Method: The PD-ECGF/TP expression was evaluated by immunohistochemistry using a monoclonal antibody to PD-ECGF/TP in 117 patients with IBC.

Results: PD-ECGF/TP immunoreactivity was observed in both cancer cells and stromal cells (mainly in macrophages) in the invasive margin. Therefore, we evaluated the PD-ECGF/TP expression separately in cancer cells and stromal cells. Sixty-one (52.1%) cases were defined as PD-ECGF/TP-positive in cancer cells and 44 (37.6%) were positive in stromal cells. The PD-ECGF/TP expression in cancer cells did not correlate with any prognostic factors. However, the expression in stromal cells positively correlated with tumor size and microvessel count, and inversely correlated with estrogen receptor status. Significantly decreased relapse-free survival (RFS) and overall survival (OS) were found in patients with the positive expression of PD-ECGF/TP in stromal cells. A multivariate analysis using the Cox proportional hazards model showed that the PD-ECGF/TP positivity in stromal cells independently correlated with OS as well as nodal status and tumor size.

Conclusion: The PD-ECGF/TP expression in stromal cells correlates with tumor angiogenesis and may predict the prognosis of patients with IBC.

464 POSTER

Tumour angiogenesis by vascular grading is of prognostic significance in breast cancer

S. Hansen, D.A. Grabau, M. Bak, F.B. Sørensen. *Dept. of Oncology and dept. of Pathology, Carsten Rose; Dept. of Pathology, Odense University Hospital, Aarhus University Hospital, Denmark*

Purpose: The aim of the study was to evaluate the prognostic value of angiogenesis by vascular grading of primary tumours from breast cancer patients.

Material and Methods: The investigation included 841 patients. The median follow-up time was 12.5 years. Recurrence free survival (RFS) and breast cancer specific overall survival (OS) were observed. Adjuvant systemic treatment was given to 40% and radiation therapy to 42% of the patients. The microvessel endothelium was immunohistochemically stained by antibodies against CD34. Angiogenesis was determined by semiquantitative vascular grading, by subjective scoring into three groups according to the number of stained microvessels in the tumour section.

Results: The reproducibility of vascular grading between-observers was estimated to 0.59 (0.41; 0.83), (95% CI). Vascular grading was significantly associated with histologic type, histologic grade, tumour size, vessel invasion, and axillary nodal status. Vascular grading significantly predicted RFS and OS in both the node-negative and node-positive patients. The 5-years RFS probability ± SE for all patients was 78 ± 2% in vascular group one, 56 ± 3% in vascular group two, and 44 ± 3% in vascular group three. The Cox multivariate regression analysis showed that vascular grading contributed with independent prognostic value in all patients.

Conclusion: The angiogenesis determined by vascular grading has independent prognostic value both for patients with node-negative and node-positive breast carcinomas.

465 POSTER

Multivariate analysis of prognostic factors in lymph node negative breast cancer

J.S. Ross, P.J. Muraca, D. Jaffe, T. Morgan, J.A. Fletcher. *Albany Medical College, Dept. of Pathology and Laboratory Medicine, 47 New Scotland Avenue, Albany, NY 12208, USA*

A series of prognostic factors were tested for the prediction of early, late (LRec) recurrence and disease related death (DRD) in lymph node negative (LN−) breast cancer (BRCA) patients.

Design: Formalin fixed paraffin embedded primary LN-BRCA specimens from 220 women (median age 63 years) followed for a minimum of 120 months were evaluated for primary tumor size, histologic grade, estrogen receptor status (competitive binding assay), and HER-2/neu gene amplification by fluorescence in-situ hybridization (FISH). FISH was performed using the Oncor unique sequence HER-2/neu probe (Oncor, Inc. Gaithersburg, MD). No patients received adjuvant treatment prior to the first episode of disease recurrence.

Results: On univariate analysis HER-2/neu gene amplification by FISH independently predicted ERec; LRec ($p \ll 0.0005$); and DRD ($p \ll 0.0001$). When stratified into HER-2/neu non-amplified, borderline amplified and highly amplified groups, patients with highly amplified tumors had a relative risk (adjusted relative hazard) of ERec of 8.3 (range 2.1–32.4); LRec of 4.3 (range 1.7–11.0) and DRD of 11.0 (range 3.0–40.7). Tumor size, histologic grade and estrogen receptor status did not predict ERec, LRec or DRD. On multivariate analysis HER-2/neu gene amplification by FISH predicted ERec, LRec and independent of tumor size, grade and hormone receptor status.

