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Differential diagnostic patterns of lung neuroendocrine tumours

A clinico-pathological and immunohistochemical study of 122 cases

Marzia Bonato [1], Michele Cerati [1], Alberto Pagani [2], Mauro Papotti [2], Federica Bosi [3], Gianni Bussolati [2], and Carlo Capella [1]

[1] Department of Human Pathology, II Faculty of Medicine, University of Pavia at Varese, Viale Borri 57, I-21100 Varese, Italy
[2] Department of Biomedical Sciences and Human Oncology, University of Turin, Turin, Italy
[3] Department of Pathology, IRCCS Policlinico S. Matteo, Pavia, Italy

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Summary. A series of 3 tumourlets (TLs), 81 typical carcinoids (TCs), 14 atypical carcinoids (ACs) (well-differentiated neuroendocrine carcinomas, WDNCs) and 24 small cell-intermediate cell carcinomas (SCC-ICCs) of the lung were studied. HIstopatological features were correlated with amine and peptide hormone immunoreactivity and with clinical data. All types of tumours expressed general neuroendocrine (NE) markers: Grimelius positivity and chromogranins were detected more frequently in well-differentiated (TLs, TCs) than in less well differentiated tumours [ACs (WDNCs) and SCC-ICCs] whereas neuron specific enolase (NSE) was prominent in the latter tumours. TLs and peripheral TCs were benign, often showing a paraganglioid pattern and frequently expressing gastrin-releasing peptide (GRP), which is present in the peripheral airways of normal lung. Central TCs were associated with lymph node metastases in 8.5% of the cases, frequently had a trabecular architecture, often associated with human milk fat globule 2 (HMFG2)-positive acinar and rosette-like structures, and were mainly immunostained for the α-subunit of human chorionic gonadotrophin (α-hCG) and serotonin. ACs (WDNCs) were associated with intrathoracic and/or extrathoracic metastases in 57.1% of the cases with a mortality rate of 35.7%. Their histological and cytological features were intermediate between those of TCs and SCC-ICCs. ACs (WDNCs) expressed serotonin and α-hCG less frequently than TCs. All SCC-ICCs were surgically treated and displayed a mortality rate of 91.6% with a mean survival of 10.2 months after operation. These tumours were characterized by high expression of HMFG2 and NSE, while the expression of both orthotopic (serotonin, GRP) and ectopic (ACTH) specific NE substances was very low. Since all TCs (either central or peripheral) had a favourable outcome, while about 36% of ACs (WDNCs) were fatal, the latter seem more appropriately designated "well-differentiated NE carcinomas". The differential diagnosis between different NE tumours of the lung is important and is mainly based on morphology. Both panendocrine and specific immunohistochemical markers are helpful in distinguishing the less aggressive, mostly benign varieties from the more malignant varieties.

Key words: Panendocrine markers – Endocrine markers – Immunocytochemistry – Carcinoids – Lung tumours

Offprint requests to: C. Capella

Introduction

There is substantial morphological, histochemical, ultrastructural and functional evidence suggesting that well-differentiated neuroendocrine (NE) tumours (tumourlets, TLs; typical bronchial carcinoids, TCs) and NE-type small-cell and intermediate-cell carcinomas (SCC-ICCs) represent the two ends of a wide spectrum of lung tumours which share similar NE characteristics. Despite this common feature it has been demonstrated that their biological behaviour is quite different; some consider SCC as the malignant counterpart of TC (Bensch et al. 1968; Hattori et al. 1972). Tumours with intermediate histological and biological patterns, designated "atypical carcinoids" (ACs; Arrigoni et al. 1972; De Caro et al. 1983) or "well-differentiated neuroendocrine carcinomas" (WDNCs; Gould et al. 1983, 1988) are not very well defined. The incidence of metastases and survival rates of these latter tumours have been reported to lie between those of TCs and SCCs (Arrigoni et al. 1972; Mills et al. 1982; Gould et al. 1988). However, others (Smith 1969; Salyer et al. 1975) have failed to detect any morphological pattern in bronchial carcinoids that might be significantly correlated with prognosis. In an attempt to address the as yet unresolved problems of differential diagnosis and prognosis inherent to these tumour types we performed a detailed study on a large series of patients affected with NE tumours of the lung [3 TLs, 81 TCs, 14 ACs (WDNCs) and 24 SCC-ICCs]. The morphological characteristics and immunohistochemical reactivities of the various tumours

were studied. For the latter, antisera to the general pan-endocrine markers and regulatory peptides were used. The aim of the study was to determine whether different staining patterns could be demonstrated, and if so whether they bear any diagnostic and/or prognostic significance.

Materials and methods

The neoplasms that comprise this series were identified during routine evaluation of surgical pathology specimens and consultations seen in the Departments of Pathology of the University of Pavia, I Medical Faculty, the University of Pavia at Varese, II Medical Faculty and the University of Turin, from 1966 to 1987. Clinical records for each patient were reviewed for age, sex, tumour size and site and lymph node and/or visceral metastases (see Table 1). In addition, data from a minimum follow-up of 2 years were collected for each case. The slides of all cases were reviewed. Paraffin-embedded specimens of formalin or Bouin's fixed tissue were available for each case. Serial sections were cut at 5 μm from representative blocks of each case and stained with haematoxylin and eosin (H&E) and Grimelius stains.

The tumours had been diagnosed as TLs, TCs, ACs (WDNCs) and SCC-ICCs on histological grounds, using the criteria of Azzopardi (1959), Arrigoni et al. (1972) and Gould et al. (1983, 1988).

Table 1. Summary of clinical and follow-up data

	Mean age (years)	Range	Male/female	Metastases		Follow-up			
				LN	Distant	Mean length of follow-up	DOD	AWD	Mean survival of DOD
Tumourlets	50.6	44–64	3/0	0	0	7.5 years	0	1/3 (33.3%)	
TCs									
a) central	42.2	11–73	38/32	6/70 (8.5%)	1/70 (1.4%)	7.1 years	1/68 (1.4%)	1/68 (1.4%)	3 years
b) peripheral	54.8	36–71	6/5	0	0	8.5 years	0	0	
ACs (WDNCs)	57.3	43–73	8/6	5/14 (35.7%)	4/14 (28.5%)	4.4 years	5/14 (35.7%)	2/14 (14.2%)	2.7 years
SCC/ICCs	57.5	44–73	22/2	13/24 (54.1%)	2/24 (8.3%)	10.7 months	22/24 (91.6%)	2/24 (8.3%)	10.2 months

LN, Lymph node; DOD, died of disease; AWD, alive with disease; TCs, typical carcinoids; ACs, atypical carcinoids; WDNCs, well-differentiated neuroendocrine carcinomas; SCC-ICCs, small-intermediate cell carcinomas

