunately, in the last WHO classification of lung tumours [56], this tumour entity was included in the large cell carcinoma group, generally encompassing non-NE carcinoma subtypes and having nothing to do with LCNEC, with the possible exception of cell size. The main differential diagnostic problem is represented by ACs. LCNECs share the classical solid

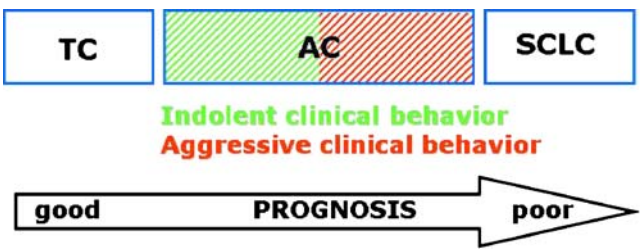

Fig. 1 Spectrum of NETs of the lung: AC was separated from TC, based on high-grade morphological features [4]. AC had a more aggressive behaviour than benign carcinoid, but in this group, cases with indolent clinical course co-existed with highly aggressive neuroendocrine carcinomas

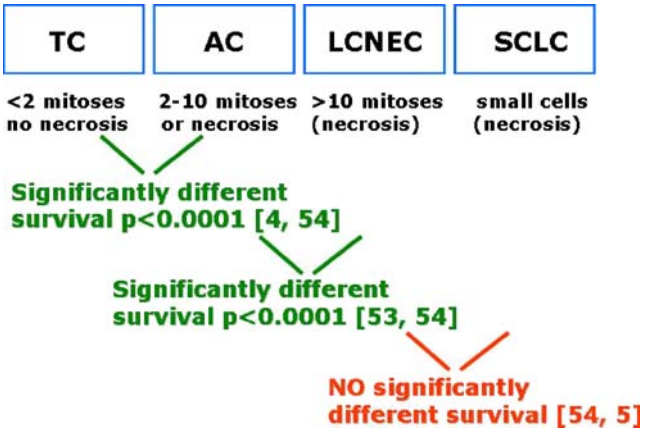

Fig. 2 Spectrum of NETs of the lung: the intermediate group of NETs, i.e. the ACs, was further split into two entities, having a LCNEC subtype recognised [54]. The latter had a significantly different clinical behaviour from carcinoid tumors [55, 54], but no differences in outcome were seen between LCNEC and small cell carcinoma [5, 55]

organoid and lobulated NE morphology but have an extensive, geographic-type necrosis, with a prominent infarct-like appearance and a mitotic count exceeding 10 mitoses per 10 HPF. Large tumour size, marked pleomorphism, lower nuclear to cytoplamic ratio and large nuclei with prominent nucleoli are additional features. The cell size distinguishes large cell NE from small cell carcinoma, but cases of co-existing small and large cell components are well documented. In such instances, an uncommittal report of poorly differentiated NE carcinoma with combined small land large cell features may be sufficient for the purpose of selecting the appropriate therapeutic strategy (chemotherapy; Figs. 3, 4). Two other highly malignant tumours enter into the differential diagnosis with LCNEC: basaloid carcinoma and large cell carcinomas. Minor morphological features (solid growth in large cell type, peripheral palisading in basaloid carcinoma) and, more importantly,

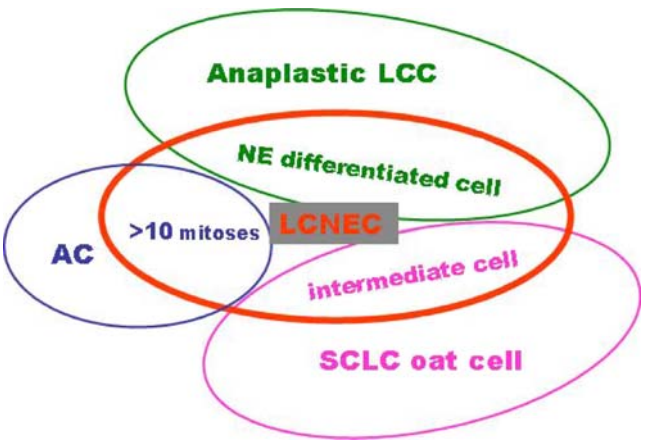

Fig. 3 The LCNEC subtype [54] (*red circle*) includes former anaplastic large cell carcinomas (*LCC*) with NE differentiation, former small cell cancers (*SCLC*) of the intermediate cell type and those former *AC* having >10 mitoses/10HPF

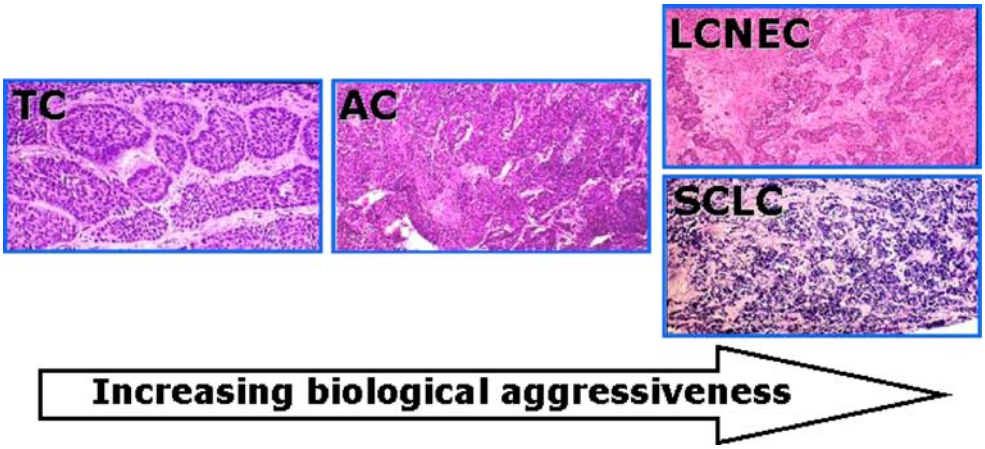

Fig. 4 Proposed spectrum of NE lung tumors based on biological behaviour

expression of NE markers help to discriminate between these entities. The diagnostic immuno-histochemical profile of LCNEC includes more or less extensive (depending also on the use of antigen retrieval procedures) expression of cytoplasmic CgA and synaptophysin as well as neural cell adhesion molecule at the cell membrane level [25]. The simultaneous absence of high molecular weight cytokeratin (types 1, 5, 10, 14 of the Moll's catalog) reactivity is an additional finding supporting a diagnosis of NET. These cytokeratins are in fact restricted to non-NE carcinomas of the lung [50, 59] and of other organs [36].

Small cell lung carcinoma

Small cell lung carcinoma (SCLC) usually arises as a rapidly growing tumour of the major airways. It is made up of small cells with scanty cytoplasm and condensed small nuclei, approximately three lymphocytes in size. Neoplastic cells grow in diffuse sheets loosely connected in a thin stroma. The distinction in three groups, according to the cell size, as oat cell, intermediate and combined cell type, has poor correspondence to clinical features and seems, at least for the first two forms, to be related more to tissue preservation, especially in biopsy specimens, than to true cell characteristics. In addition, several cases of intermediate cell variant are now reclassified as LCNEC. High mitotic rate and large infarct-like necrosis are additional main features.

Small cell carcinoma should be differentiated from several small cell proliferations, both of epithelial and mesenchymal origin. With regard to the latter, pulmonary lymphomas may resemble small cell carcinoma, and several nuclear and cytologic features may overlap, making immuno-histochemistry mandatory for a correct diagnosis. Other small cell tumours, which may be confused with small cell carcinoma include basaloid carcinoma, poorly differentiated squamous cell carcinoma and peripheral neuroectodermal tumours of the thoracic wall (so-called Askin tumours). The differential diagnosis with the former three is based on immuno-histochemical findings and on few morphologic features, such as loose connective tissue, nuclear condensation and scanty cytoplasm, which are more prominent in small cell carcinoma. The distinction from Askin tumour may be more difficult because of several shared features. In Askin tumour, however, the nuclei have a more dispersed chromatin, one or more small nucleoli, and specific immuno-histochemical markers, such as CD99, are constitutively expressed.

Although absent in TC and AC [50], TTF-1 is positive in the majority of both large and small cell NE carcinomas and is therefore useful to differentiate these lesions from poorly differentiated NE carcinomas of other sites. High molecular weight cytokeratins are not expressed in small cell lung cancer, in contrast with non-NE carcinomas [59].

Combined NE carcinomas

A small proportion of NE carcinomas are histologically heterogeneous. This occurs mostly with poorly differentiated carcinomas, both small and large cell types, which may grow in association with squamous cell carcinomas and adenocarcinomas, or, less frequently, with sarcomatous tumours. Combined carcinomas share many clinical, epidemiologic and prognostic features of the NE counterpart, so that, in the WHO classification of lung tumours, they are generally considered as variants of small and LCNECs (codes 8045/3 and 8013/3, respectively). The morphology of such tumours recapitulates the specific features of the individual components. The type of mixture of the two cell populations may either be represented by an intimate intermingling of cell types, with divergent differentiation along NE and "exocrine" lineages, or may represent a sort of "collision" tumour with a small/large cell carcinoma growing adjacent to a non-small cell lung carcinomas (NSCLC), with minimal interplay between the two components. Immuno-histochemistry is helpful for the correct identification of combined tumours and in our opinion a panel of markers, including CgA and CD56 (for NE cells) and high molecular weight cytokeratins (for NSCLC types),

is sufficient to detect the differential reactivity of the two cell populations.

NE differentiation in NSCLC

More or less extensive areas of NE differentiation can be easily detected by CgA immuno-histochemistry in otherwise conventional NSCLC, as also observed in carcinomas of several other organs. This finding does not address to a diagnosis of mixed or combined NE-exocrine carcinoma, unless the extent of NE cells exceeds 30% of the whole tumour (according to the definition of mixed exocrine–endocrine carcinomas) and is generally restricted to a minor cell population. In fact, the recognition of a NE phenotype is possible with immuno-histochemistry only, in the absence of light microscopical features of the NE origin. NE features may be recognised in all lung carcinoma types, most frequently in adenocarcinomas but also in squamous and large cell carcinomas or in sarcomatoid carcinomas/blastomas [1, 6, 10, 47]. The percentage of NE cells may range from 3 to 25%, either as single cells scattered among non-NE glandular or squamous neoplastic cells, or more rarely as small clusters of neoplastic cells admixed within the non-NE component. Little is known about the significance of NE differentiation in NSCLC, and controversial data exist in the literature about the different behaviour and response to chemiotherapy for NE differentiated vs conventional NSCLCs. A recent study [19] indicates that NE differentiation has no impact on survival of a series of NSCLC, at variance with other studies [38], which found a worse prognosis in stage I NSCLC with NE differentiation. This is not surprising because NE differentiation is a well-known indicator of poor prognosis in prostate cancer [7] and in gastric adenocarcinoma [22].