Conclusions: In this series of LN BRCA patients, HER-2/neu gene amplification by FISH significantly predicted early and late disease recurrence and disease related death independent of tumor size, grade and estrogen receptor status.

466 POSTER

Independent role of p53 expression and reduction in kinetic cell activity in predicting clinical complete response (CR) to primary chemotherapy in breast cancer (BC) patients

A. Bottini, A. Berruti, A. Bersiga, M.P. Brizzi, A. Brunelli, F. Cirillo, S. Aguggini, G. Bertoli, P. Alquati, L. Dogliotti. *Centro di Senologia, Azienda Ospedaliera Istituti Ospitalieri, Cremona; Unviversità degli Studi di Tornino, Dipartimento di Scienze Cliniche e Biologiche, Azienda Ospedaliera S. Luigi, Oncologia Medica, Orbassano, Italy*

Mutant p53 and reduction in proliferative activity have been evaluated in order to assess whether they may play a role in modulating response of BC to primary chemotherapy. 145 patients bearing T2–4, N0–1, M0 primary BC were submitted to 2 different chemotherapeutic regimens before surgery. The first 64 received the CMF regimen (days 1.8 every 28) associated with tamoxifen (30 mg daily) in those with estrogen receptor positive (ER+) BC, the remaining 81 were submitted to single agent epirubicin (120 mg/m2 every 21 days). The expression of p53, bcl-2, Ki67, ER, progesterone receptor and c-erbB2 was evaluated in BC specimens obtained at diagnosis by incision biopsy and at post-chemotherapy surgery. 35 patients (24.1%) attained a clinical CR, 72 a partial response (PR) (49.7%), and 37 a stable disease (SD) (25.5%). p53 expression was significantly associated with a lower CR, the maximum difference being observed for a cut off of >20% positive cells (7.1% vs 28.2%, respectively; $p < 0.02$). p53 was a stable phenotype, only 4 cases with p53 negative BC before chemotherapy became positive afterwards. In multivariate regression analysis, p53 expression was an independent variable inversely associated with clinical CR, while reduction in Ki67 expression (>50%) was an independent variable directly associated. Menopausal status, T, N, histology grade, ER, PgR, c-erbB2, Ki67 and bcl-2 did not enter the model. The clinical CR mainly confined to p53 negative BC confirms in vivo the finding that the responsiveness of BC to chemotherapy in part derives from the capability of tumor cells to undergo apoptosis. The chemotherapy induced antiproliferative effect concurs independently in obtaining the tumor responsiveness.

467 POSTER

Menstrual phase and timing of breast cancer surgery: Statistical aspects

G. Haeusler, C. Tempfer, H. Heinzel, Ch. Kainz. *Dept. of Gynecology, Vienna University, Austria*

Purpose: The impact of menstrual cycle dependent timing of surgery on long-term outcome of breast cancer patients is still discussed controversially. It is our experience that many colleagues are dissatisfied with the uncertainty related to this potentially simple and beneficial therapeutic tool. New arguments would be helpful to explain the contradictory data reported in the literature. In accordance with McGuire (1991) and Jager and Sauerbrei (1995). we feel that the statistical side of the problem is of the utmost interest, since looking for the optimal splitting of the menstrual cycle is equivalent to cut-point searching involving a cyclic covariate. Referring to this problem Altman et al. (1994) described "optimal" cut-point searching in connection with a simple continuous prognostic factor. They reported an approximative formula by Lausen and Schumacher to be a useful tool for correction of the obtained minimum p-value.

Methods: Since the mathematical theory for an analogous correction in case of a cyclic covariate such as the menstrual cycle is not yet available, we designed a simulation study using randomly generated exponentially distributed survival data. We randomly assigned a menstrual cycle value between 1 and 28 days to every survival time. We varied the sample size ($n = 140$, $n = 280$, $n = 1400$), the amount of censoring (33% and 67%) and the minimum selection interval (between 7 days and 14 days). When using minimum lengths of the selection interval ranging from 7 days to 14 days, a total of 210 different partitions were possible. We generated 2000 simulated samples for each of these 210 scenarios.