Table 2. Source and working dilution of antisera used

Polyclonal antisera (AS) or monoclonal antibodies (mAb)	Working dilution	Code Source	
Chromogranin A (CGA) (mAb)	1/100	LK2H10	Dr. R.V. Lloyd, Ann Arbor, Mich., USA
	1/500	Phe-5	Enzo, New York, USA
Chromogranin B (CGB) (AS)	1/2000	–	Prof. H. Winkler, Innsbruck, Austria
Synaptophysin (SYN) (AS)	1/400	–	Dr. F. Navone, Milan, Italy
Serotonin (5-Hydroxytryptamine, 5HT) (AS)	1/500	43H27	Immunonuclear, Stillwater, Minn., USA
S-100 Protein (S-100) (AS)	Undiluted	590100	Ortho, Raritan, Minn., USA
Neuron-specific enolase (NSE) (mAb)	1/100	M16-N3	Sanbio, Am Uden, The Netherlands
Gastrin-releasing peptide (GRP) (AS)	1/1000	B43-1	Milab, Malmö, Sweden
α-Subunit of hCG (α-hCG) (mAb)	1/1000	5E8	Dr. S. Ghielmi, Brescia, Italy
Adrenocorticotrophic hormone (ACTH) (AS)	Undiluted	596010	Ortho
Human milk fat globule 2 (HMFG-2) (mAb)	1/100	MCA291	Serotec, Kidlington, UK
Cytokeratin (CK) (AS)	1/200	KLI	Immunotech, Marseille, France
Calcitonin (CAL) (AS)	Undiluted	596015	Ortho
Calcitonin gene-related peptide (CGRP) (AS)	1/1200	B41-1	Milab
Enkephalin (ENK) (AS)	1/640	B15-1	Milab
Bovine pancreatic polypeptide (BPP) (AS)	1/840	B32-1	Milab
Substance P (AS)	1/320	B45-1	Milab
Neurofilament (NF)			

For TLs, TCs and ACs (WDNCs) six growth patterns were considered: (1) trabecular with thin gyriform ribbons or rather broad irregular cords; (2) acinar or glandular, with well-formed acini, tubules or rosettes lined by a single row of columnar or cuboidal cells forming true lumina; (3) paraganglioid, with well-defined nests of polygonal clear cells (*zellballen*) separated by highly vascularized fibrous septa; (4) solid-nest, with ill-defined nests of cells; (5) diffuse, with sheets of cells; (6) mixed, in which several patterns of growth were variously combined.

Serial paraffin sections were processed for immunohistochemistry. Immune reactions were revealed by the avidin-biotin-peroxidase complex (ABC) technique according to Hsu et al. (1981) and developed with diaminobenzidine. Monoclonal antibodies and polyclonal antisera were employed; their respective working dilutions are listed in Table 2. All primary antibodies were incubated for 16–48 h at 4° C. Controls for immunostaining included incubation of adjacent sections with antiserum preadsorbed with 1–50 μg/ml of related antigen, its substitution by rabbit or mouse non-immune serum and its omission as first layer. The antigenic profiles of the tumour cells as defined by the immunoperoxidase technique were related to histological types, structural patterns and central or peripheral site of primary tumours. In addition, the immunophenotypes of TL, TC and AC (WDNC) type tumours were compared with those of a selected series of SCC-ICCs. The mean score of the different NE markers (Table 3) for each type of tumour [TL, TG, AC (WDNC), SCC-ICC] was calculated, dividing the sum of positive cells of each case (expressed as a percentage) by the number of tumours belonging to the same group.

All differences in this study were tested for statistical significance by means of the Fisher exact test and were considered significant at a probability level of 0.05.

Results

The 122 NE tumours studied were divided according to their histological features into: 3 (2.4%) TLs, 81 (66.3%) TCs, 14 (11.4%) ACs (WDNCs), and 24 (19.6%) SCC-ICCs.

All TLs were subpleural, multiple and minute (less than 3 mm). A prevalent paraganglioid pattern was found in 2 cases, while in 1 case a dominant broad trabecular architecture was observed. The histochemical and immunostaining results are summarized in Table 3. All cases expressed general NE markers widely including Grimelius silver positivity (Fig. 1), chromogranin A (CGA) and synaptophysin (SYN). Chromogranin B (CGB) was absent. All cases stained for more than one hormone. The most frequently and widely distributed hormones were gastrin-releasing peptide (GRP) (Fig. 2), the α-subunit of human chorionic gonadotrophin (α-hCG), and serotonin (5HT), while calcitonin, adrenocorticotrophic hormone (ACTH) and enkephalin (ENK) were present in only a few cells. All TLs tested were negative for pancreatic polypeptide (PP) (1 case) and substance P (3 cases). S-100-protein-immunoreactive sustentacular cells were well represented in the 2 cases with paraganglioid features, while they were very few in the only case with trabecular architecture. The mean score for GRP expression was statistically higher in TLs than in TCs ($P=0.0045$), ACs (WDNCs) ($P=0.0049$) and SCC-ICCs ($P=0.0024$).

Seventy of the 81 (86.4%) TCs were central and 11 (13.5%) were peripheral. The tumour was found in the right lung in 53 cases (65.4%) and in the left lung in 27 cases (33.3%); in 1 case the side was not indicated.

Table 3. Relationship between positivity for histochemical-immunohistochemical markers and histological type in 122 neuroendocrine tumours of the lung

Marker or stain	Histological type									
	Tumourlet		Peripheral TC		Central TC		AC (WDNC)		SCC-ICC	
	I	MS	I	MS	I	MS	I	MS	I	MS
Grim	3/3(100%)	93.3	11/11(100%)	70.9	69/69(100%)	63.4	12/14 (85.7%)	48.4	6/23 (26%)	4.1
NSE	2/2(100%)	90	10/10(100%)	86.9	63/63(100%)	86.9	10/10(100%)	77	10/10(100%)	91
CGA	3/3(100%)	76.6	11/11(100%)	47.2	70/70(100%)	51.8	10/13 (76.9%)	37.3	18/23 (78.2%)	24.9
CGB	0/3		5/11 (45.4%)	2	25/66 (37.8%)	3.8	2/13 (15.3%)	1.2	1/23 (4.3%)	0.04
SYN	2/3 (66.6%)	50	10/10(100%)	71	67/67(100%)	65.8	11/12 (91.6%)	24	9/23 (39.1%)	7
5HT	2/3 (66.6%)	53.3	6/10 (60%)	15.7	47/69 (68.1%)	7.4	7/14 (50%)	1.5	1/24 (4.1%)	0.4
α-hCG	2/3 (66.6%)	11.6	9/10 (90%)	4.8	49/69 (71%)	10.7	9/14 (64.2%)	15.7	1/24 (4.1%)	0.8
GRP	3/3(100%)	67.3	3/10 (30%)	4.2	9/68 (13.2%)	1.3	2/14 (14.2%)	2.5	0/24	
CAL	2/3 (66.6%)	2.3	1/9 (11%)	0.1	6/64 (9.3%)	0.3	4/10 (40%)	0.9		
CGRP	0/3		0/2		1/13 (7.6%)	0.07	0/1			
ENK	1/3 (33.3%)	0.3	3/4 (75%)	0.7	4/27 (14.8%)	0.1	1/5 (20%)	0.2		
ACTH	3/3(100%)	1	4/10 (40%)	0.4	10/68 (14.7%)	1	2/14 (14.2%)	0.1	0/24	
HMFG2	2/3 (66.6%)	16.6	4/11 (36.3%)	10.4	44/67 (65.6%)	16.3	11/12 (91.6%)	26.4	23/24 (95.8%)	50.4
CK	2/2(100%)	87.5	11/11(100%)	74.5	60/60(100%)	83.7	13/13(100%)	66.1		
NF	0.3		1/10(10%)	0.1	16/64 (25%)	6	0/13			
S-100	3/3(100%)	1.6	6/11 (54.5%)	2.5	28/69 (40.5%)	1.6	7/14 (50%)	0.7		

