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

A.C. Hardy-Bessard, A. De Roquancourt, P.H. Cottu, P. De Cremoux, M. Marty, M. Espie. *Dpt of Medical Oncology Hopital Saint Louis, and Institut Curie, Paris, France*

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

A. Makar, H. Van Leuven, S. Declerq, W. Beelaerts, J. Gerris, M. Kockx. *Dept. Gynec + Pathol, A.Z. Middelheim, Antwerp, Belgium*

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

B. Linderholm, B. Tavelin, K. Grankvist, R. Henriksson. *Depts of Oncology and Clinical chemistry, Umeå Universal Hospital, Sweden*

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

B. Venizelos, P. Blair[1], J. Holland[1], A.F. Freemont[1], N.J. Bundred. *Dept of Surgery, University Hospital of South Manchester, UK; [1]Dept of Pathological Sciences, University of Manchester, UK*

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

P. Hupperets[1], E. Thunnissen[2], J. Peterse[3], H. Schouten[1]. *[1]Dept. Internal Medicine, University Hospital Maastricht, The Netherlands; [2]Dept. Pathology, University Hospital Maastricht, The Netherlands; [3]Dept. Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands*

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

associated with clinical outcome in these heavily treated pts, whereas the absence of CIP-1 expression seems to be associated with good prognosis.

P37 Multivariate prognostic index with emphasis on proliferation adds specificity to standard prognostic factors in operable breast cancer

R. Huovinen, Y. Collan, T. Kuopio, P. Kronqvist, S. Mänttäri, P. Jalava, L. Juntti. *Departments of Oncology and Pathology, University of Turku, Turku, Finland*

The selection of breast cancer patients for systemic adjuvant therapy stands on multiple clinico-pathological factors with proven prognostic value. Although the toxicity of the standard forms of adjuvant therapy is not great, the question more often asked is, which patients could be left without the therapy. For patients with obviously high risk of recurrence, the standard forms of adjuvant therapy certainly are not sufficiently effective, and consequently more aggressive chemotherapy protocols are under evaluation. There is an urgent need for standardized, easily accomplished model according to which the patients could be selected for appropriate treatment, taking together the individual risk-benefit profile.

We re-evaluated the prognosis of 230 breast cancer patients (mean age 59, SD13), with median follow-up of 6.6 years; all invasive tumors, ductal 85%, lobular 9%, other 6%, N+ 34%, N– 66%, Gr1 22%, Gr2 55%, Gr3 23%, estrogen receptor status positive 87%, negative 13%. The patients were divided in groups of low and high risk of mortality according to three different prognostic models: the former clinical model M1 (stage), the present model M2 (stage, grade and estrogen receptor status), and model M3 based on multivariate prognostic index (MPI) of Baak et al. (1985) (tumor size, lymph node status, mitotic activity index). 68 patients were actually given adjuvant systemic therapy. All three models unanimously suggested 73 patients to have low risk and 62 patients to have high risk. Among the rest of the patients (95) the models diverged.

The disease-specific survival at the end of the follow-up among the patients in the low risk group by each model was 87%, 90%, 88% in M1, M2, M3 respectively. Corresponding figures in the high risk group were 75%, 79%, 72%. The models showed quite similar power as prognostic models, but M2 was the most sensitive (0.83) and M3 the most specific (0.64). When premenopausal patients were analyzed separately, M3 showed high efficiency in identifying patients with high risk, the absolute difference in survival in the low and high risk groups being 44%, meanwhile the difference by grouping according to M1 and M2 was 31% and 27%, respectively.

The results speak in favor of using the present clinico-pathological factors which appeared to construct a sensitive model, in decision making about adjuvant therapy. The multivariate model, in which mitotic activity is stressed, helps to create different prognostic categories with higher specificity.

P38 Immunohistochemical detection of tumor cells in lymph nodes and bone marrow aspirates in node negative (N0) breast cancer (BC)

B. Gerber, A. Krause, E. Rohde, D. Richter, K. Friese. *Dept. Ob/Gyn, University of Rostock, Germany*

Simultaneous immunohistochemical (IHC) examination of axillary lymph nodes and bone marrow aspirates from patients (pts.) with nodal negative BC have not been published yet.

Pts. & Methods: 180 pts. with pT1-2 N0 M0 BC were subjected to bone marrow aspiration. Lymph nodes as well as bone marrow aspirates were retrospectively examined for tumor cells using a cytokeratin antibody and ABC-technique. The immunohistochemical results were correlated with the histological findings and other prognostic factors (ER, PR, S-Phase, Ploidy, Ki-67, EGF-R, HER-2/neu, p53, Cathepsin-D and pS 2. The mean follow up time was 56 ± 18 months.

Results: Totally in 58 of 180 pts. (32%) tumor cells were detected in lymph nodes (12.7%; N1a-IHC) and/or bone marrow aspirates (27.7%; M1-IHC).

	Lymph nodes	
Bone marrow	N0-IHC (n = 157)	N1a-IHC (n = 23)
M0 (n = 130)	122 (67.8%)	8 (4.4%)
M1-IHC (n = 50)	35 (19.4%)	15 (8.3%)

Disease free survival and overall survival showed a prognostic disadvantage for women with tumor cell detection in any site and number compared to women without any tumor cells ($p < 0.05$). Differences between IHC-positive and IHC-negative pts. were found in tumor size, grading, vessel invasion, ER, S-phase, Cathepsin D. By multivariate analysis tumour size and grading, but not the detection of tumor cells, were confirmed as independend prognostic factors in node negative BC.