Results: Neither the sample size nor the amount of censoring had any remarkable influence on the inflation of type I error rate (Fig. 1a + 1b).

Choosing a selection interval of 14 days yielded a type I error rate of about 27% at the common 5% nominal level. In other words there were significant results in I out of 4 cases, although there was nothing to detect according to the design of this simulation study. At a nominal level of 10% and 1% the false positive rates were 47% and 7.3%, respectively. The multiple testing problem became more evident allowing a minimum selection interval ranging from 7 to 14 days. The type I error rate increased to 63% at a nominal level of 5%. Even if we used the 'impressive' 1% nominal level, we got a significant result in 25% of the tests. The simulation study showed that for achieving an actual type I error rate (significance level) of approximately 5%, a P-value < 0.006 was necessary, using a selection interval of 14 days. This barrier dropped to a P-value < 0.001, when we used minimum selection intervals ranging from 7 days to 14 days.

Results: In view of the data described above and the works of Altman et al. (1994) it seems absolutely necessary to integrate the number of performed tests in the evaluation of a prognostic factor, which was (even if more tests have been performed) at least not mentioned by those authors who found a statistically significant benefit for menstrual cycle dependent timing of surgery on long-term outcome of breast cancer patients. Our results underline the necessity of cautious statistical interpretation when dealing with a cyclic covariate such as the menstrual cycle.

468 POSTER

A comparison of biochemical quantitative and immunohistochemical detection methods for the detection of the erbB2 oncoprotein in breast cancer tissue

C. Giersig, F. Opri, U. Torsten. *Dept of OB and GYN at the University Hospital Benjamin Franklin, Free University of Berlin, Germany*

Aim: The management of breast cancer depends upon prognostic factors which allow separation of patients into high and low risk groups. Reviews of studies concerning the overexpression of the c-erbB2 oncogene in breast cancer tissue attribute different prognostic value to DFS and OST. Why so?

Material and Methods: The quantitatively and qualitatively analyzed grade of overexpression of the c-erbB2 oncoprotein has been evaluated in 101 breast cancer samples: biochemically via Western Blot analysis, immunohistochemically with the ABC on paraffin sections using two different monoclonal antibodies (CB 11 and 3B5). The quantification of the immunoblots was performed by densitometric measurement.

Results: With the CB-11 mAb, the biochemical detection showed an overexpression of the c-erbB2 oncoprotein in 34.6%, the immunohistochemical procedure in 27.7% of the cases. The 3B5 mAb has given a weak staining signal in the stroma of all examinated sections. Additinal 5 tumors with a strong staining signal in the tumour cells were found positive on 3B5 mAb, however showed to be negative in the biochemical procedure or by the use of CB-121 Ab in either method.

Conclusion: The difference in statements concerning the prognostic value of the c-erbB2 oncoprotein in breast cancer may in great parts be attributable to the different technical procedures used in the studies.

469 POSTER

Expression of cytosolic thymidine kinase in the proliferative breast carcinoma after primary chemotherapy: Therapeutic indication

F. Gachon, S. Charrier, F. Kwiatkowski, G. Besse, H. Curé, J. Chassagne, J.P. Ferrière, F. Penault-Llorca, J. Dauplat, P. Chollet. *Centre Jean Perrin and INSERM U484, BP 392, 63011 Clermont-Ferrand Cedex 1, France*

Thymidine kinase (TK) activity has been assessed in breast cancer after primary chemotherapy in relation to clinical and pathological response, proliferative activity and hormonal receptors. TK was assayed in the cytosol of 76 patients treated by induction chemotherapy: 26 AVCF/M, 32 NEM and 18 TNCF (Ecco 8, 95, S13, 53). After surgical resection of the remaining tumor, the enzyme activity was measured using a radioenzymatic method. High levels of TK (>30 U/mg protein) were found with 89% specificity and 64% sensitivity in residual invasive carcinoma. Conversely, TK activity was lower with presence of only *in situ* carcinoma or alterated cells residual after treatment. TK rate was positively correlated to the remaining tumor size ($p < 0.0001$) and aneuploidy ($p < 0.001$). TK was negatively correlated to the clinical complete response with a mean rate of 55 versus 92 U/mg protein for partial responses or no change ($p < 0.02$). Moreover, the mean TK activity measured for patients treated by TNCF, the most intense and effective regimen in breast cancer (51% of complete clinical and 30% of complete pathological responses), was lower. 57 *versus* 90 U/mg protein for the other two protocols ($p < 0.01$). TK was also increased in tumors with positive estrogen and progesterone receptors ($p < 0.001$ and $p < 0.04$).

