P32 Prognostic parameters in node negative breast cancer receiving adjuvant CMF: Analysis of a randomized trial with emphasis on p53 and HER 2 neu

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Objectives: to better define prognosticators in node negative breast cancer as well as indicators of chemosensitivity.

Patients (pts) and Methods: p53 (DO7 MoAB) and HER 2-neu (CB11 MoAB) expression were studied by immunohistochemistry in 282 and 225/330 pts with N-BC prospectively treated with no or 6 months CMF adjuvant chemotherapy with a median follow-up of 11 years. Correlation with initial presentation and outcome were studied with SAS software.

Results: 23% of the tumors overexpressed p53 and or HER 2-neu. This expression was associated with high grade tumors ($p = 0.01$) and low ER expression ($p = 0.01$). There was also a strong association between p53 and HER-2 neu expression ($p = 0.001$).

In multivariate analysis neither p53 or HER-2 neu expression affected event-free, overall survival or positive effect of adjuvant CMF.

Alternatively both were predictive of local relapses in those pts with conservative therapy (66%) (odds ratios 4.13 and 4.14 respectively).

Conclusions: In this large series of N-BC patients prospectively treated with no or adjuvant CMF, classical prognosticators were confirmed, while we failed to confirm that of p53 and HER 2 neu (over)expression. The incidence of p53 and HER-2 neu overexpression was low, in keeping with other studies. Those expressions were strongly associated; they had no prognostic significance in terms of survival or effect of adjuvant chemotherapy but could be associated with radioresistance when conservative therapy was conducted. We are conducting a case-control study to confirm those findings.

P33 Apoptosis and related proteins in ductal breast carcinoma

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Object: Bcl-2 expression in infiltrating ductal carcinoma of the breast is associated with a better prognosis in node positive tumors.

Patients and Methods: A group of 100 infiltrating ductal breast carcinomas was examined by histological techniques and immunohistochemistry for bcl-2. We could dissect a group of bcl-2 negative versus a group of bcl-2 positive tumors. From each group randomly selected cases (from each group $n = 10$) were studied by markers for cell replication, apoptotic cell death (DNA in situ and labeling), p53 and the bcl-2 antagonist bax. As an internal control for bcl-2 and bax immunoreactivity we used the cytoplasmatic expression of normal lymphocytes. All tumors were graded according to the Nottingham modification of the Bloom-Richardson system. A non-parametric Mann-Whitney U test was used to compare both groups.

Results and Discussion: In a group of 100 infiltrating ductal breast carcinomas 74% were positive for bcl-2. Bcl-2 was expressed in normal adjacent breast tissue and in the carcinoma in situ component. In these regions, bcl-2 was expressed in the ductal structures. Based on these results we have compared ten randomly selected cases of each group for different markers. The Bloom Richardson grading was significantly higher ($p < 0.03$) in the bcl-2 negative group. Cell replication as demonstrated by the nuclear immunoreactivity for Ki-67 and apoptotic cell death (ISEL) were not statistically different between both groups. Bax which opposes bcl-2 in the cell was equally expressed in both groups. The results demonstrate that bcl-2 expression in breast tissue is related with cell differentiation. A loss of differentiation in the carcinoma cells is associated with a loss of bcl-2 expression that may help to explain the controversies in this field.

P34 Prognostic value of vascular endothelial growth factor protein in node-negative breast cancer

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This study was an effort to evaluate if the cytosolic level of vascular endothelial growth factor (VEGF) protein measured in primary tumours of node-negative breast cancer is an indicator of prognosis, and the clinical significance when compared to established prognostic factors. 575 consecutive patients with primary tumours T1–T2, no distant metastasis were included in the study. The cytosolic levels of VEGF were measured by a quantitative sandwich enzyme immunoassay technique. The patients were followed for a median of 45 month. The median age was 58 years. Univariate analysis was performed with VEGF as a dichotomous variable with the cut-off at the median value. Differences in overall survival were estimated using the Log Rank test. Other established predictive factors as age, oestrogenreceptor, tumour size, histologic grade, and histologic type were tested in the same way. Multivariate analyses were performed using the Cox Regression model.