Grim, Grimelius technique; I, immunoreactivity; MS, mean score; for other abbreviations, see Tables 1 and 2

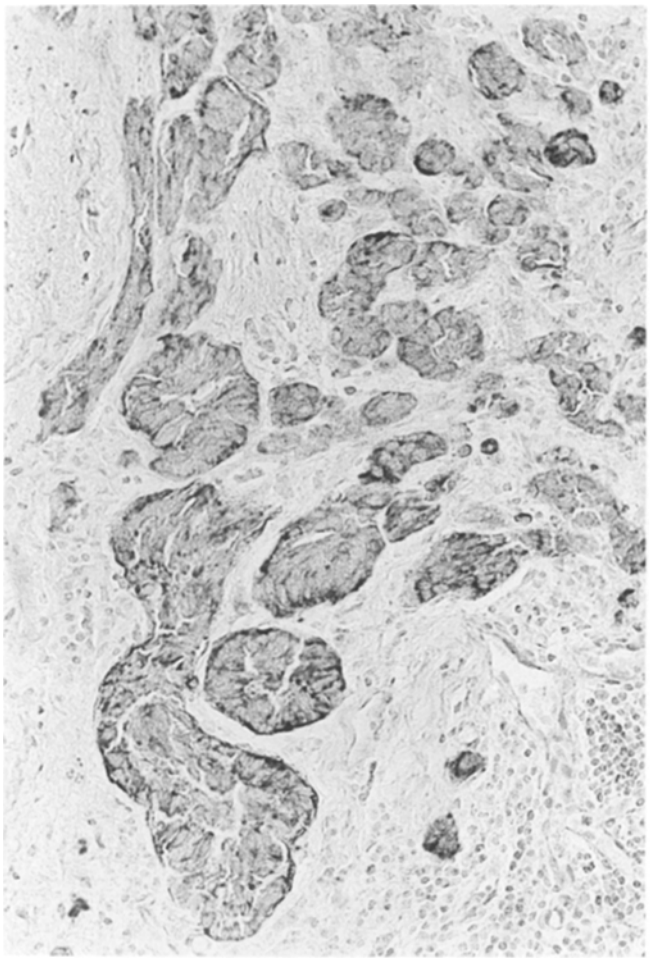

Fig. 1. Tumourlet. Grimelius silver, ×200

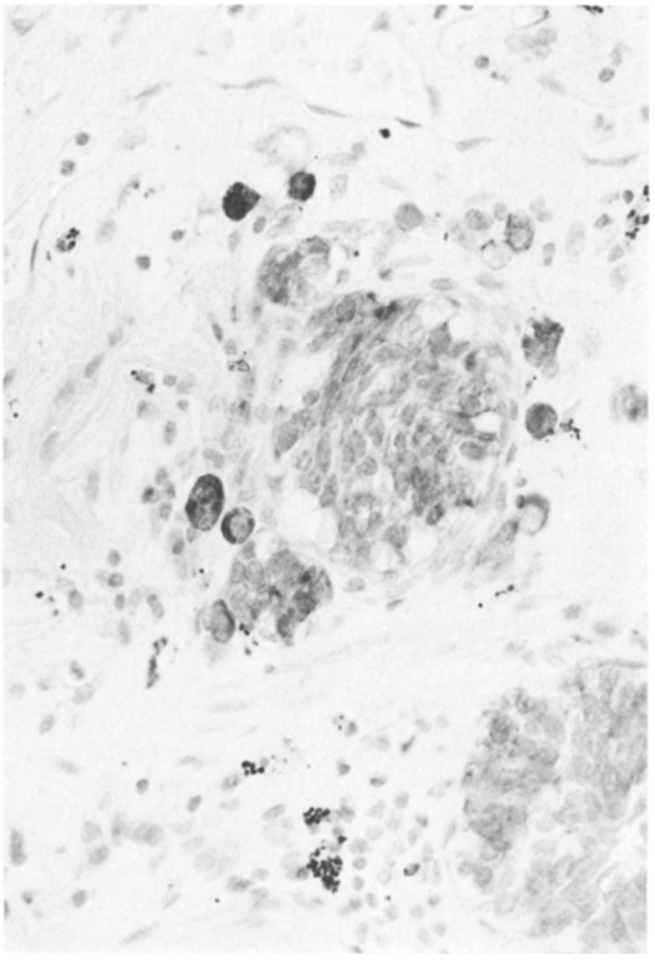

Fig. 2. Gastrin-releasing peptide-positive cells in a tumourlet. Immunoperoxidase, haematoxylin counterstain, ×200

The exact site of centrally located tumours was as follows: main bronchus, 10 cases (14.2%); upper lobar bronchus, 16 cases (22.8%); right bronchus intermedius, 11 cases (15.7%); lower lobar bronchus, 32 cases (45.1%). In 1 case the exact site was not indicated. The site of peripheral tumours was recorded in 8 cases: 3 tumours were located in the lower lobe, 3 others in the right middle lobe and 2 were in upper lobes. The size of the tumour was specified in 68 instances and ranged from 1 to 5 cm (mean 2.9 and 3.1 cm for central and peripheral tumours, respectively).

In 50 of 70 (71.4%) central and 8 of 11 (72.7%) peripheral carcinoids the histological pattern of the tumour was predominantly trabecular (Fig. 3). A paraganglioid aspect (Fig. 4) predominated in 3 of 11 (27.2%) peripheral and in 10 of 70 (14.2%) central tumours. An acinar component (Fig. 5) was found in 37 of 70 (52.8%) central and in 6 of 11 (54.5%) peripheral tumours. Solid nests were prevalent in 11.4% of the central and in 9% of the peripheral tumours. Diffuse sheet-like cell masses were prevalent in only 1 tumour, which was centrally located. In only 1 peripheral carcinoid was the tumour mass associated with multiple TLs and with hyperplasia of endocrine cells in the bordering bronchial epithelium.

Round to polygonal cells were prevalent in both central (71.4% of the cases) and peripheral (72.7%) TCs. Fusiform cells were observed in 12.3% of the tumours and were prevalent in 5.7% of the central and 27.2% of the peripheral lesions respectively. The cells showed a minor degree of pleomorphism in about one-third of the cases (34.2% for central and 27.2% for peripheral tumours respectively). Prominent nucleoli were found in only 4 cases. Mitotic activity was generally low, ranging from 0 to 3 mitoses/10 high power fields (HPF).