In conclusion, after primary chemotherapy, TK expression was directly related to the residual active tumor amount (aneuploidy, invasion and size). We intend to increase our experience to examine more clearly if a high residual TK is rather a marker of residual proliferation capacity (i.e. resistance), or a biological factor linked to hormonal sensitivity.

470 POSTER

In vivo bromodeoxyuridine (BrdUrd) labeling index as a prognostic marker in human breast cancer

W.H. Goodson[1], D.H. Moore[1,2], B.-M. Ljung[3], K. Chew[2], B. Mayall[2], H.S. Smith[1], F.W. Waldman[2]. *[1]California Pacific Medical Research Institute, San Francisco, CA, 94115; Depts [2]Laboratory Medicine; [3]Pathology, University of California, San Francisco, CA, 94143, USA*

Background: A practical prognostic index that works in 100 percent of cases is needed to measure cell proliferation in human breast cancers.

Methods: With informed consent, 133 women received 200 mg/M^2 BrdUrd preoperatively. IU4 antibody was used to measure the Labeling Index (LI) of DNA-incorporated BrdUrd in 2,000 cells. Ki-67 LI was determined with the MIB1 antibody (121 cases) and S-phase by flow cytometry (95 cases). Follow up was 2 to 8 years years. Patients were divided into groups above and below the median for each LI. Survival was compared between groups of women with each LI above and below the median with the Mantel-Cox test and univariate and multivariate analysis.

Results: Follow up was 100 percent. Women in the low BrdUrd LI group had significantly better disease free survival (DFS; $p = 0.0008$) and overall survival (OS; $p = 0.0004$). Ki-67 predicted a trend ($p = 0.06$) for better DFS and OS. Low S-phase predicted better OS but not DFS.

Conclusions: BrdUrd LI is a significant prognostic index which is superior to Ki-67 and S-Phase by flow cytometry.

471 POSTER

Tc-99 Tetrofosmin scintimammography in determining prognostic characteristics of breast cancer

F. Flanagan, D. Bergin, N. Phelan, J.T. Ennis. *Mater Hospital, Breast Screening Unit, Dublin, Ireland*

Purpose: To compare scintigraphic features of breast lesions imaged with scintimammography with tumour prognostic factors.

Materials & Methods: 36 consecutive patients with a clinically palpable abnormality requiring tissue diagnosis were examined with scintimammography prior to surgical intervention. Histological features were compared to scintigraphic characteristics.

Results: Out of the 36 cases evaluated 19 were benign and 16 were malignant. The average tumour to background ratio (TBR) in malignant cases was 2.1 (range = 1.4–4.0). There was a good correlation between size of tumour measured pathologically and by scintimammography ($r = 0.8$). There was no correlation between TBR and size of tumour or TBR and tumour grade. Patients with ER negative tumours tended to have a higher TBR. Scintimammography correctly categorised lymph node status in 10 out of the 13 patients who had axillary lymph node dissection. Unsuspected substernal lymph nodes were detected in a single patient.

Conclusion: Scintimammography may not only play a role in discriminating benign from malignant lesions but may also be useful in determining tumour prognostic factors in-vivo.

472 POSTER

HER-2/neu oncogene amplification in breast cancer

T.P. Brien, C.E. Sheehan, P.J. Muraca, S. Stylos, R.L. Johnson, J.S. Ross. *Albany Medical College, Dept. of Pathology and Laboratory Medicine, 47 New Scotland Avenue, Albany, NY 12208, USA*

The HER-2/neu oncogene is localized to chromosome 17q and shares significant homology with the epidermal growth factor receptor. HER-2/neu protein overexpression has been associated with poor prognosis in breast cancer.

Design: Formalin-fixed paraffin-embedded primary breast cancer tissues from 128 women (mean age 60 years) were tested for HER-2/neu gene amplification by automated (Ventana Gen II, Tucson, AZ) fluorescence in-situ hybridization (FISH) using the Oncor unique sequence probe (Oncor, Inc., Gaithersburg, MD). The tumors were also evaluated immunohistochemi-