Conclusion: In 32.2% of all conventionally as pT1-2 N0 M0 staged breast cancer pts. tumor cells are detectable in axillary lymph nodes and/or bone marrow aspirates. A prognostical disadvantage of tumor cell detection has been proven, but they do not represent independent prognostic factors.

P39 Prognostic value of plasminogenaktivator inhibitor type 1 and 2 in primary breast cancer

N. Fersis, U. Krainick, H. Frise, S. Thron, M. Szekelyi, G. Wittmann, D. Wallwiener, G. Bastert. *Gynecology and Obstetric University of Heidelberg, Germany*

Introduktion: Elevated concentrations of urokinase-type plasminogenaktivator (uPA) and his inhibitor PAI-1 in cytosolic extracts obtained from breast cancer patients are associated with a poor prognosis. Data about the prognostic value of another uPA inhibitor PAI-2 are incomplete. In order to substantiate the prognostic value of PAI-1 and PAI-2 in primary tumor we have measured the concentrations in the cytosol from 252 breast cancers.

Material and Methods: We used ELISA to test PAI-1 and PAI-2 in tumor extracts. The relation of this data to know prognostic factors and other variables such s-phase fraction and ploidy was studied. Disease-free and overall survival were analyzed according to Cox's proportional hazard model.

Results: The median PAI-1 value was 20.3 μg/g protein and for PAI-2 1.63 μg/g protein. Ductal invasive breast cancer has a greater concentration of PAI-1 than lobular invasive cancers. No differences was found for PAI-2. Patients with negative lymph node status had significantly higher PAI-2 values than those with affected lymph nodes ($p = 0.015$). After a median observation of 32 months in the univariate analysis showed that high levels of PAI-1 are correlated with short DFS (RR:1.56; 95% CI; $p = 0.005$) and OAS (RR: 186; $p = 0.001$).

Summary: The present study indicates that PAI-1 is an independent prognostic factor and high PAI-2 concentrations exercise a protective function in tumor metastasis via the lymphatic system.

P40 Prognostic factors predictive of lymph node status in breast cancer

V. Distante, S. Bianchi, R. Simoncini, A. Petrolo, A. Valiani, L. Orzalesi, L. Cataliotti. *Istituto Clinica Chirurgica I, University of Florence Florence, Italy*

In T1 tumours the incidence of lymph node metastases ranges from 21 to 35%. The Authors developed a case-control study to individualize a predictive factor of positive nodes. One hundred thirty one patients with T1a-b breast cancer were studied. All underwent axillary dissection and the pathologic status of the nodes was T1a: 29 N–, 9 N+ and; T1b: 103 N–, 33 N+. The factors evaluated were necrosis, p53, CerbB2, Bcl2, NM23 and Mib1. All of these were categorized in three levels but necrosis and the worst category in terms of prognosis were compared with the others two. The percentage of each worst factor is reported: T1a p53 (14% N– vs 12.5% N+); CerbB2 (54% N– vs 14% N+); Bcl2 (90% N– vs 71% N+); NM23 (0% N– vs 33% N+) and Mib1 (0% N– vs 12.5% N+); necrosis (80% N– vs 90% N+). T1b: p53 (4% N– vs 17% N+); CerbB2 (10% N– vs 20% N+); Bcl2 (39% N– vs 54% N+); NM23 (35% N– vs 43% N+); Mib1 (2% N– vs 0% N+); necrosis (91% N– vs 92% N+). The only statistically significant factor was p53 (<0.05) but only in the T1b category. The Authors concluded that these factors are not able to predict the axillary lymph node status.

P41 Urokinase plasminogen activator and cathepsin D in micrometastatic cells of patients with primary breast cancer

E.-F. Solomayer, I.J. Diel, Ch. Gollan, G. Meyberg, S. Bode, D. Wallwiener, G. Bastert. *Dept. of OB/GYN, University of Heidelberg, Voss-Str. 9, 69115 Heidelberg, Germany*

Proteases in tumor tissue may play an important role in metastasis and invasion. This study evaluated the prognostic relevance of urokinase plasminogen activator (UPA) and cathepsin D detection in disseminated tumour cells in bone marrow.

Bone marrow was sampled intraoperatively from both iliac crests in 280 patients with primary breast cancer. Interphase cells were enhanced and stained immunocytologically. Three antibodies were used: 2E11, detecting tumor associated glycoprotein (TAG 12, which is typically expressed by almost all breast cancer cells), anti-UPA and anti-cathepsin D antibodies.

87 women (31%) developed distant metastatic disease after a median follow-up of 68 months. Patients without tumor cell detection in bone marrow had a significantly longer metastasis-free interval (MFI = 70 months, $p < 0.001$) as well as a significantly longer survival time (median 72 months). Women with cathepsin D positive tumor cells in bone marrow (n = 27; 10%) had a significantly shorter MFI (38 months) compared with cathepsin D negative women (64.5 months; $P = 0.003$). Patients with UPA-positive tumor cells in bone marrow (n = 98; 35%) had a significantly shorter MFI (44 months) compared with UPA-negative patients (MFI = 60 months; $p < 0.001$). The worst prognosis was