

Furthermore, we found that in absence of Bcl-2 overexpression both Bax and/or Bak overexpression (both present in 60% of cases), increased the apoptosis detected in those tumors ($p = 0.017$ and $p = 0.085$ respectively).

Our results further stress the role of Bcl-2 overexpression blocking the apoptosis of breast cancer tumors and thus controlling the Bax and Bak death facilitator activities.

PP-1-17 Circulating Tumor Markers (CEA, MCA, CA 15.3, CA 549) in the Diagnosis Breast Cancer Recurrence after Surgery: 5-Year Results

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Concomitant measurement of 4 serum markers (CEA, MCA, CA 15.3 and CA 549) were performed every 3–6 months in 128 breast cancer patients with no evidence of disease after surgery. After a median follow-up of 5 yrs (range 4–5 yrs) 30 pts (23%) relapsed. In 25 of these at least one marker was abnormal (sensitivity: 83%); the 5 pts with normal marker value at the time of relapse had only local recurrence (soft tissue metastases). The sensitivity of CEA and MCA (32% and 47%) was significantly lower than the sensitivity of CA 15.3 (80%) and CA 549 (81%) ($p = 0.02$). Ninety-nine pts did not relapse: 90 have normal marker values (specificity: 92%). The predictive value of a positive test and of a negative test is 76% and 95%, respectively. The combination of 2 or more markers does not increase the sensitivity ($p = 0.5$) and the positive predictive value of CA 15.3 or CA 549 alone. The 5-year results confirm that a single marker determination (CA 15.3 or CA 549) is recommended in the follow-up of pts after surgery for breast cancer.

PP-1-18 Plasma c-erbB2 Concentrations and Response to Chemotherapy in Breast Cancer

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The c-erbB2 oncogene is amplified and/or overexpressed in about 25% of breast cancer. The c-erbB2 overexpression has been related to a poor prognosis and a lower response to chemotherapy [1]. Using an enzyme-immunoassay (Triton Diagnostics, Ciba-Corning France) we determined plasma c-erbB2 concentrations in patients with metastatic [2] and inflammatory breast cancers and examined the potential value of plasma c-erbB2 as a predictive indicator. The cut-off value, determined in 30 healthy women between 20 and 80 years, was 27 U/ml. Patients with a c-erbB2 concentration higher than 27 U/ml were considered as c-erbB2 positive (c-erbB2+). 10 out of the 33 metastatic and 9 out of the 25 inflammatory breast cancer patients were c-erbB2+. The response to chemotherapy was not significantly different between c-erbB2+ and c-erbB2– patients with metastatic (4/10 vs 10/23) and inflammatory (6/9 vs 11/16) breast cancer. Plasma c-erbB2 assay has no predictive value in metastatic and inflammatory breast cancer patients.

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[1] Gusterson et al. 1992, *J Clin Oncol*, 10, 1049–1056.

[2] Révillion et al. 1996, *Eur J Cancer*, in press.

PP-1-19 Determination of Cytosol ERBB-2 Protein in Primary Breast Cancer

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To determine over-expression of c-erbB-2 gene in breast cancer, ELISA using anti-c-erbB-2 MoAb was performed with cytosol fractions of 139 resected breast cancer specimens from patients with stage I–III B in 1994–1995. Cut-off value was set at 18 ng/mg protein to correlate with gene amplification. The median and mean value of erbB-2 protein were 7.1, 18.7 ng/mg protein, respectively. The positive rate was 18.7%. Positive erbB-2 was associated with histological grade and serum CEA level, but not with tumor size, stage, vessel invasion, nodal status, intraductal component, serum CA15-3 level and PR. There was a weak inverse relation in erbB-2 level and ER. The prognostic importance will be evaluated in future.

PP-1-20 Psychosocial Correlates of Oestrogen and Progesterone Receptors in Breast Cancer: Results of Three Consecutive Studies

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Psychosocial correlates of hormone receptor status (assessed by DCC assay) were investigated in 93 consecutive patients attending a radiotherapy service. Life event, coping style, and psychological adjustment self-report scales were completed. The 75 patients with receptor-positive (RP) (oestrogen and/or progesterone) were better adjusted psychologically than the 18 patients with receptor negative (RN) lesions. The results of this first study have been replicated in a sample of 89 consecutive patients hospitalized for breast surgery. The 73 patients with RP were better adjusted psychologically before and after surgery than the 16 patients with RN lesions. The high level of distress found in the first and second study and the high prevalence of psychiatric history in RN group of patients led us to design a third study matching 11 patients with RN lesions with 11 patients with RP lesions for medical and sociodemographic data and comparing the two groups for life events and psychological adjustment. The results of this third study are showing that patients with RN lesions are reporting significantly more long lasting stressful life events before cancer diagnosis. These three studies are indicating that the main psychosocial variables related to RN status is a significant psychological distress related to long lasting stressful events preceding cancer diagnosis. The relationship found here between hormone receptor status and psychosocial variables contributes to the understanding of possible effects mediated by the central nervous system on breast cancer initiation and progression.

