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).