The tumour stroma was always sparse and was represented by richly vascularized fibroconnective tissue septa. Calcification or ossification of bronchial cartilage involved by tumour growth was detected in 14 central TCs.

The staining results in TCs are summarized in Table 3. Grimelius positivity and CGA, NSE and SYN were seen in all carcinoids with most tumour cells intensely positive (see positivity scores in Table 3). In contrast, less than 40% of all TCs had positive results for CGB. The expression of specific NE product was variable from tumour to tumour. Many TCs expressed multiple NE markers simultaneously. α-hCG (Fig. 6) was demonstrated in 73.4% of the cases. This was followed by 5HT (Fig. 7) (69.6%), PP (26,6%), ENK (22.5%), ACTH (17.9%), GRP (15.5%), calcitonin

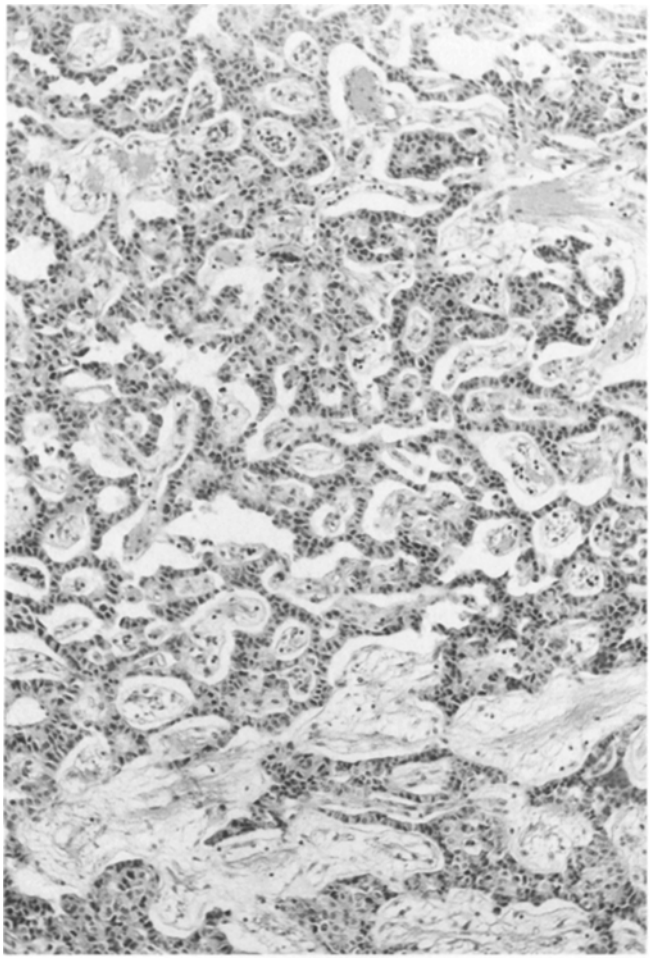

Fig. 3. Trabecular structure with vascular pseudorosettes in a central typical carcinoid. Haematoxylin and eosin (H&E), ×100

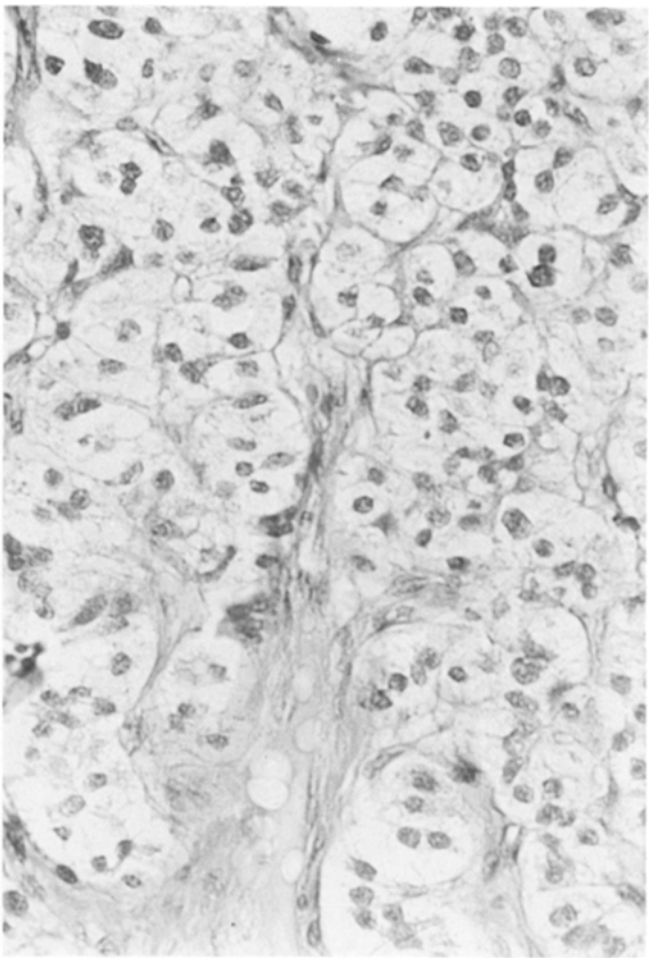

Fig. 4. Paraganglioid aspect with characteristic *zellballen* in a peripheral typical carcinoid. H&E, ×400

(9.7%) and calcitonin gene-related peptide (CGRP) (6.6%). All these substances, with the exception of 5HT and PP, appeared to be more frequently expressed in peripheral than in central TCs; however, a statistically significant difference was found only for ENK ($P=0.0278$). The mean score for 5HT and α-hCG was statistically higher in central than in peripheral TCs ($P=0.011$ and $P=0.019$, respectively). All tumours tested (19) were negative for substance P.

HMFG2 immunoreactivity was present both in the cell cytoplasm and in the lumina of acini and rosettes (Fig. 4) of 48 of 78 (61.5%) cases. The expression of this marker was more evident in central (65.6% of the cases with a mean score of 16.3) than in peripheral (36.3%; mean score 10.4) TCs. All tumours were strongly and diffusely immunostained by cytokeratin antibodies; in most cases the staining was stronger in the perinuclear cytoplasm; in 4 cases a punctate "fibrous-body"-like staining was observed. Sixteen of 64 (25%) central and 1 of 10 (10%) peripheral tumours reacted with antineurofilament antibodies. The pattern of cytoplasmic staining was diffuse in the majority of the cases; in 9 tumours a dot-like positivity was detected. S-100-positive sustentacular cells (Fig. 8) were more frequently detected in peripheral (54.5% of the cases; mean score 2.5) than in central (40.5% of the cases; mean score 1.6) TCs.

Some correlations between structural morphology and distribution of immunoreactivity for the various substances could be demonstrated in the present series of TCs. Tumours with a prevalent paraganglioid pattern were associated with a larger number of S-100-positive sustentacular cells ($P=0.001$) and with a higher expression of 5HT-, GRP- ($P=0.043$), ACTH- ($P=0.001$), calcitonin-, and ENK-positive cells when compared to tumours with a prevalent trabecular or solid nest pattern. On the other hand, neurofilament protein- and HMFG2-positive cells were more frequently detected in TCs with a prevalent trabecular or solid nest pattern, respectively.