Molecular profiling of lung NETs

The molecular profile of NE lung cancer has been extensively investigated with the goal of identifying features helpful for diagnosis, prognosis and even therapy for this special lung tumour category. The endpoint of genome, gene transcript, cell product, receptor and regulatory peptide expression analysis is ultimately to create a “molecular classification” of lung NETs that can serve as a complement or alternative to morphology to better define the different histological types and address the appropriate therapeutic strategy.

In the search for molecular markers of lung NETs, gene expression profile studies are increasing. Apart from common NE markers (such as chromogranins), insulinoma-associated (IA-1) gene and human achaete-scute homolog-1 (hASH1) gene overexpression were observed [8, 15, 51]. Among them, hASH1 is a recently described marker for NE differentiation in lung tumours [21] and was also suggested as a therapeutic target because of its cell growth modulation effect in lung cancer cell lines [33]. Other reports [3] found that carcinoids (typical and atypical) had genes that clustered with gliomas, while SCLC clustered with bronchial epithelium, suggesting a different histogenesis of these two tumour subtypes. Subsequent studies confirmed such distinct molecular subgroups [8, 23]. Moreover, by immuno-histochemistry, an expression profile of positive carboxipepditase E (CPE) and negative gamma-glutamil hydrolase (γGH) was preferentially detected in TC and AC thus significantly predicting good prognosis, whereas the opposite phenotype (negative CPE and positive γGH) was a feature of LCNEC and SCLC [18].

Comparative genomic hybridisation identifies genome alterations of chromosome copy number, gains and losses, as well as gene amplifications, although the precise localisation of the altered loci cannot be obtained with this methodology. The most common genetic feature in both TCs and ACs is the allelic deletion of the long arm of chromosome 11. Different loci in 11q are altered in these tumours, being 11q13 (linked to the MEN1 gene locus) lost in nearly one third of sporadic carcinoids, both typical and atypical. It was recently demonstrated that the main genetic defect of MEN1 consist in loss of heterozygosity (LOH) or micro-satellite instability (MSI), which lead to the consequent absence of the gene product menin in TC and AC [58]. However, the development of lung carcinoids in MEN1 syndrome is a rare occurrence. MEN1 gene alterations are also virtually absent in poorly differentiated NE carcinomas, and MEN1 kindreds do not present these types of tumours as inherited carcinomas. Interestingly, familial lung carcinoid syndromes different from MEN1 have been described, and still need to be better defined [31].

With regard to SCLC, the vast majority shows deletions of the short arm of chromosome 3, being the putative tumours suppressor genes still to be completely identified. The von Hippel Lindau (3p25) and FHIT (3p14.2) genes seem to be the most likely candidates, and LOH of both has been detected in a high percentage of SCLC. However, although the frequency of LOH at 3p was higher in the group of poorly differentiated NE carcinomas, large and small cell types had different LOH patterns at 3p and also at 22q), and a significantly different genetic background was observed in these two forms [53]. Finally, DNA gain in 17q24–25 was demonstrated as markers of brain metastases in SCLC [41].

Among alternative mechanisms of gene inactivation, hypermethylation contributes to the silencing of genes acting as tumour suppressors (DNA repair, cell cycle regulation, angiogenesis and invasion modulators, etc). As compared to other lung cancer types, this mechanism is

rarely acting in lung NETs. In fact, DNA methylation rarely involves TC, whereas RASSF1A and Caspase 8 are more frequently altered in AC and SCLC [48].

Among genetic alterations involving cell cycle regulators, *p53* gene point mutations are frequently present in LCNEC and SCLC, while absent in all TCs and most ACs [32]. In this context, immuno-histochemical detection of *p53* protein has been proposed in the differential diagnosis of NE lung tumours. Mutations in *RB1* gene with loss of function of the retinoblastoma gene product have also been detected in most SCLC [13, 24]. Similarly, mutations of the *PTEN* gene were also mainly restricted to SCLC [61].

Concerning key pathways that regulate the cell cycle, altered expression patterns of proteins acting in several steps have been described, with diagnostic or prognostic implications. Rb/*p16*/cyclin D1 pathway aberration is one of the most common in NETs, with a variety of molecules and different mechanisms involved, probably reflecting variable genetic divergency among individual tumours [20]. Signalling pathway of the E2F family of transcriptional factors (E2F1, E2F3) was studied in lung NETs [11]. Recently, Salon et al. [46] provided evidence of a direct and functional interconnection between the E2F-1, Skp2 and cyclin E oncoproteins in poorly differentiated lung NE carcinomas, but not in TC and AC, suggesting their co-operation in the development of these tumours.

Telomerase activity was widely studied in NE lung tumours. Telomerases are the enzymes that synthesise telomeric DNA strands, thereby compensating the DNA losses during each cell division. A high intra-cellular telomerase activity can be considered the compensation to telomere length shortening resulting from uncontrolled proliferation. SCLCs have high telomerase activity in nearly 90–100% of the cases, confirming the model of a high proliferation with extensive genetic instability, as demonstrated by their numerous allelic losses [26]. Moreover, a significantly higher telomerase activity in LCNEC than in TC was recently described [30, 62]. In lung cancer, a significant correlation between telomere length and diagnostic/prognostic factors has only occasionally been documented. Telomerase inhibitors are being investigated in the clinical setting, in combination with cytotoxic chemotherapy for advanced lung cancers, although anti-telomerase therapies need a better comprehension of the telomere elongation machinery and of the response to telomere shortening induced-DNA damage [27].

Finally, limited data have been generated on molecules regulating invasive growth in pulmonary NETs. Salon et al. [45] described a correlation between progression and levels of E-cadherin and β-catenin. In particular impaired expression of E-cadherin and β-catenin correlate with lymph node metastasis and advanced stage disease in carcinoids and LCNEC [39].

Tumour profiling for therapeutic purposes

Novel therapeutic strategies are arising from a better understanding of cancer biology. Innovative therapies targeted to peptide receptors, signalling molecules or catalytic enzymes proved to be helpful in patients with several tumour types, including NETs. An accurate expression profile of the various peptide receptors in lung NETs can be helpful for therapeutic purposes, including the choice of radioligands or non-radioactive peptide analogues [40, 44]. Among them, somatostatin receptors (SSTRs) have been demonstated in NE and non-NE lung tumour [37]. The tissue localisation of SSTRs in lung tumours was widely analised by means of auto-radiography [43], in situ hybridisation, immuno-histochemistry and RT-PCR [35, 37]. Recently, the SSTR type 2 immuno-histochemical expression was investigated in 58 NETs of the lung (12 TC, 20 AC, 19 LCNEC and 7 SCLC) and correlated with follow-up [40]. A strong membrane reactivity was detected in 91% of TC, 65% of AC, 57% of LCNEC and 43% of SCLC.

Thymidylate synthetase (TS) is an enzyme playing an important role in cellular proliferation and growth [29], catalysing the methylation of fluorodeoxyuridine monophosphate to deoxythimidine monophosphate, an essential precursor for DNA synthesis [14]. Five-fluorouracil (5-FU) is an anti-cancer agent largely used in various human neoplasms, that inhibits TS and blocks DNA synthesis [49]. The predictive role of TS quantification in tumours treated with anti-folate drugs such as 5-FU has been extensively described in a variety of human tumours. NETs represent potential targets of anti-folate agents, and we have recently conducted a study on the quantification of TS mRNA and protein levels in these tumours, showing a differential expression of TS in the spectrum of pulmonary NETs, being higher in poorly differentiated NE carcinomas, and indicated TS as a predictive marker of clinical response in a group of patients treated with 5-FU (Ceppi et al., submitted).

The signalling pathway involving mTOR (the mammalian target of rapamycin) is one of the main regulators of cell growth and proliferation and is located at the crossroad of several major signal transduction molecules (including, PTEN/Pi3-kinase, AMKP, Ras/Raf). Its functions in mammalian cells include the control of mRNA translation and metabolism. All mTOR functions are blocked by rapamycin as well as by other mTOR inhibitors under development, such as everolimus and temsirolimus [12, 63]. The only available literature data on mTOR in NE lung tumours are represented by experimental models in SCLC cells. In fact, it has been shown that blocking PI3k/Akt/mTOR pathway with rapamycin is sufficient to overcome chemoresistance and promote apoptosis in SCLC cells [57]. A recent preliminary report on phase II clinical trial, in which the

rapamycin derivative temsirolimus was evaluated in SCLC patients after chemotherapy, failed to show any beneficial effect for the patients [34]. No data exist on the distribution of mTor protein (with special reference to its Serin 2448-phosphorylated active form) and its downstream targets (S6K and 4EBP1) in the spectrum of pulmonary NETs. The immuno-histochemical expression of these molecules was analysed in the same series of 58 lung NETs. The preliminary data indicate that well-differentiated tumours had a higher expression level of mTor (75% of TC vs 43% of SCLC), but no correlation with the expression of the target proteins S6K and 4EBP1 (implicated in protein synthesis and cell proliferation) was observed. With regard to the expression of hypoxia-related factors, HIF1alpha (which is targeted by mTor via the initiation factor eIF4E), but not HIF2, was strongly expressed in the nuclei of mTor positive tumours only, although no differential reactivity was observed between well-differentiated tumours and high-grade NETs (unpublished data).

Conclusive remarks

A spectrum of NETs of the lung exists including pure forms and mixed forms. In the former group, a continuum of lesions is recognised ranging from benign TCs to ACs (having a low-grade behaviour, although often associated with regional and distant metastases), to the highly aggressive poorly differentiated carcinomas of the small and large cell types, which share the same poor prognosis. In the mixed tumour group, again several tumour types are recognised, being the NE component extensively represented in association with any of the NSCLC subtypes (so-called combined carcinomas), or being the NE component restricted to a cell population scattered among conventional adenocarcinoma cells (or more rarely within squamous or large cell carcinomas).

The correct morphological identification of all these tumour types together with the definition of a molecular profile may allow to better characterise individual NETs, with the ultimate goal of providing a combined morphological and molecular classification of lung NETs, useful for the choice of the most appropriate therapeutic strategy.

