the ability of immunohistochemical markers to predict survival in a large series TN breast cancer.

**Materials and Methods:** We identified 147 TN breast cancers among 625 consecutive invasive breast cancers by immunoprofiles for ER, PR and HER2 and compared clinicopathologic characteristics and patients' survival between TN and non-TN breast cancers. The TN cancers were further subclassified by staining for cytokeratin (CK) 5/6, epidermal growth factor receptor (EGFR), vimentin, c-Kit, p63, P-cadherin, CK8 and CK18. We then applied 4 different criteria to define basal-like phenotype in TN breast cancers (criteria 1: CK5/6+ only, criteria 2: CK5/6+ and/or EGFR+, criteria 3: CK5/6+ and/or EGFR+ and/or vimentin+, and criteria 4: one or more marker(s) positive among CK5/6, EGFR, vimentin, c-Kit, p63 and P-cadherin). Each of these criteria, as well as each individual marker, was then evaluated for prognostic significance by survival analysis.

**Results:** Among the 147 (23.5%) TN breast cancers, 138 (93.9%) patients received chemotherapy and median overall survival (OS) of TN patients was 64 months (range, 8–185 months). Compared with non-TN breast cancers, TN cancers showed larger tumor size and higher histologic grade, but fewer lymph node metastasis. In addition, patients with TN breast cancer had reduced OS within 6 years of diagnosis but not thereafter. Using immunohistochemical markers to define basal-like cancers among the TN breast cancers, we noted positive staining for CK5/6+ in 35.4%, EGFR+ in 16.3%, vimentin+ in 28.6%, c-Kit+ in 11.6%, p63+ in 8.0% and P-cadherin+ in 43.8%. Using the criteria outlined above, we defined 52 (35.4%) cases as basal-like by criteria 1, 65 (44.2%) by criteria 2, 82 (55.8%) by criteria 3 and 113 (76.9%) by criteria 4. Remarkably, basal-like phenotype defined by any of these criteria did not show survival difference from non-basal phenotype in TN breast cancers. Interestingly, however, luminal CKs, 8 and 18 were also commonly expressed in TN breast cancers (55.1% and 45.6%, respectively), and TN breast cancers expressing CK8 and/or CK18 showed reduced OS ($p = 0.002$) and disease-free survival ($p = 0.011$).

**Conclusions:** In our series, TN breast cancers showed poorer prognosis within 6 years of diagnosis than non-TN breast cancers. However, there was no survival difference between basal and non-basal phenotypes as defined by immunohistochemical profiles of 6 basal markers in TN breast cancers. By contrast, expression of luminal CKs appears to identify a more aggressive subgroup of TN breast cancers.

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**Prognostic significance of triple-negative phenotype in breast cancer**

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**Background:** Triple-negative breast cancers (TNBC) are defined as a lack of expression of estrogen, progesterone receptors and HER2. Recently gene expression analysis identified several breast cancer subtypes including basal-like, ER−/HER2+, luminal type and this transcriptionally basal type is compatible with histological TNBC. This TNBC subgroup accounts for 15% of all types of breast cancer and high relapse rate with poor prognosis. The aim of this study was to evaluate the clinico-pathological characteristics and prognosis in breast cancer patients who expressed triple-negative phenotype on immunohistochemistry and to compare this TNBC subgroup to the others.

**Materials and Methods:** Three hundred forty-eight patients who underwent curative breast cancer surgery from January 2000 to December 2005 were analyzed retrospectively. The IHC method was used to define ER, PR and HER2 expression status. HER2 was scored positive if 3+ result was found or amplified gene expression on FISH. According to this method, total patients was divided to three subgroups; Hormone receptor positive (HR+), HR negative/HER2 positive (HR−/HER2+) and TNBC.