There were 14 ACs (WDNCs). Ten cases were centrally located tumours ranging in size from 1.5 to 5 cm (mean 3.5 cm). Four (28.5%) were peripherally located and ranged from 2.5 to 10 cm (mean 5.1 cm). Nine (64.2%) tumours had a prevalent broad trabecular pattern which was combined with acinar or rosette structures in 4 cases. Solid nests and sheet-like masses prevailed in 2 cases. Ten tumours were composed of polygonal cells only, while 4 also exhibited spindle cells which predominated in 2 cases. Necrosis was present

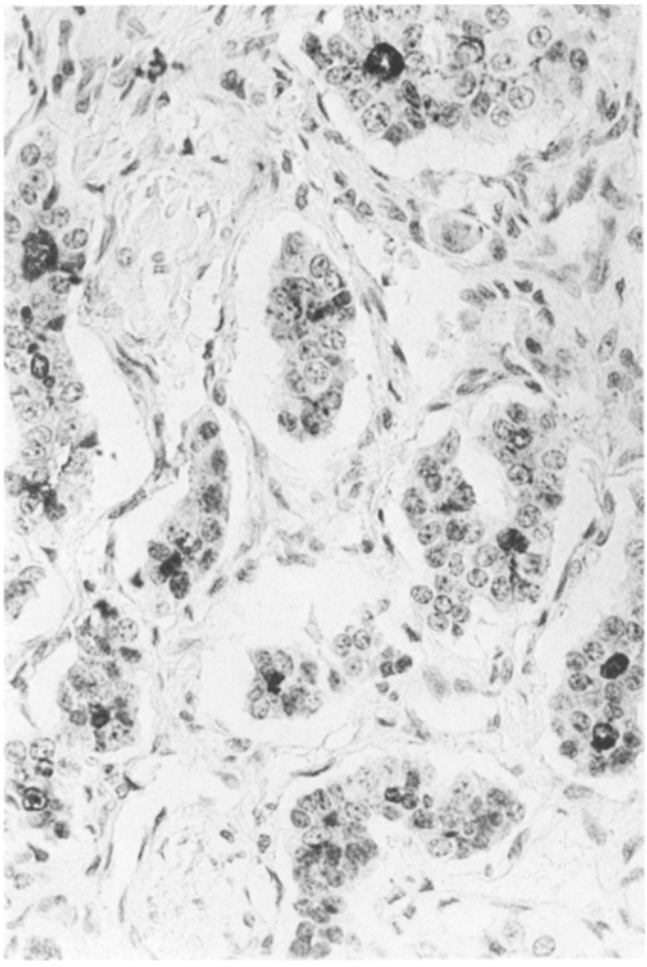

Fig. 5. Tubuloacinar structure in a central typical carcinoid, human milk fat globule 2 antibody, immunoperoxidase, haematoxylin counterstain, ×200

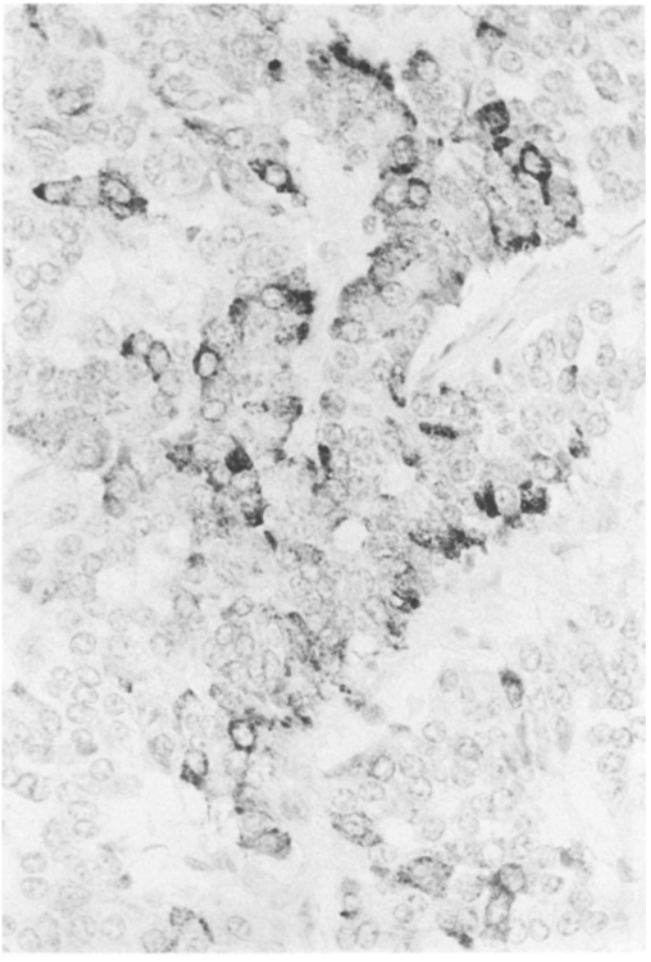

Fig. 6. Intense immunoreactivity for α-hCG in a trabecular typical carcinoid. Immunoperoxidase, haematoxylin counterstain, ×400

in cell trabeculae and nests in 10 (71.4%) cases. Haematoxyphilic encrustation of vascular walls (Azzopardi's phenomenon) was encountered focally in only 2 cases. Mitotic activity was generally high, ranging from 0 to 72 mitoses/10 HPF. The mean number of mitoses/10 HPF was 24.3. This value was statistically different from that of TCs ($P=0.001$). The nuclei were predominantly round and moderately hyperchromatic and showed a finely granular chromatin pattern where sparse coarse chromatin aggregates were present (Fig. 9). Marked nuclear hyperchromasia was found in 7 cases. An increase in the nuclear/cytoplasmic ratio was detected in all cases.

The histochemical and immunostaining results are summarized in Table 3. A positive staining for Grimelius, NSE, CGA and SYN was found in 85.7%, 100%, 76.9% and 91.6% of the cases respectively. CGB expression was detected in only 2 (15.3%) cases. The most frequently represented hormone was α-hCG in 9 of 14 (64.2%) cases with a mean score of 15.7, followed by 5HT in 7 of 14 (50%) cases with a mean score of 1.5, calcitonin in 4 of 10 cases with a mean score of 0.9, GRP in 2 of 14 cases with a mean score of 2.5 and ACTH in 2 of 14 (14.2%) cases with a mean score of 0.1. All tumours tested were negative for substance P (3 cases) and PP (3 cases). Diffuse cytokeratin staining was present in 100% of the cases, while HMFG2 positivity was confined to a discrete number of cells (mean score = 26.4) and was more often encountered in acinar areas. All tumours were neurofilament negative. S-100-positive sustentacular cells were detected in 7 of 14 cases with a mean score of 0.7.