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REVIEW AND PERSPECTIVE

Goblet cell carcinoids and other mixed neuroendocrine/nonneuroendocrine neoplasms

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Abstract Within the spectrum of neuroendocrine tumors arising in different organs, intermediate and controversial entities exist displaying a coexistence of neuroendocrine and nonneuroendocrine cell populations, and that are grouped under terms such as "goblet cell carcinoid", "mixed endocrine-exocrine carcinoma", "combined carcinomas", or "adenocarcinoma with neuroendocrine differentiation". These tumors may display variable amounts of the two components, potentially ranging from 1 to 99%, and variable structural patterns, ranging from single scattered neuroendocrine cells to a well-defined neuroendocrine tumor cell component organized in typical organoid, trabecular, or solid growth patterns. Although variably included in the site-specific World Health Organization classification schemes, clear definitions and diagnostic features are still missing, as well as a definite knowledge of their biological properties and histogenesis. In the present report, the main characteristics of tumors showing mixed neuroendocrine and nonneuroendocrine features will be described, using morphological patterns and site of origin as schematic guidelines. Moreover, molecular and clinical aspects, which might help to understand their possible histogenesis and biological behavior, will be reviewed.

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Background

Cells belonging to the neuroendocrine system may be divided into those which give raise to neuroendocrine glands, such as pituitary, parathyroids, paraganglia, and adrenal medulla, and those which are disseminated in different organs and systems, such as the respiratory, gastrointestinal, biliary and urogenital tracts, the pancreas, the thyroid, the skin, and others. The neoplastic counterpart of the former group is represented by the characteristic pure neuroendocrine tumors, specific for each gland (pituitary and parathyroid adenomas/carcinomas, paraganglioma, and pheochromocytoma), whereas the latter may give origin to either pure neuroendocrine tumors (both well and poorly differentiated) or neoplasm showing combined aspects of both neuroendocrine differentiation and nonneuroendocrine features specific to the site of origin.

Neuroendocrine tumors originating from diffuse neuroendocrine cells are only partially classified under the same scheme [63], while in part follows the classification of the tumors of the specific site of origin (i.e., in the respiratory and urogenital tract); such almost contradictory state of affairs concerns also the group of mixed neuroendocrine and nonneuroendocrine neoplasms. Major points are represented by the morphological background which should be recognized in these lesions, the extent of the two components to be recovered, and even their clinical implications (Table 1). The role of immunohistochemical markers in the identification of mixed neuroendocrine nonneuroendocrine neoplasms is not different from the diagnostic approach of

Table 1 Clinical relevance of the recognition of mixed neuroendocrine and nonneuroendocrine neoplasms

Variant of mixed NE/non-NE tumor	Clinical behavior
Goblet cell carcinoid	More aggressive than typical carcinoid
Goblet cell carcinoid + adenocarcinoma	Dictated by the adenocarcinoma component
MEEC with well-differentiated neuroendocrine component	Dictated by the exocrine (usually adenocarcinoma) component
MEEC with poorly differentiated (small or large cell) neuroendocrine component	Dictated by the neuroendocrine component
(Adeno)carcinomas with focal neuroendocrine differentiation	Same as ordinary adenocarcinomas (except in the prostate, having worst prognosis)
Mixed medullary/follicular carcinoma	Usually dictated by the medullary component (radioiodine therapy however indicated)

NE Neuroendocrine, *MEEC* mixed exocrine-endocrine carcinoma

pure neuroendocrine tumors and we will focus only on peculiar aspects related to specific entities or histopathogenetic studies.

Pathological features

In general terms, two major morphological patterns in mixed neuroendocrine and nonneuroendocrine neoplasms are encountered: as a first possibility, the two components grow strictly intermingled, to the point that individual cells co-express both exocrine products and endocrine granules (Fig. 1); this pattern is typical of the so-called goblet cell carcinoid. As an alternative, the two components are clearly distinguishable by morphology, although growing within the same nodule, and this type of tumors is generally grouped under the term mixed exocrine-endocrine carcinoma (MEEC). A third possibility is represented by the occurrence of focal neuroendocrine differentiation in otherwise nonneuroendocrine tumors (mainly adenocarcinomas from various sites). Although this latter aspect is of pathogenetic and possibly clinical relevance, it represents in our opinion a separate part of the spectrum although it enters in the possible differential diagnosis of MEEC [74]. It is worth noticing that focal neuroendocrine differentiation in non-neuroendocrine tumors has been described in a variety of different locations (breast, prostate, lung, colon, stomach, and so on) with a frequency which is greatly related to the method (and corresponding sensitivity) employed to detect the neuroendocrine counterpart. In nonsmall cell lung carcinoma, neuroendocrine differentiation has been reported in up to 36% of cases, depending on the method used to identify neuroendocrine cells [1, 28]. Focal neuroendocrine differentiation, although not mentioned in the WHO classification of tumors of the digestive system [23], has been reported in esophageal [24], gastric [51], colorectal [3, 5, 13, 21, 22, 30, 53], and extrahepatic duct carcinomas [27]. In the breast, more or less extensive neuroendocrine differentiation foci were described in conventional lobular or ductal carcinomas [62, 68, 73], and are kept separate from the exceptional "carcinoid tumors" of the breast and the neuroendocrine carcinoma subtype, both of which display neuroendocrine features in more than 50% of tumor cells [68]. In the prostate and bladder, neuroendocrine differentiation has been described in a fraction of prostatic and bladder carcinomas [6, 25]. The interest for neuroendocrine differentiation in nonneuroendocrine tumors embraces histogenetic, diagnostic, and clinical issues, mostly related to the correct morpho/functional (hormonal) typing of different neoplastic components in both primary and metastatic tumors. Additionally, significant clinical and prognostic correlates were proven in prostatic adenocarcinoma only, while remaining mostly controversial for cancers at other sites.

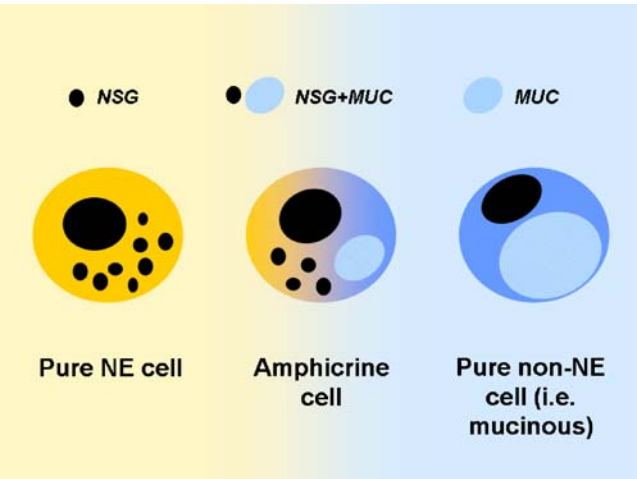

Fig. 1 Schematic illustration of amphicrine cells containing both neuroendocrine granules (NSG) and exocrine products (i.e., mucinous material-MUC)

Goblet cell carcinoids

The term goblet-cell carcinoid was introduced in 1974 for tumors of the appendix exhibiting histological features different from both ordinary carcinoid and adenocarcinoma [66]. Since the original description, they were considered low-grade malignant tumors, although aggressive cases

with extensive local spread and metastases were observed. In 1978 the term "adenocarcinoid" was coined, and despite abundant mucin production and goblet-cell arrangement, a closer relationship to carcinoid than to adenocarcinoma was postulated [76]. Morphologically, goblet cell carcinoids represent an intimate mixture of mucin-laden signet ring cells and highly granulated neuroendocrine cells in a tumor with classical organoid pattern. In addition, both aspects may be recognizable in some individual cells of the tumor. At least in the appendix, goblet cell carcinoids were the first reported examples of mixed nonneuroendocrine and neuroendocrine tumors, and are classified as their pure neuroendocrine counterparts, according to the 2000 World Health Organization (WHO) classification [63]. Indeed, the relative proportions of the two components are variable and the majority of cases present a predominance of one of the two main aspects, either limited mucinous cell population or more scant neuroendocrine cell among signet ring cells, whereas only in some cases both neuroendocrine and exocrine aspects are well represented. The distinction between goblet cell carcinoid and other types of tumor is of great importance because of the implications for treatment and prognosis. Frank adenocarcinoma can arise from goblet cell carcinoids, and tumors with both components are classified as mixed goblet cell carcinoid–adenocarcinoma (Fig. 2). The adenocarcinoma component of the latter determines their prognosis, which is generally worse than that of pure goblet cell carcinoid. Recommendation for an aggressive approach is adopted by a greater part of the surgical community, since lymph-node involvement may be observed in more than 12% of the patients [65]. Exceptional cases of combined goblet cell carcinoid and mucinous cystoadenoma of the appendix have been also reported [2].

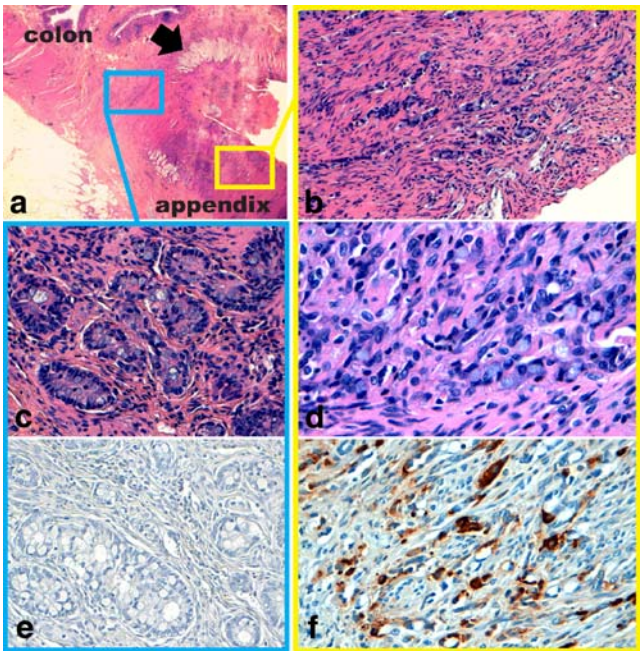

Fig. 2 Invasive goblet cell carcinoid of the appendix (**a**, bottom right); extending to the subsierosa (**b**) with typical signet ring neoplastic cells (**d**) and positive for chromogranin A (**f**), admixed with a frank adenocarcinomatous component infiltrating the wall of the cecum (**a**, top; **c**), with mucin production (**a**, arrow), negative for chromogranin A immunohistochemistry (**e**) (original magnifications: **a** 20×; **b** and **e** 200×; **c**, **d**, and **f** 400×)

Goblet cell carcinoids are mostly affecting the appendix, although reported in other sites of the gastrointestinal and biliary tract. Cases showing composite appearance of signet ring carcinoma, goblet cell carcinoid, and glandular adenocarcinoma have been described in the stomach [15]. A case of multiple microscopic signet ring cell carcinoids was reported in the gallbladder, in which many small organoid-patterned carcinoid tumors infiltrated the gallbladder wall and had focal exocrine cell differentiation, in the form of scattered mucin-laden signet ring cells in the neoplastic nests [54]. Only scant amphicrine cells were found by double stainings and the proportion of signet ring cells did not exceed 20% of the whole tumor.