**Results:** Sixty-seven cases (19.3%) of 348 patients showed triple-negative expression pattern and forty five cases (12.9%) showed HR−/HER2+ expression pattern and the others showed HR+ expression pattern. The TNBC subgroup was associated with a high tumor grade and high relapse rate. Other characteristics such as age, stage, lympho-vascular invasion, relapse site showed no difference between three subgroups. The 5YSR of HR+, HR−/HER2+ and TNBC subgroup were 94.0, 83.2 and 88.7% ($p = 0.013$) and 5 year DFS rate were 87.4, 75.1 and 79.7% ($p = 0.011$). In multivariate analysis, risk group by IHC was the only independent prognostic factor and TNBC phenotype showed the worst outcome (HR 3.85, 95% CI 1.41−10.52, $p = 0.008$).

**Conclusion:** Both TNBC subgroup and HR−/HER2+ subgroup showed significantly worse prognosis than HR+ subgroup and there was no significant difference between these two subgroups. In multivariate analysis, TNBC showed the significant prognostic variable in breast cancer and to overcome these characteristics of TNBC, it is need to find another powerful molecular targeted agents or new cytotoxic drugs.

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**Activated Leukocyte Cell Adhesion Molecule (ALCAM/CD166) predicts response to adjuvant chemotherapy in breast cancer**

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**Background:** Activated Leukocyte Cell Adhesion Molecule (also known as ALCAM or CD 166 and MEMD) functions as cell surface immunoglobulin and is recently reported as possible prognostic marker in breast cancer. Its predictive value regarding adjuvant chemotherapy has not yet been evaluated.

**Material and Methods:** To evaluate ALCAM expression levels in the primary tumor tissue ALCAM mRNA expression was analyzed by cDNA microarray analysis in 162 patients (100 of them received adjuvant chemotherapy) and ALCAM protein expression was analyzed by Western Blot analysis in 160 patients (among them 87 received adjuvant chemotherapy). Both results were obtained in 133 cases. A strong positive correlation between protein and mRNA expression ($p < 0.001$) was observed.

**Results:** Using statistical analysis a stratified subgroup analysis showed positive correlation of high ALCAM mRNA expression with longer overall survival (OAS; $p = 0.0012$) in patients treated with adjuvant chemotherapy regimens ($n = 100$). In contrast, patients with high ALCAM mRNA expression who did not receive chemotherapy tended to have a worse prognosis than those with low ALCAM mRNA levels. Similar but statistically weaker correlations were found regarding ALCAM protein expression data. The predictive impact of ALCAM mRNA expression in chemotherapy treated patients was corroborated by multivariate Cox regression analysis also including histopathological markers like histological grading, nodal involvement, ER status, clinical stage and the logarithmic values of ALCAM mRNA expression ($p = 0.001$ for OAS).

**Conclusion:** High ALCAM expression levels in primary breast cancer might be a suitable marker for prediction of response to adjuvant chemotherapy.

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**Urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 for tumor-biological risk assessment in node-negative breast cancer patients – The multicenter trial NNBC 3-Europe**

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**Background:** Various groups started clinical trials aimed at a feasible way to improve risk-assessment by testing biological parameters based on RNA or protein level. Current ASCO guidelines allow the use of biological risk assessment by the invasion factors uPA/PAI-1 (Harris et al. 2007). In 2003 we launched the NNBC 3-Europe trial with the following questions:

1. Is risk-assessment by the invasion markers uPA and its inhibitor PAI-1 more effective than by clinico-pathological factors (St. Gallen 2005) with regard to identification of low-risk patients?
2. Is adjuvant chemotherapy using an anthracycline-taxane sequence (FEC-Docetaxel) superior to standard FEC in high-risk patients?

**Study Design:** Risk assessment was performed either by St. Gallen 2005 or by the invasion markers uPA/PAI-1. In low-risk patients, no adjuvant chemotherapy is given. High-risk patients receive adjuvant chemotherapy according to randomisation: FEC-Doc versus FEC. Adjuvant endocrine therapy is given according to current AGO guidelines.

**Results:** To date, 109 centres participate, 2308 patients have been registered. Overall, in 811 patients chemotherapy could be avoided, 687 were randomized to receive FEC-Doc, 683 to FEC. Biological risk assessment was performed in 1710 patients. Using both, grading and uPA/PAI-1 results, the low-risk group comprised 39% of the patients.