The median VEGF level was 2.40 ng/mikrog. DNA (range 0.11–144.79). A significant difference in survival was found with a worse outcome for patients with higher levels of VEGF ($p = 0.0012$). In addition to VEGF, age (<58 vs >58 years), tumour size (T1 vs T2), grade (I + II vs III) and oestrogenreceptor (neg vs pos) all were statistically significant for overall survival in univariate analyses, ($p = 0.047$), ($p = 0.042$), ($p = 0.010$) and ($p < 0.001$) respectively. To evaluate the joint prognostic value of the variables, a multivariate analyses was performed. Variables included were: age, VEGF, histologic grade, tumour size and oestrogen receptor. Histologic grade ($p = 0.0070$), age ($p = 0.0330$), and VEGF ($p = 0.0348$) were prognostic for overall survival. The results suggest that the cytosolic level of VEGF protein is an independent prognostic factor for survival in node-negative breast cancer.

P35 Expression of hepatocyte growth factor/scatter factor in primary breast cancer

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Hepatocyte growth factor/scatter factor (HGF/SF) is normally a fibroblast derived cytokinase which acts as a mitogen and motogen for epithelial cells. High tumour concentrations of HGF/SF has been shown to correlate with reduced relapse-free and overall survival in women with primary breast cancer. Breast epithelial cells have been shown to possess the HGF receptor but which cells produce HGF in the breast is unclear.

To determine the expression of the HGF/SF in invasive breast cancer, in the adjacent non-malignant breast epithelium and mesenchymal cells, we have performed *in situ hybridisation* using cDNA probes labelled with 35S and control slides treated with crude RNA. To score the slides the intensity of the signal (0–3) and the number of positive cells (1 = <20%; 2 = 20–80%; 3 = ≥80%) were multiplied together to give a final score which is representative of total mRNA expression.

	Normal Breast Fibroblast (n = 24)	Endothelium (n = 24)	Non-malignant Breast = 20) Epithel = 20) (n = 20)	Inv Ductal Carcinoma (n = 18)	Inv Lobular Carcinoma (n = 6)
No positive	18	8	19	16	6
HGF expression	(1.58)	(0.708)	(3.05)	(4.05)	(4.66)
Score Range	0–3	0–3	0–9	0–9	3–6

Values are means $^{*}p < 0.05$ (Ca vs endothelium: Normal breast vs endothelium). In the breast the majority of HGF/SF is produced by epithelial cells rather than fibroblasts ($p < 0.001$; Wilcoxon Rank Sign Test).

Epithelial cell production of HGF/SF by breast cancers may explain their propensity for metastasis and the finding that HGF/SF is a strong prognostic factor in breast cancer.

P36 CIP-1 protein expression in node-positive breast cancer patients

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CIP-1 is a cyclin dependent kinase inhibitor which negatively controls cell proliferation. Since chemotherapy may affect cell cycle regulation, in this study the hypothesis was tested that increased levels of CIP-1 may be associated with poor response to chemotherapy and with dismal clinical outcome. CIP-1 protein was assessed by immunohistochemistry (IHC) in 26 node-positive breast cancer patients (pts) (≥10 tumor containing axillary nodes or tumor containing infraclavicular node). All 26 pts had been treated with 4 cycles of conventional chemotherapy followed by high-dose chemotherapy supported by bone marrow stem cells. In 1 pt no tumor was left in the paraffin section for IHC. Nuclear staining for CIP-1 was observed in tumor cells in 18/25 of tumors (with usually moderate (+) and sometimes equal intensity (++) compared to internal controls). Nine of the pts with this staining had no evidence of disease (NED) after a median follow-up of 3 yrs, whereas 8 had recurrent disease. Five pts without this staining pattern (intensity 0 or +/−) had NED, whereas 2 pts died, one with, and one without disease.

Nuclear staining for CIP-1 in an estimated area of >50% of tumor area was observed in 18/25 of tumors. No differences in clinical outcome could be detected: 10 pts with nuclear staining of >50% of tumor area had NED, whereas 8 pts had recurrent disease. Those pts with minimal or absent nuclear staining (≤10% of tumor area) (3 pts) had NED.

CIP-1 expression is found in a high percentage of nuclei in breast cancer tumor cells of pts with bad prognosis breast cancer. CIP-1 expression is not