Immunohistochemical studies determined similar patterns of E-cadherin and beta-catenin in goblet cell carcinoids and conventional carcinoids of the appendix, as compared to colon adenocarcinoma [42], although higher proliferation rate and dysregulation of several cell cycle modulators (cyclin D1, p21, and p16) were observed in goblet cell carcinoids [33], a finding which could explain their more aggressive behavior as compared to usual carcinoid tumors. It is interesting to note that a differential pattern of S-100 protein expression was observed in classical and goblet cell carcinoids of the appendix, being the former invariably positive, and the latter negative. These features suggest a possible origin from different progenitor cells, namely subepithelial neuroendocrine cells and epithelial crypt stem cells for classic and goblet cell carcinoids, respectively [19].

Mixed exocrine–endocrine carcinomas

In the literature, the definition of MEEC and the distinguishing criteria from carcinomas with neuroendocrine differentiation are not uniform: some take into account the extension of the neuroendocrine component only, while others consider type and extent of the observed morphological patterns [7, 9, 14, 41, 62, 73]. This lack of standardization created several controversies in both fields of tumor recognition and treatment of these lesions. In addition, especially in pulmonary and gastroenteropancreatic locations, a relatively wide spectrum (and possibly a continuum) of neuroendocrine differentiated tumors exist including tumors with well-represented (>30% of tumor area) neuroendocrine cell component and tumors with scattered neuroendocrine cells only [1, 31, 49, 51, 53, 62].

With regard to the criterion of neuroendocrine component extension within the tumor, the "rule" of at least 30%

of neuroendocrine differentiated areas, mentioned in the 2000 WHO classification of endocrine tumors [63], allows to consider as true MEECs those tumors with a well-represented or significant neuroendocrine cell population only. On the other hand, however, no reasonable explanation is provided for this limit from both a pathogenetic and histogenetic point of view. By morphology, MEECs are the result of intermingling of frankly glandular areas with typical organoid neuroendocrine areas (in the case of a well-differentiated tumor) or classical small cell carcinoma areas. The criterion of the structural pattern in the definition of MEEC is relevant to allow separation of conventional adenocarcinoma with a less represented neuroendocrine cell population randomly spread in the exocrine tumor, in the absence of carcinoid-like or small cell areas (see above).

MEEC can be encountered virtually at all sites, with either common or peculiar aspects according to the site of origin. Some of the main pathological features of MEECs according to their localization are summarized below.

MEEC in the pancreas, gastrointestinal, and biliary tract The WHO classification of endocrine tumors has incorporated MEEC in the section on endocrine tumors of the pancreas and briefly commented these forms in the paragraphs on stomach and appendiceal endocrine tumors [63]. In the pancreas and stomach, these were defined as epithelial malignant tumors characterized by a combination of a predominant exocrine component and a neuroendocrine cell subpopulation represented by at least one third of the tumor area [10, 23, 63]. This same definition has been applied in the 2004 classification of tumors of endocrine organs, concerning pancreatic endocrine tumors [10]. In the pancreas, MEEC include mixed ductal–endocrine carcinoma and mixed acinar–endocrine carcinoma. Exocrine tumors, in which the neuroendocrine component, is represented by scattered individual cells constituting mostly a tumor-associated but non-neoplastic cell population, were not defined as truly mixed exocrine–endocrine tumors [37]. As for most other locations, mixed ductal–endocrine and mixed acinar-endocrine carcinomas are exceptionally rare [37]. Mixed ductal-endocrine carcinomas account for approximately 0.5% of all ductal adenocarcinomas [49] and 15% of all acinar carcinomas of the pancreas [36]. As for conventional pancreatic carcinomas, the majority of mixed ductal–endocrine carcinomas occur in the head of the pancreas [37, 49], while acinar–endocrine carcinomas are equally divided between the head and tail [36, 50]. Endocrine-related symptoms may be present, and a case with hypergastrinemia has been reported [69].

In the case of ductal–endocrine carcinoma, the exocrine component of these tumors is represented by moderately to poorly differentiated ductal or glandular structures or mucinous (colloid) carcinomatous structures, whereas the neuroendocrine component can be well or poorly differentiated. The well-differentiated neuroendocrine component is represented by solid, trabecular, or acinar structures formed by small- to medium-sized cells, positive for neuroendocrine markers and negative for mucin stains. The cell population and growth pattern of the poorly differentiated neuroendocrine component are similar to those of small cell carcinoma of the lung [49].

In many mixed acinar–endocrine carcinomas, the two different tumor components can be convincingly identified only with immunohistochemical stainings. In some cases, the acinar component can be recognized at morphological level because of the organization of neoplastic cells in small glandular units and tintorial properties different from the surrounding neoplastic component. The neuroendocrine component, on the contrary, consists most frequently of solid nests or trabeculae of cells with randomly oriented nuclei and amphophilic cytoplasm.

Metastases affect the liver and regional lymph nodes, although distant spread to other organs is reported in some cases [36, 69]. The behavior of mixed exocrine–endocrine carcinomas is dictated by the exocrine component; so duct–endocrine carcinomas have the same poor prognosis as ductal adenocarcinomas and the same is true for mixed acinar–endocrine carcinomas [36, 37].

Among the variety of MEEC in the gastrointestinal tract (Fig. 3), variable combination of morphological aspects have been reported, and an attempt to list all reported cases would fail to be comprehensive. In the stomach peculiar aspects include cases showing at immunohistochemical and ultrasctructural levels the presence of amphicrine features [77], also in liver metastases [57], and pancreatic-like acinar differentiation in the exocrine component [16, 31]. As well, in the small and large intestine MEEC cases have been documented, showing both well-differentiated [32] and poorly differentiated features [61, 71] of the neuroendocrine component. Gallbladder carcinomas with combined adenosquamous and large-cell neuroendocrine features have also been reported [48]. MEEC may also occur in the ampullary region, and we recently observed a case located in the periampullary region predominantly in the submucosa and extending to the main pancreatic duct, showing two distinct architectural patterns intermingled within the same lesion, composed of a predominant large cell neuroendocrine carcinoma component admixed with moderately differentiated neoplastic glandular structures. The presence of both neoplastic counterparts was evident at preoperative brushing cytology, which displayed the coexistence of mucin-laden cells and synaptophysin positive cells (Fig. 4). The patient is currently alive with disease 11 months after surgery.

Lung A small proportion of neuroendocrine lung tumors, in most instances poorly differentiated carcinomas of the small and large cell types, may grow in association with

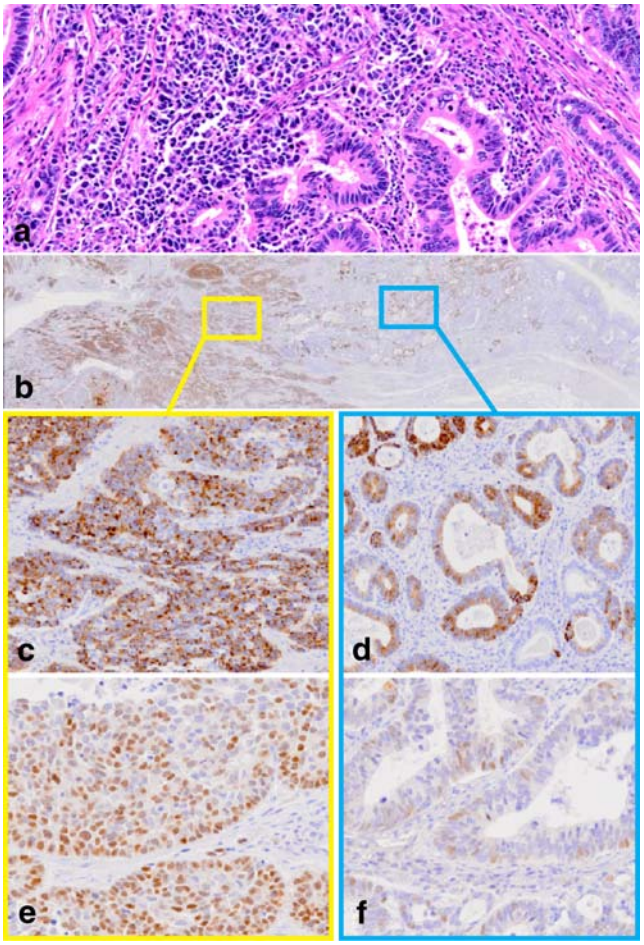

Fig. 3 MEEC of the stomach, with poorly differentiated-large cell-neuroendocrine component (**a**, left) and adenocarcinoma (**a**, right) features. Chromogranin A (**b**) was positive in the neuroendocrine component (**c**) but also in adenocarcinoma glands close to the neuroendocrine areas (**d**). Human ASH-1, a transcription factor involved in neuroendocrine differentiation, paralleled chromogranin A immunohistochemical pattern (**e** and **f**, neuroendocrine and adenocarcinoma components, respectively) (original magnifications: **a**, **e**, and **f** 400×; **b** 20×; **c** and **d** 200×)

squamous cell carcinomas and adenocarcinomas, or, less frequently, with sarcomatous tumors. According to the WHO classification of lung tumors [70] they are classified under the term "combined carcinomas". The morphology of such tumors, as well as their clinical behavior, recapitulate the specific features of the individual components, and this tumor group is described in detail elsewhere in the current issue [60].