**Discussion:** The NNBC-3 Europe trial shows that risk assessment based on biological testing of fresh frozen tumor material is feasible. The study is planned to recruit 5.700 patients and it is performed in cooperation

with the EORTC PathoBiology Group, the German AGO Breast Group, and the GBG GmbH (German Breast Group).

Unrestricted grants by Sanofi-Aventis and Pfizer and NBL Funding Martin-Luther-University Halle-Wittenberg (KFZ 15/29).

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**Serum lactate dehydrogenase (LDH) is a significant prognostic variable for survival in patients with metastatic breast cancer – a multivariate analysis**

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**Background:** Advanced breast cancer that metastasizes to bone increases the risk of skeletal-related events that can be debilitating and potentially life-limiting. However, detailed evaluations of prognostic factors for survival in patients with advanced breast cancer and bone metastases (mets) have not been reported. Therefore, we conducted a retrospective analysis of data from a pamidronate-controlled trial of zoledronic acid (ZOL) in patients with bone mets from breast cancer to investigate potential prognostic variables for overall survival.

**Material and Methods:** Only patients treated with ZOL (n = 435) who had assessments of biochemical markers of bone metabolism and complete data sets of baseline (BL) variables were included in this analysis. The 23 BL variables assessed included demographics, disease history and distribution, FACT-G, ECOG performance status (PS), type of bone lesions, bone mets history, and laboratory assessments of bone markers, blood counts and markers of renal and hepatic function, including LDH. Multivariate analyses were used to assess risk ratios (RRs) for death over 24 mo. A reduced model was generated by stepwise backward elimination until only significant ($P < 0.05$) variables remained.

**Results:** Although many variables, including bone marker levels, were significant prognostic factors in univariate models, only 7 factors remained significant in the reduced multivariate model. In this model, advanced age correlated with increased risk of death vs patients $\leqslant$50 yr old (50−60: RR = 1.83, $P < 0.01$; 60−70: RR = 1.78, $P = 0.01$; >70: RR = 2.53, $P < 0.01$). Other significant variables included: impaired vs fully active ECOG PS (RR = 1.74; $P < 0.01$), prior vs no prior chemotherapy (RR = 1.97; $P < 0.01$), FACT-G score <65 units ($P < 0.05$ for comparisons with $\geqslant$75 units), presence of lytic vs mixed bone lesions ($P = 0.02$), weight <60 kg ($P < 0.02$), and LDH levels. Patients with LDH $\geqslant$ULN but <2$\times$ULN had a 2-fold increased risk of death, and LDH >2$\times$ULN correlated with a 6-fold increased risk of death vs LDH <ULN ($P < 0.0001$ for both). Although on-treatment levels of bone markers have been correlated with survival previously, bone marker levels at BL were not significant in the multivariate model.

**Conclusions:** This model confirms the significance of previously described prognostic factors such as age and ECOG PS. However, the finding that LDH level correlates strongly with survival is new in the breast cancer setting and merits further investigation.

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**Re-evaluation of the old preoperative prognostic markers CA 15-3 and CEA using a cohort of 1093 patients treated for breast cancer 1998–2006 at a single institution**

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**Background:** Prognostic markers are essential for choosing the individual therapy for patients with newly diagnosed breast cancer. Using our own cohort from 1998–2006 we studied this group concerning preoperative serum CA 15-3, CEA and outcome.

**Material and Methods:** Since 1998 all patients with breast cancer were entered in our tumor registry. The data base (in SPSS) is maintained by a study nurse. Once yearly follow-up is obtained using our own out-patient clinic data, information from general practitioners and the general cancer registry. Informed consent is taken from the patients at time of diagnosis. CA 15-3 or CEA are defined elevated if above 25 U/ml or 4.6 $\mu$g/l respectively.

**Results:** 1093 patients with newly diagnosed primary breast cancer were evaluated in this study. Mean follow-up was 34 months. Clinico-pathological features as well as survival pattern of the cohort were found similar as those described in the literature. Independent prognostic factors were nodal status (N), hormone receptor status (HR), tumor grading, tumor size and age. Twenty-five or five percent of the patients showed elevated preoperative CA 15-3, or CEA, respectively, whereas in 5% of the patients the levels of both tumormarkers were increased.