Statistically significant differences ($P=0.05$) between TCs and ACs (WDNCs) were found for the frequency of Grimelius, CGA, calcitonin and neurofilament expression. The mean score for 5HT ($P=0$), CGB ($P=0$), CGA ($P=0.0074$), ENK ($P=0$), ACTH ($P=0$), S-100 ($P=0.001$) and neurofilaments ($P=0.001$) was statistically lower in ACs (WDNCs) than in TCs.

There were 24 SCC-ICCs, 7 in the left and 13 in the right lung; in 4 cases the side was not indicated. Seventeen tumours were central and 4 peripheral; the site of 3 was not recorded.

The predominant histological pattern was trabecular in 15 (62.5%) cases, solid-nested in 5 (20.8%) cases and diffuse in 4 (16.6%) cases. Acini, rosettes with central lumina and paraganglioid aspects were seldom encountered. The cells were small in size in 18 (75%) cases, intermediate in 3 (12.5%) cases and mixed small-inter-

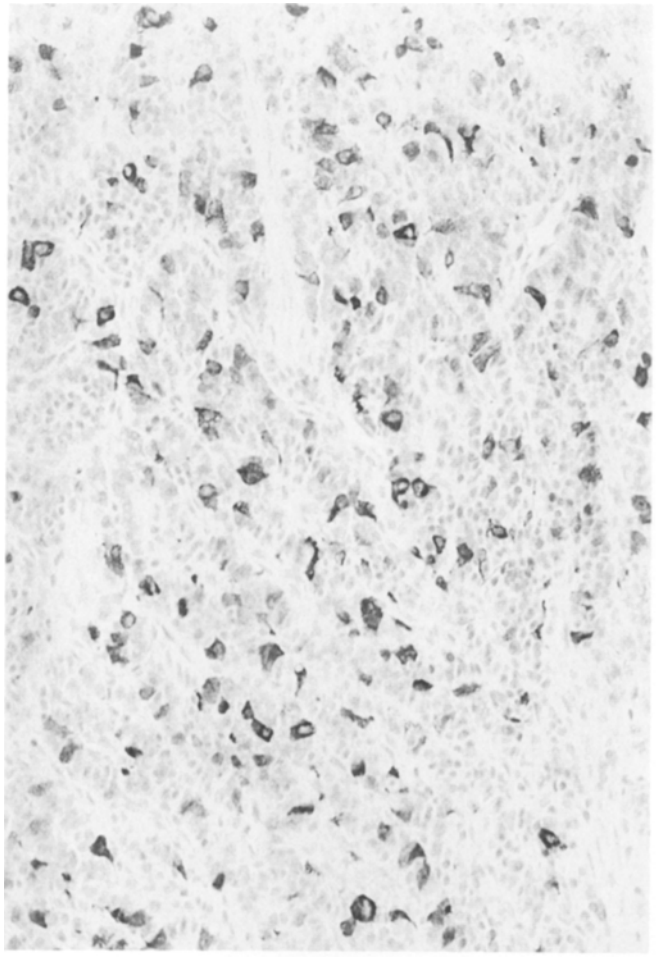

Fig. 7. Numerous serotonin-immunoreactive cells in a central typical carcinoid. Immunoperoxidase, haematoxylin counterstain, ×200

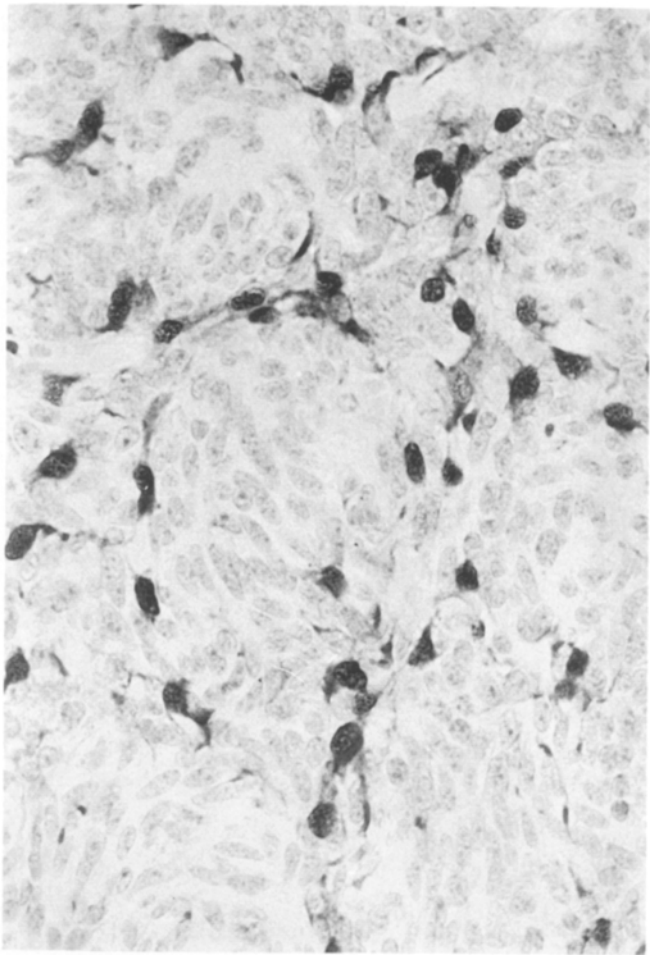

Fig. 8. Peripheral typical carcinoid, paraganglioid-type with protein S-100-positive sustentacular cells around the cell nests. Immunoperoxidase- haematoxylin counterstain, ×400

mediate in 3 (12.5%) cases. Round and fusiform cells were predominant in 17 (70.8%) and 7 (29.1%) cases respectively. The nuclear-cytoplasmic ratio was high and mitoses were numerous (mean value: 74/10 HPF) in all cases. Hematoxynophilic deposits in vascular walls ("Azzopardi phenomenon") were encountered in 20 (83.3%) cases and in these they were present in foci of tumour necrosis. Only 4 cases did not show any significant tumour cell necrosis. The stroma of SCC-ICCs was frequently considered to be abundant, with areas of stroma equal to or greater than the area of epithelial cells. Lymphatic invasion was detected in all cases. Twenty-three cases were tested with the Grimelius silver technique and 6 cases (26%) were positive. Diffuse intracytoplasmic staining for NSE was present in most neoplastic cells in all cases tested, while positivity for CGA, CGB and SYN was found in 78.2%, 4.3% and 39.1% of the cases respectively. α-hCG and 5HT were both detected in only 1 case. No immunoreactivity could be observed with the other anti-regulatory-peptide antibodies used. Twenty-three out of 24 (95.8%) cases expressed strong cytoplasmic immunostaining with the HMFG2 antibody, with a mean score of 50.4.

Statistically significant differences ($P=0.05$) between TCs and SCC-ICCs were found for frequency of Grimelius, HMFG2, CGA, CGB, SYN, 5HT, α-hCG, GRP and ACTH expression and between ACs (WDNCs) and SCC-ICCs for Grimelius, SYN, 5HT and α-hCG expression. The mean score for NSE and HMFG2 was statistically higher in SCC-ICCs than in TCs ($P=0.001$) and than in ACs (WDNCs) ($P=0.006$).