Thymus A variety of tumors including neoplasms showing neuroendocrine and thymic epithelial features, representing less than 1% of all thymic tumors, has been introduced in the WHO classification of thymic tumors [70]. The neuroendocrine component may be represented by either thymic carcinoid (usually atypical) or small cell carcinoma and the thymic epithelial component by variable thymoma/

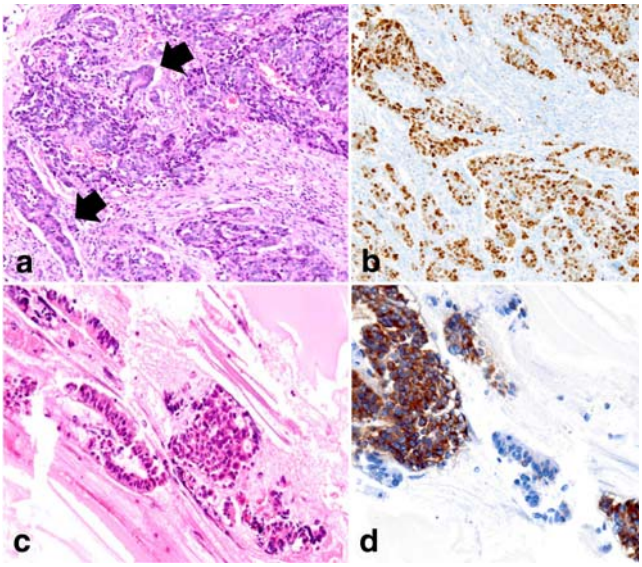

Fig. 4 MEEC of the periampullary region, with poorly differentiated-large cell-neuroendocrine component (**a**), highly proliferating by ki-67 immunohistochemistry (**b**), admixed with adenocarcinoma features (**a**, arrows). The lesion was preoperatively diagnosed by endoscopic brushing sampling and displayed in the cytological specimen the presence of a double component, made of glandular structures and aggregates of medium to large-sized cells (**c**). The latter is positive for synaptophysin (**d**) (original magnifications: **a** and **b** 200×; **c** and **d** 400×)

thymic carcinoma types [44, 46, 58]. Ectopic hormone (i.e., ACTH) secretion in thymic neuroendocrine tumors is quite common, and, although not reported in the literature, it should be expected also in thymic mixed neuroendocrine nonneuroendocrine neoplasms. Mixed neuroendocrine carcinoma-thymoma should not be confused with conventional thymic carcinoma which may display focal areas of neuroendocrine differentiation in a large proportion of cases [26, 39, 40].

Thyroid Peculiar endocrine–neuroendocrine differentiation in thyroid carcinoma occurs in the so-called mixed medullary–follicular carcinomas (MMFC), which are defined as tumors showing both the morphological features of medullary carcinoma together with immunoreactive calcitonin, and the morphological features of follicular (or papillary) carcinomas together with immunoreactive thyroglobulin [10]. MMFC are very rare tumors, accounting for less than 0.2% of all thyroid tumors [34, 56]. As for conventional medullary carcinoma, MMFC may arise as sporadic tumors, in the vast majority of reported cases, or associated with MEN2 syndrome [38, 43, 47, 55], both type 2A and type 2B. MMFC is an extremely heterogeneous group of tumors in terms of morphological features. The vast majority of cases present as a predominant medullary carcinoma admixed with follicular-derived structures, in a variable degree of extension, atypia, and differentiation. The medullary component generally comprises 70–80% of the tumor, and the follicular derived cell

population is predominant in very few cases. No specific morphological features have been reported on the medullary carcinoma side, whereas the follicular population may be represented by any follicular cell-derived carcinoma entity. The spectrum of morphological pictures of the follicular counterpart, in fact, comprise single follicles admixed in an otherwise classical medullary carcinoma, which should be present deep into the tumor and display cytological atypia (to differentiate them from entrapped follicles at the periphery of a conventional medullary carcinoma), or cluster of cells arranged both in follicular or papillary structures presenting the diagnostic nuclear features of papillary carcinoma. Other morphological patterns of growth in the follicular component include oxyphilic carcinoma, poorly differentiated solid/trabecular carcinoma, and anaplastic carcinoma [56], as well as amphicrine tumors with calcitonin and mucin coproduction [20]. Immunohistochemistry (mainly thyroglobulin and calcitonin) is helpful to highlight the two different components, although the diagnosis relies mostly on morphology.

Their clinical behavior is not different from classic medullary carcinoma, although it has to be remembered that metastatic cases may present both components in the secondary localizations, thus supporting the evidence that a combination of therapeutic strategies (including radioiodine) has to be considered in such cases.

Urogenital tract In the prostate, apart from the prognostically relevant neuroendocrine differentiation in conventional adenocarcinoma (described above), pure neuroendocrine neoplasms may be present, both well differentiated (carcinoids) and poorly differentiated (small and large cell carcinomas). The former are almost invariably pure neuroendocrine neoplasms, whereas in up to 50% of small cell carcinomas an adenocarcinoma component may be present [11], thus representing mixed neuroendocrine and nonneuroendocrine tumor entities. The frequency of such finding strongly suggests a common origin of the two different tumor components.

The same holds true for the urothelial tract, which may very rarely develop pure well differentiated neuroendocrine tumors, and more frequently poorly differentiated small cell carcinomas (usually in the urinary bladder) [11]. Again, in about 50% of cases, areas of nonneuroendocrine urothelial carcinoma are mixed within the lesion, thus representing mixed neuroendocrine and nonneuroendocrine tumors. A contradictory issue, however, is based on the agreement that the diagnosis of small cell carcinoma of the bladder could be addressed even in the absence of immunohistochemical evidence of neuroendocrine differentiation (therefore just based on morphological ground).

Skin Primary Merkel cell-neuroendocrine carcinoma of the skin, may present biphasic differentiation with squamoid, eccrine, or even melanocytic components. The association with squamous cells carcinoma has been frequently described as synchronous closely intermixed tumors or metachronous lesions [4]. Furthermore, cases of Merkel cell carcinoma have been described to contain glandular, melanocytic, striated muscle, and lymphoepithelioma-like features [8, 12, 14, 55]. These latter findings further support the hypothesis of tumors showing divergent differentiation. As also stated for other sites, these entities should be separated from nonneuroendocrine tumors showing neuroendocrine differentiation, as frequently observed in basal cell carcinoma [18] or sweat gland apocrine and eccrine carcinomas [67].

Molecular findings

Few reports in the literature have appeared addressing the histopathogenetic issue of mixed neuroendocrine/nonneuroendocrine tumors, and mostly reported heterogeneous and controversial data, thus making the different histopathogenetic hypothesis still open.

The genetic background of goblet cell carcinoids has been explored in few studies. By gene expression analysis, differential expression of genes involved in adhesion, metastasis and apoptosis were found in goblet cell carcinoids and malignant carcinoids, as compared to classic carcinoids discovered as incidental findings [45].

A rather higher frequency of 11q (25%), 16q (38%), and 18q (56%) losses was found in goblet cell carcinoids of the appendix as compared to other gastrointestinal carcinoids; by contrast, no mutations of K-ras, beta-catenin, or DPC4 genes were detected in any case [64]. In another study, p53 mutations were found in 25% of cases, as compared to 44% of classic carcinoids [59].

In gastrointestinal MEEC, genetic studies applying alternative techniques (loss of heterozygosity, mutational analysis, clonality analysis, and others) have been reported. A common genetic origin of the two tumor components have been proved by most of them [17, 29, 35, 61, 75] especially in those cases in which the neuroendocrine component is represented by poorly differentiated small or large cell carcinoma, whereas rebutted by others [71].

Detailed genetic studies on mixed exocrine–endocrine carcinomas of the pancreas have never been reported so far. No somatic or germline point mutations in the MEN1 gene were detected in the neuroendocrine component of four cases we analyzed (Volante M and Bussolati G, unpublished observation).

Concerning MMFC, very few cases reported in the literature were analyzed for the presence of specific genetic alterations. Excluding the patients affected by known MEN2 syndromes, somatic point mutations in the *RET* protoonco-

gene have been detected in some sporadic tumors [52, 72]. The majority harbored the M918T mutation in exon 16, but an A883F point mutation in exon 15 was also detected in a single case. In the larger reported series [72], the percentage of somatic *RET* point mutations was similar to that of conventional medullary carcinoma (25%, 3/12 cases). In this same study, the genetic analysis on the two different components, isolated by means of laser-based microdissection, revealed that somatic *RET* mutations were restricted to the medullary counterpart. Moreover, the analysis of three female patients by means of the HUMARA method showed differential clonal patterns in the medullary and follicular-derived tumor components, thus disproving the hypothesis of a common progenitor stem cell.

Concluding remarks

The group of mixed neuroendocrine and nonneuroendocrine neoplasms includes rare tumors, located in an intermediate position between pure neuroendocrine and nonneuroendocrine tumors, virtually present in any location, and which display highly heterogeneous morphological patterns. In general terms, to achieve their diagnosis, the two components—neuroendocrine and nonneuroendocrine—should be recognized at morphological level and both components should be present in a tumor cell population representing at least one third of the whole lesion. Such definition separates these forms from exocrine tumors, which present focal areas of neuroendocrine differentiation, recognizable exclusively by immunohistochemical stainings for neuroendocrine markers and in less than one third of the tumor.

The histogenesis of mixed neuroendocrine/nonneuroendocrine tumors is not completely understood, being molecular data either proving or disproving a unique origin of the two components from a common progenitor cell. Still controversial is also the clinical meaning of their recognition, although their clinical behavior is generally related to the more aggressive component.

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Conflicts of interest statement We declare that we have no conflict of interest.

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REVIEW AND PERSPECTIVE

Treatment of gastroenteropancreatic neuroendocrine tumors

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Abstract Neuroendocrine tumors are rare; thus, individual experience with the diagnosis and treatment of these tumors is mostly low, except in specialized centers. For histological diagnosis, standards have been described recently. Pathological classification and clinical staging influence diagnostic and therapeutic decisions. This chapter aims at demonstrating the importance of pathological and clinical classification of neuroendocrine tumors on therapeutic decisions, indicating the appropriate therapy for different stages of the disease. Surgical therapy will be discussed shortly, including palliative surgical strategies. However, the focus of the manuscript is medical therapy. Biotherapy, its effects, and remaining uncertainties are presented as well as different chemotherapeutic schemes. Finally, new options of palliative medical therapies like kinase inhibitors and anti-angiogenetic drugs will be discussed.

Keywords Gastroenteropancreatic neuroendocrine tumors · Biotherapy · Somatostatin analogue · Interferon · Chemotherapy · Streptozotocin · Tyrosine kinase inhibitor · Anti-angiogenic therapy

Introduction

Neuroendocrine tumors are classified according to their differentiation, localization, and functionality. The pathological classification and the stage of the tumor disease have important prognostic implications and, thus, influence therapeutic decisions. This chapter starts with a brief discussion of surgical options, then focuses on the established indications of bio- and chemotherapy in gastroenteropancreatic neuroendocrine tumors of the foregut and midgut. Finally, the evolving new therapeutic options are discussed.