PP-1-21 Correlational Study of Microangiographic and Immunohistochemical Techniques for Tumour Vascularisation

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Tumour vascularity is an important predictor of prognosis in breast cancer. We have correlated microvessel density with prognosis in 177 with primary breast cancer. In a smaller prospective series of 21 mastectomy patients we have studied the pattern of neovascularisation and vessel density by the new technique of microangiography and immunohistochemistry (IHC) using monoclonal antibodies to CD34, basic fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) by immunoperoxidase IHC. Microangiograms were done on the gross specimens and there after IHC was done on 4 μ m thin sections of tumours. Microangiograms revealed two distinct vascular patterns — an anastomosing pattern (15/21) and a radial pattern (4/21). In 2/21 there was no distinct pattern. Six angiograms were graded 1 (lowest vessel count), 11 graded II and 4 were graded III (highest vessel count). IHC vascular counts correlated with the angiogram grade. Correlation between vascular counts, angiogram grade and angiogenic growth factors will be presented.

PP-1-22 Non-Invasive Measurement of Antioestrogen Activity in the Breast

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Tamoxifen, an antioestrogen with agonist and antagonist properties, is currently being assessed as a long-term chemopreventative agent in patients at high risk of breast cancer. Surrogate markers of Tamoxifen action on the normal breast are needed to assess its action in individual women. The protein pS2 is stimulated by oestrogen in cancer cell lines in vitro, whilst the breast cyst protein Apolipoprotein D is inhibited by oestrogen also in vitro. Both proteins can be measured in breast secretions. Healthy women ($n = 63$) and women with breast pain ($n = 15$) provided breast secretion samples. Sequential samples were collected in women treated for breast pain with Tamoxifen ($n = 6$) and Zoladex ($n = 9$) to determine if measurement of these proteins could be used as antioestrogen markers. Apo D and pS2 were measured by radioimmunoassay and total protein by the Bradford method.

Results Premenopausal secretion levels of pS2 ($p < 0.02$) were significantly higher and Apo D significantly lower ($p < 0.03$) than postmenopausal values. Women with breast pain had significantly higher pS2 (median 19.6 vs 8.5 ng/mg protein, $p < 0.04$) and lower Apo D (median 59.9 vs 159.9

ug/mg protein, $p < 0.03$) than pain-free premenopausal women. After Zoladex and Tamoxifen treatment, pS2 levels fell, (both $p < 0.02$) and Apo D rose significantly ($p \leq 0.03$ and $p < 0.02$ respectively). Apo D and pS2 may prove useful intermediate markers of antioestrogen action in the breast. Zoladex = luteinising hormone releasing hormone analogue. Stats: Mann Whitney, Kruskal Wallis tests.

PP-1-23 Expression of Thymidine Phosphorylase in Mammary Carcinomas and its Relationship to Angiogenesis

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Human thymidine phosphorylase (dThdPase) activity is indispensable for the angiogenic activity of the platelet-derived endothelial cell growth factor. dThdPase has been reported to increase in some types of malignant tumor, and the role of dThdPase in the progression of tumors is unknown. In this study, we examined dThdPase expression and angiogenesis in 139 mammary carcinomas and 54 benign mammary disorders, using biochemical and immunohistochemical methods. dThdPase expression was much common in mammary carcinomas, compared with benign mammary disorders, and dThdPase expression of mammary carcinoma cells was correlated with microvessels density of stroma in mammary carcinomas of 3–4 cm in diameter, in p53 negative mammary carcinomas. In c-erbB-2 negative mammary carcinomas and in mammary carcinomas of premenopausal women.

PP-1-24 Prediction of the Effect Of 5'-Deoxy-5-Fluorouridine (5'-DFUR) by the Status of Angiogenic Enzyme Thymidine Phosphorylase Expression in Advanced Breast Cancer Patients

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5'-DFUR is known to appear antitumor activity through the conversion to 5-Fu by thymidine phosphorylase (TP). Recently, TP was demonstrated to be identical to angiogenic molecule PD-ECGF. The relationship between the clinical response of 5'-DFUR and TP/PD-ECGF expression was determined in 24 advanced breast cancer patients. 13 were TP/PD-ECGF positive and 11 were TP/PD-ECGF negative. In 13 TP/PD-ECGF positive patients, 4 showed objective response (OR) and 3 showed stable disease (SD) by 5'-DFUR, however only one case showed OR and no case showed SD in 11 TP/PD-ECGF negative patients, suggested that 5'-DFUR was likely to be effective for TP/PD-ECGF positive patients. An angiogenic enzyme TP/PD-ECGF expression might be a predictor of the effect of 5'-DFUR treatment in breast cancer.