The Cox model and subgroup analysis revealed that patients of the age group 35−50 years with elevated CEA levels had a significantly poorer overall survival (OAS) ($p = 0.0005$) than patients with normal CEA levels. An increase in CA 15-3 levels even negatively affected both disease-free (DFS) ($p = 0.0002$, for age group 35−50 yrs) and overall survival ($p = 0.0002$, for age group 35−65 yrs). Multivariate analysis further showed that CEA is an independent prognostic factor for overall survival adjusted for T, N, HR and age ($p = 0.012$). CA 15-3 was found to be an independent prognostic factor for both overall and disease-free survival adjusted for the same factors ($p = 0.003$ and $p = 0.006$). The outcome of a small group of patients with elevated CEA levels alone or in combination with higher CA 15-3 levels is poorer than the outcome of patients with elevated CA 15-3 levels alone (DFS $p = 0.021$, OAS $p = 0.008$).

**Conclusions:** Preoperative levels of CA 15-3 and CEA are of prognostic value for patients that have been newly diagnosed with breast cancer. More patients showed elevated CA 15-3 levels than higher CEA levels. Since measuring CA 15-3 and CEA is easy and not expensive, this method could be recommended as a routine method to obtain additional information on the patient's prognosis.

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**Persisting risk after BCT in N+ and N− patients – a single institution analysis from 1485 patients**

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**Background:** To find risk groups for local recurrence (LR) and disease free survival (DFS) for node-negative (N−) and node-positive (N+) patients after breast-conservation surgery (BCS) + radiotherapy (RT) $\pm$ adjuvant systemic therapy (ST).

**Material and Methods:** From 1984 to 1997, 1485 patients with breast cancer underwent BCS, ST and RT in our institution. Complete data were available in 1347 patients: N−: 958, N+: 389 patients. Recursive partitioning analysis was carried out to find cut points in the prognostic variables with a $p \leqslant 0.05$ for the end points local recurrence (LR) and DFS. Co-variates included were age, T-stage, N-stage, ratio of involved lymph nodes and excised nodes (NR), location of the index tumour (Loc), ST (chemo/horm), ER/PR status, menopausal status and the presence of surgical marker clips. The relative hazard ratio (RHR, HR relative to median patient) was estimated in sub-groups of at least 20 patients, as well as the 10 yr. DFS.

**Results:** After a mean f/u of 107 months the rate of LR at 10 years was 6.3% (N−: 5.5%, N+: 8.6%), and the DFS was 79.8% (N−: 83.9, N+: 68.4). For N−: For LR hormone therapy was the most relevant variable, followed by Loc and age. For DFS again the application of hormones is most important, followed by age, T-stage and Loc. For N+: For LR age is the most relevant prognostic factor, followed by T-stage and PR. For age the cut point is at 38.5 years. The 10 yr. LR rate for the elder ones is 93.5 years, for the younger ones 63.3 years. For DFS the N-ratio is the most significant prognostic factor: cut off at 48.5%, 10 year results: elderly: 72%, younger: 32%. In the next level age follows, with a cut off at 37.5 years and the most extreme difference in the 10 year DFS of 45% (75.6 vs. 30.6 years). In the 3rd level PR becomes relevant. All results presented are significant: $p \leqslant 0.05$.

**Conclusion:** After standard therapies for BCT certain situations in prognostic factors result in an unfavourable outcome. In the N neg group the lack of hormone therapy results in a negative outcome. Tumour location may influence local control and disease free survival; the medial tumours reveal the worst prognosis. They may need more aggressive systemic therapy and probably radiotherapy to the internal mammary chain.

In N+ patients age is the most relevant factor followed by tumour size and PR for LR; for DFS the NR is the most significant variable followed by age, tumour size and PR. The NR has an impact on survival only, but not on LR. Compared to the common prognostic factors more attention should be paid to the application of hormones, to PR neg patients and to medially located tumours in terms of a more aggressive therapy.