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Surgery

Surgery is the only curative therapy available in gastroenteropancreatic neuroendocrine tumors. Unfortunately at diagnosis, most tumors have already metastasized, and thus, surgery is rarely curative. Surgery is rarely indicated in patients with small (<1 cm) gastric neuroendocrine tumors type 1 or type 2, due to the mostly benign course of the disease. In contrast, oncological resection of the tumor is imperative in malignant type 3 gastric neuroendocrine tumors [53, 54, 58]. Duodenal and pancreatic primaries are both indications for surgical therapy. However, palliative surgery of the primary, in patients with inoperable hepatic metastasis, has no positive effect on survival. It has been suggested, that palliative surgery of hepatic metastases, either in combination with surgical therapy for the primary or following R0-resection of the primary tumor, does prolong survival in patients with pancreatic or midgut neuroendocrine tumors. All series so far are, however, retrospective analysis of monocentric experiences, and selection bias has to be taken into account. Surgical intervention for hepatic metastasis, without removal of the primary, is not recommended. In general, surgical therapy adheres to oncological principles [12, 22, 30, 31, 49, 53]. However, for appendiceal neuroendocrine tumors due to the excellent prognosis of small (<2 cm) tumors, appendectomy is now considered curative, and right hemicolectomy is indicated only in tumors larger than 2 cm with deep mesoappendiceal invasion [53].

Further, more palliative treatment options such as radiofrequency ablation, chemo-embolization of liver metastases, and peptide radio-receptor therapy have been reviewed recently and will not be discussed here [53].

Medical therapy

Biotherapy can be used as a symptomatic treatment in patients with functioning neuroendocrine tumors, i.e., in patients with the carcinoid syndrome, watery diarrhea-, or glucagonoma syndrome. Symptomatic therapy for insulinomas is rarely necessary, as most of these tumors are benign, and thus, surgery is curative. The objective of symptomatic biotherapy is to reduce signs, symptoms, and complications of hormone hypersecretion syndromes and, thus, to increase the quality of life and overall survival. In addition, biotherapy has been used as an antiproliferative treatment in slow-growing malignant, well-differentiated (World Health Organization classification), metastasized neuroendocrine carcinomas. While tumor regression is rare, stabilization of tumor growth has been demonstrated.

Most gastroenteropancreatic neuroendocrine tumors present with malignant, metastatic disease. Chemotherapy is a palliative option in both slow-growing, well-differentiated, or rapidly proliferating, poorly differentiated, neuroendocrine tumors. In slow-growing, well-differentiated tumors, tumor growth is unpredictable, and slow tumor progression may alternate with long intervals of stable disease. As the quality of life is good in most patients with metastasized well-differentiated neuroendocrine tumors, antiproliferative therapy should only be initiated whenever progressive disease has been demonstrated according to standard criteria. While different treatment regimens have been shown to be effective in well-differentiated pancreatic neuroendocrine carcinomas, chemotherapeutic options for tumors of the small bowel are poor. Localization of the primary is not as important for poorly differentiated carcinomas, as these rapidly growing tumors respond to different chemotherapeutic agents, irrespective of the localization of the primary. These tumors may grow rapidly, and medical treatment should not be withheld to demonstrate progressive disease.

Biotherapy

Biotherapy is defined as the therapy for hormonal hypersecretion syndromes and/or tumor growth with substances or pharmacological derivatives thereof occurring naturally in the body. Despite the widespread therapeutic use of biotherapy in neuroendocrine tumors, data fulfilling the criteria of evidence-based medicine are rare. The interpretation of study results has to be done with some caveats. Data referring to the therapeutic efficacy of biotherapy only rarely give primary endpoints like mortality or the time to progression. Most studies include a variety of neuroendocrine tumors, the number of patients is low, and most studies represent single center experience. The results of different studies are difficult to compare, as dosage, treatment duration, and a variety of different pretreatments were employed. There are only few prospective, randomized multicenter studies in therapy-naïve patients with documented progress before initiation of biotherapy. No placebo group was ever included in these studies. However, despite these drawbacks, there are indications of the benefit of biotherapy on symptoms of hormone hypersecretion, while on the other hand, definite data on the antiproliferative effectiveness and its positive effect on survival are still lacking.

Somatostatin analogues (SSA)

Endogenous somatostatin (SS) circulates in two biological active forms, i.e., SS-14 and SS-28. SS binds with high affinity to five G protein-coupled membrane receptors (sst1-5). Ligand binding inhibits adenylate cyclase activity, reduces calcium influx, and negatively influences hormone synthesis and secretion. An inhibitory influence on proliferation may be due to the activation of phosphotyrosine phosphatases, the mitogen-activated protein kinase activity [38], and to the inhibition of the transcription factor complex activator protein 1. In vitro, high doses of SSA induce apoptosis in tumor cells, and this could translate into inhibition of tumor growth. Additional antiproliferative effects may be related to the anti-angiogenic activity of SS either directly or via inhibition of growth factors [11]. Most neuroendocrine tumors express a higher density of sst in tumor tissue compared to the normal tissue. This allows for specific, tumor tissue-targeted, therapeutic effects and should reduce the number of side effects, i.e., suppression of physiologically secreted hormones. However, sst-subtype expression varies considerably between different tumor types and among tumors of the same type. Even within a given tumor, sst expression is not homogenously distributed [27].

The clinically used SSA, octreotide and lanreotide, preferentially bind to sst2 and sst5 (Table 1). For these analogues, serum half-life is increased considerably compared to native somatostatin. The subcutaneous injectable octreotide has to be given three times daily, while long-acting preparations, (lanreotide long-lasting and octreotide long-acting repeatable [LAR]), allow for one intramuscular injection every two (lanreotide) to four (octreotide LAR) weeks. In the case of lanreotide autogel, an interval up to 6 weeks between injections and the possibility to be injected by the patients themselves, may increase patients' comfort and compliance. Recently, pasireotide has been introduced, a

Table 1 Binding affinities of somatostatin analogues to somatostatin receptor (sst) subtypes

sst	SS-14	Octreotide	Lanreotide	Pasireotide
sst1	0.93±0.12	280±80	180±20	9.3±0.1
sst2	0.15±0.02	0.38±0.08	0.54±0.08	1.0±0.1
sst3	0.56±0.17	7.10±1.40	14±9	1.5±0.3
sst4	1.40±0.40	>1,000	230±40	>100
sst5	0.29±0.04	6.3±1.0	17±5	0.16±0.1

Binding affinities are given as mean±SEM IC-50 (nmol/l)

SSA with high affinity for sst1-3 and sst5. It has been shown to be effective in patients who do not respond to the currently available SSA octreotide and lanreotide. However, its use is still restricted to clinical studies.

Octreotide and lanreotide both effectively inhibit autonomous hormone or neurotransmitter secretion by neuroendocrine gastrointestinal tumors. Unfortunately, tachyphylaxis develops after months or even years of treatment in virtually all patients. Tachyphylaxis may be due to desensitization, homologous agonist-induced down-regulation in sst numbers on the cell surface, heterologous regulation of SS receptor expression, or even SS receptor gene mutations [27].

Indications for SSA therapy

SSA are indicated in patients with symptoms due to excessive, autonomous hormone release by a neuroendocrine tumor or its metastases. In patients with the carcinoid syndrome, octreotide LAR is equally potent in the control of flushing and diarrhea. SSA are indicated in the therapy of the watery diarrhea syndrome, reducing the secretion of vasoactive intestinal peptide, and thus, diarrhea, dehydration, and electrolyte imbalance. In patients with insulin hypersecretion, SSA may reduce the insulin concentration in tumors expressing sufficient sst2 or sst5, i.e., mostly malignant insulinomas. However, as SSA inhibit glucagon secretion as well, patients have to be observed closely at the beginning of therapy to prevent severe hypoglycemia due to the reduced glucagon-dependent counter-regulation. SSA effectively inhibit glucagon secretion in patients with a glucagonoma syndrome; skin lesions improve, and catabolism is reduced (Table 2).

SSA induce remission and/or stabilization of tumor markers in approximately 70% of the patients [46] (Table 3). The decline of tumor markers, like chromogranin A, is due to the anti-secretory effect of SSA and should not be interpreted as evidence for tumor volume reduction. Tumor shrinkage is demonstrated in less than 10% of the patients. However, stabilization of tumor growth, after computer tomographic-documented progression before treatment, occurs in up to 50% of the patients with neuroendocrine tumors of various locations. (Table 4). The median duration of stabilization was 18–26.5 months [3]. In a highly selected group of patients with progressive disease, forty-seven percent of the patients demonstrated at least stable disease when treated with a high dose of lanreotide (3×5 g/day). This was confirmed recently in 75% of the patients with advanced midgut carcinoids, with stabilization for 6 to 24 months. There are no good predictors of the clinical outcome of SSA therapy. Patients achieving a positive response (stabilization) after 6 months of treatment maintain it throughout long-term follow-up and live longer than patients unresponsive to therapy [50]. Frequently occurring side effects like abdominal discomfort, bloating, and steatorrhea due to the inhibition of pancreatic enzymes are mostly mild and subside spontaneously within the first weeks of therapy. Persistent steatorrhea can be treated with supplementation of pancreatic enzymes. Cholestasis with subsequent cholecystolithiasis does occur in up to 60% of the patients due to inhibition of cholecystokinin and production of lithogenic bile. Prophylactic therapy with chenodeoxy–cholic acid and ursodeoxy–cholic acid may be able to prevent the occurrence of gallstone disease in patients on long-term SSA therapy. Serum vitamin B12 concentration may decline, possibly due to a direct inhibition of the intrinsic factor secretion at the parietal cell [52].

In summary, SSA effectively control symptoms of hypersecretion in patients with neuroendocrine tumors of the gastrointestinal tract. Despite the minor effects on tumor volume reduction observed so far, an antiproliferative effect does occur, with stabilization of the disease for up to

Table 2 Effects of somatostatin analogues on hypersecretion syndromes

Syndrome	Symptom	Hormone/ neurotransmitter	Tumor marker
Carcinoid syndrome	Flush≥diarrhea	Serotonin	5-HIAA, CgA
Watery diarrhea syndrome	Diarrhea, dehydration, acidosis	VIP	VIP
Glucagonoma syndrome	Migratory necrolytic erythema	Glucagon	Glucagon
Insulin hypersecretion[a]	Fasting hypoglycemia	Insulin	Insulin
Zollinger–Ellison syndrome	Peptic ulceration, GERD	Gastrin	Gastrin

5-HIAA 5-Hydroxyindol-acetic acid; *GERD* gastro-esophageal reflux disease

[a] Benign insulinomas rarely express sufficient sst2 and 5 for SSA to be effective. Thus, SSA are only indicated as second-line therapy in malignant insulinomas (see text)

Table 3 Biochemical effect of somatostatin analogues

N	CR	PR	SD	PD	Author
23	0	9	5	9	[2]
39	2	11	15	11	[3]
13	4	6	3	9	[15]
14	0	9			[4]
89	6/89 (7%)	35/89 (39%)	23/75 (31%)	29/75 (39%)	

CR Complete response; *PR* partial response; *SD* stable disease; *PD* progressive disease

25 months. Survival may be prolonged in those patients responding positively to SSA therapy. In addition, SSA significantly increase the quality of life in patients with symptoms related to hormone secretion, while side effects of SSA therapy are limited.