All patients, including those with SCC-ICC, with the exception of 3 cases underwent lobectomy or pneumonectomy. As shown in Table 1 there are marked differences in mortality, incidence of metastases, age and sex of patients with TC compared to those with SCC-ICC. AC (WDNC) has an intermediate biological behaviour. Five ACs (WDNCs) out of 14 (35.7%) patients in our series have died of their tumour, exhibiting a mean survival of 2.7 years. Four of these 5 had metastases at the time of diagnosis. Seven patients are living and were disease-free when last seen (mean follow-up 5.3 years). Only 2 of these had metastases at the time of diagnosis, both restricted to the intrathoracic lymph nodes. Three of these cases had a peripherally located tumour.

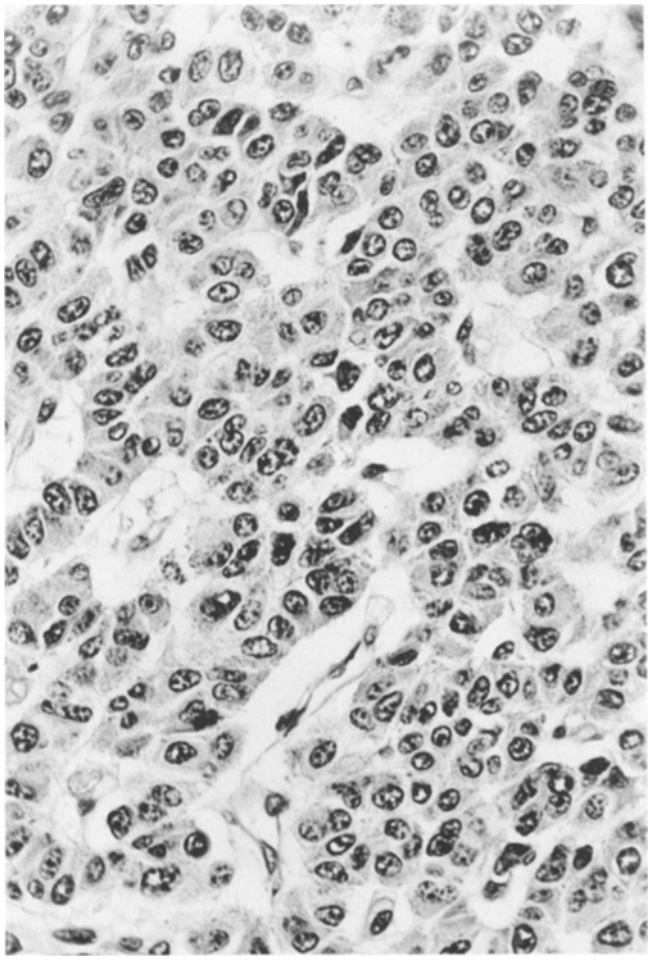

Fig. 9. Atypical carcinoid (well-differentiated neuroendocrine carcinoma) showing nuclear hyperchromasia and polymorphism. H&E, ×400

Discussion

The respiratory mucosa of several mammalian species, including man, contains NE cells occurring either as single elements or in corpuscular aggregates, the neuroepithelial bodies (Lauweryns and Peuskens 1972). The NE cells of the lung are known to contain several biologically active substances, including 5HT (Gould et al. 1983), bombesin (Wharton et al. 1978; Gould et al. 1983), or its mammalian counterpart; GRP (Tsutsumi et al. 1983a; Sunday et al. 1988) leu-enkephalin (Cutz et al. 1981), calcitonin (Becker et al. 1980; Cutz et al. 1981; Tsutsumi et al. 1983b) CGRP (Tsutsumi 1989) and the α-subunit of glycoprotein hormones (Fukayama et al. 1986, 1990). In addition, it has been demonstrated that secretory granules of lung NE cells also contain non-hormone and non-amine components such as chromogranins, which are considered to be general markers of NE elements and are mainly responsible for the Grimelius silver positivity of these cells (Rindi et al. 1986; Lauweryns et al. 1987). It is now widely held that bronchopulmonary NE neoplasms are histogenetically related to pulmonary NE cells and that the majority of them display immunoreactivity for more than one NE marker, including NSE, 5HT and various peptides (Gould et al. 1983, 1988).

The expression of these products appears to be variable. The specific NE products include both indigenous substances such as 5HT, α-subunit of glycoprotein hormones, GRP, as well as many inappropriate products that have not been identified in normal human lung, such as ACTH, and neurotensin (Gould et al. 1988).

NE neoplasms of the lung represent a heterogeneous group of tumours which can be classified according to various criteria including their biological behavior, site of origin, histological pattern and cytological characteristics (Carter and Eggleston 1980). A relatively simple classification of the spectrum of NE pulmonary neoplasma was proposed by Gould et al. in 1983. Bronchopulmonary NE tumours were subdivided into four categories: (1) carcinoids; (2) well-differentiated NE carcinomas (WDNCs); (3) NE carcinomas of intermediate cell type; and (4) NE carcinomas of small-cell type. These authors introduced the term WDNC, which embraces ACs, a term used in most laboratories. Nevertheless, they failed to mention TLs (Carter and Eggleston 1980) in their list of NE tumours. ICCs, in spite of their difference in cell size, are not easily distinguished from SCCs, as most of these are a mixture of medium and small cells. In addition, there is no difference in terms of prognosis and responsiveness to current therapeutic protocols between these two classes of tumours, which is why, in this paper, they have been grouped together. Four groups of tumours are now proposed on the basis of the several differences in different groups which have been found in the present study.

TLs are tiny, multiple peripheral carcinoids usually occurring in the setting of pulmonary fibrosis (Bonikos et al. 1976). Their neoplastic nature has been demonstrated by their occurrence in association with solitary (as in one of our cases) or multiple peripheral carcinoid tumours (Gmelich et al. 1967; Bonikos et al. 1976; Ranchod and Levine 1980), or with lymph node metastases (D'Agati and Perzin 1985). Their prevalent histological pattern was paraganglioid in 3 cases. Tumour cells, often fusiform, widely expressed eutopic substances such as 5HT, GRP and calcitonin. This further shows that TLs represent tumours composed of well-differentiated cells, imitating the peptide pattern of the NE cells present in the peripheral airways of normal lung (Gould et al. 1983; Solcia et al. 1984).

TCs were centrally located in 86.4% of cases. Differences were observed from cases located in the periphery: the mean age of patients with central carcinoids (42.2 years) was lower than that of patients with peripheral carcinoids (54.8 years), as was also seen by Bonikos et al. (1976). In addition, peripheral TCs were not associated with intrathoracic or distant metastases, while 8.5% and 1.4% of central carcinoids developed lymph node or distant metastases, respectively. A similar trend towards a poorer prognosis in centrally located tumours compared with those peripherally sited in the lung was reported by McCaughan et al. (1985). Central and peripheral carcinoids also showed differences in pattern of growth, cytological characteristics and immunopheno-

type. Prevalent paraganglioid (*zellballen*) arrangement and fusiform shape of the cells are more frequently detected in peripheral than in central TCs. Accordingly, S-100-protein-positive sustentacular cells which peripherally delineate the *zellballen* (Barbareschi et al. 1990) are more abundant in peripheral than in central TCs. The general NE markers as well as GRP, calcitonin, enkephalin, α-hCG, and ACTH are more frequently expressed in peripheral than in central TCs. It appears that staining indices of all these markers are similar between TLs and peripheral TCs but differ from central TCs. This is in keeping with an origin of some peripheral TCs from TLs, as previously suggested by Capella et al. (1981) and Solcia et al. (1984).