PP-1-25 Subclinical Thyroid Dysfunction in Patients with Breast Cancer

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It has been recognized several syndromes of thyroid dysfunction in patients with solid malignancies. The aim of this study has been to evaluate the thyroid hormonal profile in breast cancer patients and to correlate the findings with stage of disease to know the usefulness as a potential prognostic factor. We have studied 33 patients with breast cancer (100% women; age: 49 ± 10 years) previously to any type of treatment. Ria was used to establish hormonal levels: T3 (normal range = 86–187 ng/ml), T4 ($n = 4.50$ – 12.50 mg/dl), TSH ($n = 0.4$ – 4.0 mU/ml), rT3 ($n = 20$ – 50 ng/dl) and TBG ($n = 12.90$ – 13.30 mg/ml). Results are summarized as follows (mean \pm sd): T3 = 125.3 ± 48.5 ; T4 = 9.7 ± 4.8 ; TSH = 2.3 ± 4.5 ; rT3 = 53.4 ± 29.7 ; TBG = 23.7 ± 9.1 . The 37% of patients had a low T3 and the 35% of patients had increased rT3. Patients with hypoproteinemia and/or hypoalbuminemia show higher proportion of low T3 (60%) and/or increased rT3 (58%) ($p < 0.05$). TBG was not different in the groups. According with stage of disease, patients with local disease show low T3 (22%) and/or increased rT3 (25%) that were significantly different from those found in loco-regional disease (28% and 20%, respectively) or metastatic disease (48% and 60%, respectively). In conclusion: we have found a high proportion of low T3 syndrome

(euthyroid syndrome) and this feature was related with the progression of disease and decrease of proteins.

PP-1-26 Polymorphism of Estrogen Receptors from Primary Breast Cancers

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Altered (cloned) ERs are extremely abundant in breast cancer cytosols. Whether such a frequency have some biological relevance or is a biochemical artefact is unknown. Studied reported here analyse these questions. (1) To assess the frequency of altered ER in primary breast cancers, ER-positive samples (DCC assays) were submitted to hydroxylapatite (HAP) adsorption and immunohistochemical (ER1D5 MAb) assays. Our study relies on the observation that ER1D5 positivity and strong salt-adsorbed receptor to HAP relate to ER molecules with functional AB/C domains. In a series of 18 mammary tumors, HAP assays always reveal an absence of AB/C ER domains as reflected by a permanent low adsorption with 0.5 M KCl; on the contrary, presence of such domains was clearly demonstrated by nuclear immuno-staining (ER1D5) on tissue sections. Hence, apparent high frequency of cleaved ERs lacking N-terminal region in primary breast cancer cytosols seems to be a biochemical artefact due to proteolysis at the time of tissue processing and not an index of high amount of altered (variant) receptors in these tissues. Whether such a property also holds for metastases is unknown (such tumors are characterized by peculiar ERs without E2 binding affinity but recognized by anti-ER monoclonal antibodies and [³H] tamoxifen aziridine). (2) On the other hand, gel shift experiments suggested that E and/or F domains may impede the association of the C domain to [³²P] ERE: ER immunoprecipitation with H222 (epitope in E) increases the intensity of the radiolabeled band corresponding to ERE bound to A/BC domains (cleavage products).

PP-2. Surgical Aspects (September 11)

ORAL PRESENTATIONS

PP-2-1 Localisation of Impalpable Breast Lesions — A Surgical Approach

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The conventional approach to localisation of impalpable breast lesions employing a hooked wire with either stereotaxis or a perforated plate has potential disadvantages for the operating surgeon. Often the entry point of the wire lies some distance from the site of projection of the lesion on the skin. The guide-wire should pierce the skin at or close to the site of any proposed surgical incision and proceed along the shortest and most direct course towards the lesion. Ideally, the wire should lie within a radial distance of between 1 and 2 cm from its target. A method is described which achieves these objectives and involves both radiological and clinical measurements. A total of 665 guide-wire localised biopsies have been carried out at the above institutions over the periods 1-11-87 to 31-3-95² and 1-1-94 to 3-3-96¹. In only 4% of cases was re-positioning of the wire required. Excision of the radiological lesion was obtained with a single biopsy in 99% of cases. A second or third biopsy was indicated in 0.7% and 0.3% of cases respectively. Migration of the wire occurred in 2 patients and no cases of wire transection or pneumothorax were reported. This method of localisation facilitates subsequent excision and permits the most appropriate incision consistent with optimal cosmesis.

PP-2-2 A Prognostic Index for Breast Ductal Carcinoma in Situ (DCIS)

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We have developed a prognostic index (PI) for patients with DCIS utilizing three statistically significant predictors (by multivariate analysis) of local