Interferon

Interferon-α 2a, or 2b (IFN) production is a physiological response to substances as microbes, tumor cells, and antigens. IFN react with specific cell surface receptors to activate a cytoplasmatic signal transduction cascade, inducing the transcription of multiple IFN inducible genes, which act as tumor suppressor genes. IFN-α acts on 2′ 5′-A-synthetase and p-68 kinase. Both enzymes induce the degradation of peptide hormone and growth factor messenger RNA, inhibiting protein synthesis. The induction of 2′ 5′-A-synthetase correlates with clinical efficacy. The antiproliferative effect of IFN is probably due to a blockade of the cell cycle in the transition of $G_0 \rightarrow G_1$ [45]. This is due to the inhibition of cyclin B expression, resulting in reduced CDC 2 kinase activity and, thus, inhibition of the cell cycle [13]. Furthermore, induction of apoptosis, as well as increased expression of class I antigens on the tumor cell surface (which marks the cell as a target for cytotoxic T lymphocytes) may add to the antiproliferative effects. In addition, an anti-angiogenic effect has been suggested.

IFN-α has been widely used for the treatment of solid tumors. In neuroendocrine tumors, the indications for IFN are comparable to those of SSA, with carcinoid crisis being the exception. However, there are only few data on the effect of IFN therapy in patients with pancreatic neuroendocrine tumors. Most investigations used recombinant IFN-α 2a or 2b.

Indications for IFN-α therapy

Symptomatic remission is seen in 30–70% of the patients with carcinoid syndrome, with a better effect of IFN therapy on flushing compared to diarrhea. While the control of symptoms of hypersecretion by IFN is comparable to SSA, its onset of response is delayed. A biochemical response is observed in 50% of the patients. Tumor marker remission or stable 5-HIAA concentration occurred in 36 and 35% of the patients, respectively. Analyzing results of ten clinical studies with mixed tumor populations (N=255), a partial remission or stabilization of tumor markers occurs in 44 and 30% of the patients, respectively. These data are comparable to a recent meta-analysis, with median response rates of biochemical markers in up to 44% of the patients

Table 4 Antiproliferative effect of somatostatin analogues in patients with progressive disease and in patients without documented progression

SSA	*N*	CR	PR	SD	PD	Author
In patients with progressive disease						
Lanreotide	22	0	1	7	14	[23]
Lanreotide	35	0	1	20	14	[2]
Octreotide	52	0	0	19	33	[5]
Octreotide	58	0	2	27	29	[15]
Octreotide	10	0	0	5	5	[3]
Lanreotide	24	1	1	11	11	[23]
	201	1 (0.5%)	5 (3%)	89 (44%)	106 (53%)	
In patients without documented progression						
Lanreotide	31	–	2 (7%)	25 (81%)	4 (13%)	[68]
Lanreotide	39	–	4 (10%)	19 (49%)	16 (41%)	[18]
Lanreotide	19	–	1 (5%)	12 (63%)	6 (32%)	[20]
Lanreotide	18	–	–	14 (78%)	4 (22%)	[66]
Octreotide	16	–	–	14 (88%)	2 (12%)	[65]
Octreotide	15	–	1 (7%)	6 (40%)	8 (53%)	[56]
Octreotide/lanreotide	13	–	4 (31%)	1 (8%)	8 (61%)	[1]
	183	0 (0%)	12 (8%)	91 (60%)	48 (32%)	

[60]. Tumor shrinkage occurs in 10% of the patients, whereas stable disease is observed in up to 70%. Progressive disease was seen in 23% of the patients. Table 5 gives data on a large cohort of patients with evaluable results on tumor mass (N=274) treated with IFN in ten studies. The median survival was >80 months. Again, the data should be interpreted with caution. Information on spontaneous tumor growth is lacking in most of these studies. Patients with different pretreatment modalities consisting of surgical interventions, embolization therapy, and/or chemotherapy have been included. In addition, the dose regimen, the type of IFN-α (rIFN-α2a, rIFN-α2b, human leukocyte IFN), and treatment time differed considerably between the studies. In these slow-growing tumors, changes might only be obvious after long treatment periods (up to 30 months). No randomized, prospective multicenter studies have been performed. Endpoint analysis, i.e., overall survival or time to progression, is given in about one third of the trials. Overall, results of these investigations delineate a consistent pattern of efficacy for IFN on symptom control.

In almost all patients (97%) a "flu-like" syndrome occurs in the first 5 days. Anorexia, weight loss (59%), and fatigue (51%) may adversely affect well-being. Bone marrow toxicity like anemia, leukocytopenia, and thrombocytopenia have been observed in 31, 7, and 18% of the patients, respectively, as well as hepatotoxicity (31%). These effects are dose dependent. Autoimmune reactions occur in 20% of the patients [46]. Rare side effects are depression, mental disturbances, and visual impairment.

In summary, IFN therapy is primarily given in patients with metastasized neuroendocrine tumors of the gut. The effect on symptoms of hormone hypersecretion is comparable to SSA, while the onset of response is delayed compared to SSA. IFN treatment will not cure the disease; however, it may be able to control tumor growth over extended periods, the smaller the tumor burden, the more so. Thus, IFN should be given rather early in the course of the disease. Side effects of IFN therapy are more pronounced than with SSA. Individualized doses allow a reasonable quality of life.

Table 5 Antiproliferative effects of interferon therapy

Interferon	Evaluable pats.	CR	PR	SD	PD	Author
IFN	14	–	–	9	5	[17]
IFN-α	20	–	–	15	5	[64]
IFN-α	15	–	3	NI	NI	[16]
rIFN-α	20	–	4	NI	NI	[44]
rIFN-α	12	–	2	9	1	[29]
rIFN-α2b	17	–	–	16	NI	[47]
rIFN-α2b	14	–	–	10	NI	[61]
rIFN-α2b	26		4	17	NI	[59]
rIFN-α2b	25	–	–	16	9	[28]
hIFN/ rIFN-α	111	–	16	74	21	[48]
Total	274		29/ 274	166/ 239	41/ 182	
Percent			11%	70%	23%	

CR Complete remission; *PR* partial remission; *SD* stable disease; *PD* progressive disease

Combination therapy: SSA plus IFN-α

The combination of SSA and IFN-α was used in an effort to enhance the antiproliferative effect of IFN therapy, to add the positive effect of SSA on hypersecretion syndromes, and to reduce the dose of IFN-α and, thus, the number of IFN-related side effects.

Earlier studies gave contradictory results showing either no additional or an increased antiproliferative effect with combination therapy. However, a recent, well-designed prospective multicenter study showed no advantage of combination therapy, neither on biochemical nor antiproliferative results, while the number of side effects increased [23]. Thus, combination therapy is not recommended as a standard treatment regimen.

In summary, biotherapy is preferentially indicated for the treatment of hormone hypersecretion syndromes in patients with neuroendocrine tumors. Antiproliferative effects are not convincing, but stabilization of the disease does occur in up to 50% of the patients with either SSA or IFN therapy. Combination treatment does not provide any additional effect and is not recommended.

Systemic chemotherapy

Chemotherapy is a palliative option in metastasizing neuroendocrine carcinomas. Streptozotocin (STZ), fluorouracil (5-FU), doxorubicin, dacarbazin (DTIC), etoposide, and cisplatin have been used. As the response rate to monotherapy has been low, most chemotherapeutic regimens for neuroendocrine tumors rely on combination therapy.

STZ is an alkylating nitrosurea compound. STZ enters the pancreatic β cell via the GLUT2 glucose transporter. However, the molecular mechanism of cytotoxicity is still unknown. STZ is effective in the treatment of neuroendocrine tumors of the pancreas. It is used in combination with either 5-FU or doxorubicin. STZ induces nausea and vomiting in up to 90% of patients, and glomerular and tubular dysfunctions occur in 20–75%. Bone marrow toxicity is low; thus, combinations with 5-FU or doxorubicin are possible.

5-FU is a pro-drug and needs to be metabolized for antineoplastic action. Its metabolites inhibit thymidilate synthetase, thus blocking DNA synthesis. In addition, RNA

synthesis is reduced. 5-FU preferentially inhibits proliferating cells, resulting in bone marrow toxicity and gastrointestinal side effects. Patients with coronary heart disease or cardiomyopathy are at risk for cardiotoxic side effects.

Doxorubicin is supposed to interact with DNA base pairs, and this may result in steric inhibition of DNA synthesis. Additional antineoplastic actions are possibly due to the formation of free radicals, an effect on tumor cell membranes and the inhibition of topoisomerase II activity. Myelosuppression is a dose-limiting side effect. Immediate, reversible, or irreversible cardiotoxicity has been observed. Nausea and vomiting are seen in up to 80% of the patients.

DTIC has cytostatic effects and inhibits the cell cycle. Additional effects are reduction of the DNA synthesis and alkylating effects. Therapy with dacarbazin can induce a rare veno-occlusive syndrome. However in most patients, toxicity is low, and the side effects experienced are mostly nausea and vomiting.

Cisplatin is an alkylating agent and, thus, interferes with DNA replication. Nausea and vomiting occur in up to 75% of the patients, myelosuppression is usually mild to moderate, with high-dose therapy. Nephrotoxicity is possible, peripheral neuropathy is common and is dose and duration dependent.

Etoposide, a podophyllotoxin derivative, is used in combination with cisplatin for poorly differentiated NET. Cytotoxicity is due to breaks of DNA strands. Etoposide interacts with topoisomerase II. The inhibition of the cell cycle during S and G2 phase is cytostatic. Side effects like nausea, vomiting and diarrhea, myelosuppression, and alopecia are common.