Central TCs show positive staining for HMFG2, more frequently than peripheral TCs. The presence of HMFG2 in human SCC has been considered as a poor prognostic sign (Allan et al. 1987) and the higher rate of metastases in central in comparison with peripheral TC correlates with the higher HMFG2 expression.

ACs (WDNCs) appeared to be a specific clinico-pathological and biological group distinct from TCs and SCCs (Arrigoni et al. 1972; Mills et al. 1982; Gould et al. 1983). The main clinical features in our series of patients with ACs (WDNCs) are: (1) mean age (57.3 years) higher than that of patients with TCs (43.9 years) and similar to that of patients with SCC-ICCs (57.7 years); (2) absence of patients aged under 43 years; (3) male/female ratio (8/6) similar to that of TCs (44.37); (4) intrathoracic and/or extrathoracic metastases in 57.1% of cases; (5) mortality rate of 35.7% with a mean survival after surgical resection of 2.7 years. These are all in keeping with most reports (Arrigoni et al. 1972; Mills et al. 1982; Mark and Ramirez 1985).

ACs (WDNCs) have histological and cytological features intermediate between those of TCs and SCC-ICCs. Our findings show that the presence of nuclear pleomorphism, frequent mitotic figures and the presence of obvious necrosis are the most important criteria to distinguish ACs (WDNCs) from TCs; ACs (WDNCs) can be differentiated from SCC-ICCs by the less marked nuclear abnormality and the scarcity of cell moulding, crushing artefacts and DNA perivascular encrustation. In a recent paper, Travis et al. (1991) demonstrated that within the spectrum between TC and SCC, in addition to AC, the category of large-cell neuroendocrine carcinoma exists. This entity is characterized by large cell size, frequent nucleoli, high mitotic rate and frequent necrosis. Some of our cases of AC (WDNC) with elevated numbers of mitoses can probably be included within this new class, whose clinical significance remains to be proved with large numbers of patients.

Although we have not detected an immunohistochemical marker which specifically identifies ACs (WDNCs), the distribution of the panel of biomarkers used in this study appears to differ substantially from that of TCs and SCC-ICCs. The incidence of detection of panendocrine markers as well as of specific endocrine substances is lower in ACs (WDNCs) than in TCs but is higher in ACs (WDNCs) than in SCC-ICCs. The specific NE substances more widely expressed are 5HT, α-hCG and GRP, which represent eutopic products. Unlike other authors (Gould et al. 1983; Warren et al. 1984) we have not found a frequent expression of ectopic peptides such as ACTH and substance P in WDNC.

The biological behaviour and clinical characteristics of SCC-ICCs are markedly different from those of TCs and ACs (WDNCs) and therefore it appears that their distinction is mandatory (Bensch et al. 1968; Fisher et al. 1978; Carter and Eggleston 1980). In our series of 24 SCC-ICCs, 22 patients died of their disease with a mean survival of 10.2 months after surgical resection of the tumour. Other differences from TCs and ACs (WDNCs) include mean age, which was higher in patients with SCC-ICCs, and prevalence in males, which was more clearly evident in SCC-ICCs.

Although the histological criteria previously reported (Azzopardi 1959; Gould et al. 1983) are in most cases sufficient to distinguish SCC-ICC, in some cases the immunophenotypical profile helps in the differentiation of SCC-ICC from TC and AC (WDNC). The typical distribution of the different biomarkers immunohistochemically detected in SCC-ICCs is as follows: high expression of NSE and HMFG2, moderate expression of CGA and SYN, very low expression of both orthotopic (5HT, GRP, calcitonin) and ectopic (ACTH) specific NE substances.

Our study demonstrates the usefulness of immunohistochemical staining for markers of NE differentiation in lung tumours. Immunoreactivity for CGA as well as for SYN seems to be a specific and sensitive marker of NE differentiation. These markers are linked to different specialized submicroscopic structures: chromogranins represent a component of the matrix of NE granules (Hagn et al. 1986), while SYN is localized within the membranes of small clear vesicles with a size range akin to that of synaptic vesicles, distinct from dense core neurosecretory granules (Navone et al. 1986; Buffa et al. 1988). In the present study the expression of these two markers is widespread and intense in the TC and TL group, intermediate in the AC group and scarce in the SCC-ICC group, suggesting a link with the degree of tumour cell differentiation. CGB, unlike CGA, appears to be poorly expressed in NE tumours of the lung.

Our study demonstrates that α-hCG is more frequently detected than the major hormonal products of normal pulmonary NE cells such as 5HT, GRP and calcitonin. This result confirms previous observations based on a small number of NE tumours of the lung (Fukayama et al. 1986) and suggests that α-hCG can be used to identify and monitor pulmonary NE proliferations, since it can be detected in the serum of some patients bearing NE tumours (Braunstein et al. 1979). α-hCG has been interpreted as a marker of malignancy for all types of functioning pancreatic endocrine tumours (Kahn et al. 1977; Heitz et al. 1983). Conversely, the α-subunit does not seem to have any prognostic significance in NE tumours of the lung, since the incidence of detection of this marker is similar in TCs (73.4% of the cases) and ACs (WDNCs) (64.2%) and is higher in these tumours than in SCC-ICCs (4.1%). The exact biological function of isolated α-subunit production is not yet known; how-

ever two alternative hypotheses have been proposed (Fukayama et al. 1986): (1) that α-hCG production is unrelated to any physiological function and represents the consequence of the preferential α-hCG gene activation connected with the process of tumour transformation; (2) that α-hCG may have a growth promoter function for NE cells of the lung similar to that shown by GRP or bombesin (McGregor et al. 1983).

In conclusion, the Grimelius procedure and staining reactions for NSE, SYN, CGA, 5HT and α-hCG proved highly sensitive in revealing NE differentiation and appear to be of diagnostic value even on small biopsies. None of the markers investigated had prognostic value. Bronchial carcinoids (both central and peripheral) had a favourable outcome in all cases, even in the presence of lymph node metastases; only one case had a recurrence after several years. Differentiation of carcinoids from NE carcinomas (either the well-differentiated form or the poorly differentiated, small-cell entity), is mandatory, but at present the diagnosis rests on morphological grounds (number of mitoses, presence of nuclear atypia and necrosis). However, panendocrine markers as well as 5HT and α-hCG are less often expressed in AC and SCC-ICC than in TC and this finding is of help in distinguishing tumours with a more favourable outcome from those with a poorer prognosis. The term "atypical carcinoid" designating a tumour with malignant behaviour in 30–50% of cases seems misleading and the term "well-differentiated NE carcinoma" appears more appropriate.

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