In neuroendocrine tumors of the pancreas, overall response rates of 17, 18–26, and 21% were obtained with monotherapy using STZ, 5-FU, and doxorubicin, respectively. Combination therapy for well-differentiated, neuroendocrine tumors of the pancreas, on the other hand, resulted in a median response of 36%. Median remission lasted 17 months, while median overall survival was almost 2 years (Table 6). The combination of STZ and 5-FU (N= 147) resulted in an objective response in 21% of the patients, with a median survival time from the start of therapy of less than 8 months. A recent randomized prospective trial (N=163) failed to confirm the suggested superiority of STZ and doxorubicin over STZ and 5-FU [62]. Objective remission was 16% for both arms, progression-free survival was 4.5 and 5.3 months, respectively. Overall survival was higher with STZ/5FU (24.3 vs 15.7 months, p<0.03), arguing for the less toxic approach with STZ/5-FU. Other combination schemes like dacarbazin, 5-FU and leucoverin (response rate 27%), dacarbazin, 5-FU and epirubicin (response rate 30%), lomustine and 5-FU (response rate 21%) were comparable. In a recent trial, 84 patients with pancreatic neuroendocrine tumors were treated with STZ, 5-FU, and doxorubicin [33]. This triple combination achieved a response rate of 39% and a median progression-free survival of 9.3 months, confirming earlier data in small investigations [57, 67].

In contrast, chemotherapy is less effective in neuroendocrine tumors of the small intestine. An overview over ten trials (1979–2005) indicates a median response rate of 25% and a median survival of 11 months (Table 7). Thus, in well-differentiated neuroendocrine tumors of the gut, chemotherapy is not a preferred option.

In contrast, in 18 patients with undifferentiated, anaplastic, neuroendocrine tumors (five tumors were of midgut/hindgut origin), 67% objective responses were obtained [43] with a median duration of remission of less than 8 months.

In interpreting these data, it has to be kept in mind that most studies comprise only a small number of patients; the patients usually are heterogeneous groups with respect to the localization of the tumor, total tumor burden, and pretreatment schemes.

In summary, combination therapy with STZ, 5-FU, with the possible addition of doxorubicin, are now standard treatment schedules in patients with well-differentiated neuroendocrine tumors of the pancreas. For neuroendocrine tumors of the small bowel, there is still no convincing evidence for improved survival with chemotherapy, and thus, other palliative options should be discussed. However, in patients with anaplastic neuroendocrine carcinomas, the

Table 6 Chemotherapy for well-differentiated neuroendocrine pancreatic tumors

Chemotherapy	*N*	Response (%)	PFS (month)	Median survival	Author
STZ	42	36	17	17	[40]
DTIC	42	33		19.3	[55]
STZ, 5-FU	42	63	17	26	[40]
STZ, Dox	25	36	22		[21]
STZ, Dox	16	6	18		[10]
STZ, Dox	3	30		18	[51]
STZ, Dox	36	69	18	26	[42]
STZ, 5-FU	33	45	14	18	[42]
CLZ, 5-FU	44	36	11		[8]
STZ, 5-FU, Dox	10	40		26	[67]
STZ, 5-FU, Dox	12	55	15	21	[57]
STZ, 5-FU, Dox	84	39	18	37	[33]
5-FU, Epi, DTIC	15	27	10		[7]
5-FU, Epi, DTIC	32	25	21	38	[6]
All	436	39	16.5	25	

STZ Streptozotocin; *DTIC* dacarbazin; *5-FU* 5-fluorouracil; *CLZ* chlorozotocin; *Dox* doxorubicin; *Epi* epirubicin

Table 7 Chemotherapy in well-differentiated neuroendocrine tumors of the small bowel

	N	Response (%)	Median survival	Author
Dox	81	21	11	[19]
STZ, 5-FU	80	22	15	[19]
STZ, 5-FU	43	33	NI	[39]
STZ, Dox	33	40	11	[24]
STZ, Dox	3	30	5	[51]
STZ, cyclophosphamide	47	26	NI	[39]
5FU, Dox, DTIC	20	10	5	[14]
5FU, CCNU	16	25	16	[32]
MTX, cyclophosphamide	16	0	NI	[43]
VP16, cisplatin	13	0	NI	[41]
	352	**21**		

Dox Doxorubicin; *STZ* streptozotocin; *5-FU* 5-fluorouracil; *DTIC* dacarbazin; *CCNU* lomustine; *MTX* methotrexate; *VP16* etoposide

combination of etoposide and cisplatin yielded a response rate of 67%, making this regimen a rewarding tool in undifferentiated neuroendocrine tumors of the intestine.

Novel agents

In tumor cells, the regulation and modulation of growth pathways is disturbed or mutated. This, in turn, leads to dysregulation of signalling pathways, accelerated cell proliferation, and growth. Targeted interaction with key components of these dysregulated signalling pathways may present a new option of tumor therapy. Possible targets are growth factors, growth factor receptors, angiogenetic molecules, and kinases involved in proliferation pathways [26]. Targeted therapy aims at different levels of these proliferation pathways like antibody-induced neutralization of vascular endothelial growth factor (VEGF) ligands or the epithelial growth factor receptor to prevent ligand–receptor interaction or direct inhibition of the receptor tyrosine kinase by small molecules. The best known examples of multi-kinase inhibitors are imatinib or sunitinib, with activity against kinases of platelet-derived growth factor receptor (PDGF-R), bcr-abl, c-kit and PDGF-R, VEGF-R, RET, and c-kit, respectively. RAD001 (everolimus) a rapamycin analogue, is an inhibitor of the mammalian target of rapamycin (mTOR). Inhibition of the protein kinase mTOR may reduce cell growth, increase apoptosis, and reduce metastatic spread. It may have synergistic effects with SSA, as the mTOR pathway is stimulated by IGF-1, which is inhibited by SSA.

These novel targeted therapies are now used in clinical trials for neuroendocrine tumors of foregut and midgut origin. Theoretical background is the known expression of PDGF-R and VEGF-R in neuroendocrine tumors. Results of in vitro investigations in neuroendocrine tumors cell models demonstrated effective inhibition of cell proliferation. Most of the following data are not yet published, but have been presented in abstract form.

Tyrosine kinase inhibitors

First clinical trials in neuroendocrine tumors with tyrosine kinase inhibitors demonstrated partial remission in 5–18%,

Table 8 Novel agents: tyrosine kinase inhibitors and anti-angiogenetic therapy in patients with gastroenteropancreatic neuroendocrine tumors

Drug	*N*		PR		SD		PD		Progression-free survival		Author
	P	SB	P	SB	P	SB	P	SB	P	SB	
Tyrosine kinase inhibitors											
Gefitinib	39	57	10%	5%	14%	32%			31% (6 months)	61% (6 months)	[25]
Imatinib	27		4%		63%					24 weeks (med)	[9]
Sunitinib	66	43	13%	2%	75%	93%			43 weeks (med)	42 weeks (med)	[34]
Everolimus+octreotide	13	18	18%	13%	55%	81%	15%	6%	65% (6 months)	65% (6 months)	[70]
Anti-angiogenic therapy											
Endostatin	40		0%		80%		20%		5.8 months (med)	7.6 months (med)	[35]
Bevacizumab+octreotide	22		18%		77%					96% (18 weeks)	[69]
Bevacizumab+ temozolamide	18	16	24%	0%	70%	92%	6%	8%			[36]
Thalidomide+ temozolamide[a]	11	15	25%		68%		7%		1-y 79%; 2-y 61%[b]		[37]

[a] Plus 3 pheochromocytomas
[b] Overall survival
P Pancreas; *SB* small bowel; *PR* partial remission; *SD* stable disease; *PD* progressive disease; *med* median

stable disease in 14–93% and progressive disease in 6–15% of the patients. Progression-free survival ranged from a median of 24 to 42 weeks or up to 60% at months 6. These first data indicate a slightly better result for patients with neuroendocrine tumors of the small bowel. Unfortunately, progressive disease was not a prerequisite for inclusions in all trials, and different tumor groups were included in the studies (Table 8). Side effects were tolerable in most of these studies, and the incidence of grade 4 toxicities was low.

In summary, the antiproliferative or stabilization effect of tyrosine kinase inhibitors is probable comparable to biotherapy. These molecules may have synergistic effects in combination with established treatment regimen. Thus, they may well widen the spectrum of effective therapeutic strategies in neuroendocrine tumor diseases.

Anti-angiogenic therapy

Anti-angiogenic therapy is directed vs the formation of new blood vessels. Tumor angiogenesis is regarded as a critical point in the development of growth and metastases. Thus, VEGF, an endothelial growth factor, VEGF-R, or the VEGF-R kinase are potential targets for anti-angiogenic therapy. Neuroendocrine tumors are highly vascularized, and VEGF expression is high. A correlation of the VEGF plasma concentration with tumor growth has been suggested. Therefore, anti-angiogenic therapy targets either the VEGF-R ligand, the VEGF-R, or VEGF-R kinase activity.

Endostatin or angiostatin act as inhibitors of the VEGF-R, antibodies like bevacizumab interfere with VEGF, and multikinase inhibitors like vatalanib (PTK/ZK) [63] inhibit angiogenesis. Thalidomide inhibits angiogenesis by interference with VEGF and basic fibroblast growth factor (bFGF) pathways and the extracellular matrix. These drugs reduce the formation of new vessels and act cytostatic. In contrast, substances directed vs existing tumor vasculature-like combrestatin induce cell necrosis and act cytotoxic. Due to their serious side effects, none of these vascular targeting therapies are clinically available at the time being.

With all the different anti-angiogenic strategies used so far, partial remission was seen in 0–25%, stabilization occurred in 68–92% and progressive disease in 2–20% (Table 8). Again, results are still preliminary, as most of these data are only published in abstract form. As progressive disease was no prerequisite for inclusion in some studies, the rate of stabilization as well as the intervals for progression-free survival or overall survival may be overestimated. Most therapy regimen were well tolerated. The combination of thalidomide and temozolamide, however, resulted in high bone marrow toxicity (~70%) with opportunistic infections in 10% of the patients with infectious complications [36].

In summary, molecular targeted therapies and angiogenesis inhibitors offer a promising approach to antiproliferation in neuroendocrine tumors. Results of ongoing trials will provide more stringent data due to more homogenous tumor groups, inclusion of patients with similar pre-therapeutic regimen, and demonstrated progressive disease. It is to be expected that these new drugs will preferentially be used in combination with standard treatment regimen to improve stabilization rates in well-differentiated neuroendocrine tumors. Long-term effectiveness of targeted therapy may well be overcome by the development of alternative intracellular signalling pathways, thus reducing therapeutic efficacy of the new regimen. On the other hand, depending on the expression pattern of targeted molecules in the tumor, pre-therapeutic patient selection may improve success rates in selected patients. Thus, classifying neuroendocrine tumors according to the expression of possible targeted molecules may be the future standard for effective therapeutic strategies.

Conflict of interest statement We declare that we have no conflict of interest.

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