for tumor cells in bone marrow aspirate immunocytologically with antibody to cytokeratin KL-1 before treatment and 11 pts – after the treatment. Time to progression (TTP) and overall survival (OS) were analyzed to find out whether the presence of CTC influenced prognosis in ABC.

Results: At least one per million CTC are found in 41.3% of patients with ABC before systemic treatment and in 45.5% of patients – after treatment. The CTC level is not related to site or number of metastatic tumor, not to receptor status of the primary breast tumor. The CTC are found more frequently in menopausal than in premenopausal woman (47.8% vs 26.7%, $p = 0.044$). Patients with infiltrative lobular BC present with CTC significantly more frequently than those with infiltrative ductal BC (69.2% vs 31%, $p = 0.02$). Bone marrow involvement has no influence on CTC detection frequency. CTC-positivity has no effect on OS or TTP of patients with ABC. Analysis of CTC levels before and after systemic treatment demonstrated that patients with diminution of CTC number after treatment had significantly longer time to disease progression than those with increased CTC levels after treatment (14 vs 8 months, $p = 0.0127$). HLA-DR expression on CTC was found in 82% of patients (mean percentage of HLA-DR+CTC was 31.6%), CD95 was expressed in 33% of patients (mean percentage of CD95+CTC was 14.8%).

Conclusion: Increase in CTC levels after specific anticancer treatment may be a new useful, objective measure of response and an informative unfavorable prognostic factor in patients with ABC.

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Gene expression analysis of the insulin- and estrogen signalling system and their influence on clinical parameters of breast cancer patients

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Background: Obesity is thought to increase breast cancer risk both through high activity of estrogen metabolizing enzymes and by associated high insulin and glucose serum levels directly impacting on proliferation and apoptosis of breast cancer cells. In the present study, we investigated co-expression of genes of the insulin/IGF axis and the estrogen signaling system in correlation to clinical parameters in collected breast cancer specimen.

Materials and Methods: Breast cancer tissue and fasting serum was collected from 26 female patients. After microdissection of the frozen samples, RNA was isolated and expression of genes of the estrogen- and insulin/IGF signaling was measured by real time RT PCR. Fasting insulin, glucose and C-peptide as well as estradiol serum level were analysed by ELISA. Insulin resistance was calculated by the HOMA method.

Results: Postmenopausal women older than 70 years showed significant higher expression of estrogen receptor (ER) α as well as steroid sulfatase (STS) and were more likely insulin resistant than premenopausal women younger than 50 years. A strong significant association between vascular/lymphovascular invasion (L1, V1) and BMI as well as insulin resistance could be identified. Both, ERα and STS expression were significantly associated with expression of insulin receptor, IGFR1 and IGFBP4 but not IGFR2. Higher expression of IGFR1 was associated with a better histological grading, whereas higher expression of IGFR2 correlated with lymph node negativity.

Conclusion: In conclusion, the observed co-expression of components of the insulin/IGF signaling with ERα and steroid sulfatase supports the hypothesis that a close cross talk between both pathways is present in breast cancer cells. The observed correlation of insulin resistance with vascular invasion encourages further studies on larger case numbers to further examine the relevance of this association in the clinical situation.

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CAV1 and CAV2 are associated with breast cancer basal-like and triple negative immunophenotype

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Background: Caveolin-1 (CAV1) and caveolin-2 (CAV2) are the principal structural proteins of caveolae, sphingolipid and cholesterol-rich invaginations of the plasma membrane involved in vesicular trafficking and signal transduction. Over the recent years there has been controversy about their role in breast cancer and their suitability as markers of basal-like phenotype.

The aims of this study were threefold: (1) To assess the prevalence of CAV1 and CAV2 in a well-characterized series of invasive breast carcinoma using high-throughput tissue microarrays (TMAs) and immunohistochemistry. (2) To determine whether CAV1 and CAV2 could be used as diagnostic marker to identify basal-like phenotype or the triple negative (TN) subtype of invasive breast cancers (3) finally to identify if CAV1 and CAV2 have any prognostic effect on the patient outcome in invasive breast cancer.

Material and Methods: CAV1 and CAV2 protein expressions were assessed on a tissue microarray containing 880 unselected invasive breast cancer cases, by means of immunohistochemistry.

Results: CAV1 and CAV2 expression was observed in 13.4% and 5.9% of all breast cancer, respectively. Their high expression was strongly associated with high histological grade, lack of steroid hormone receptor positivity (ER and PR), and expression of basal markers (basal cytokeratines, P63, P-cadherin). Furthermore there was a significant association between CAV1 and CAV2 expression and basal-like phenotype. On univariate analysis only CAV2 had a prognostic effect on breast cancer-specific survival; however, this was not independent from other traditional markers on multivariate analysis.

Conclusion: Our results demonstrate that both CAV1 and CAV2 are associated with basal-like phenotype. Further studies are warranted to determine whether they play a role in basal-like/triple negative breast cancer development or are just surrogate markers for this subgroup.

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Relation of intratumoral B-Cells and response to neoadjuvant chemotherapy

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Introduction: Tumor-infiltrating immune cells have contradictory effects on tumor growth and angiogenesis. In early stage cancer, intratumoral T Cells attenuate tumor progression, as shown for node negative colorectal cancer, and are a better predictor of patient survival than the standard histopathological staging. Conversely, in later stages, cancer cells recruit macrophages to release mitogenic factors (e.g. EGF), to remodel the extracellular matrix and to facilitate angiogenesis. However, hardly any data exists on the influence of the immune response of breast cancer patients receiving neoadjuvant chemotherapy in particular in view of antibody based chemotherapy. In this study we analyzed the expression of IGHM as a marker of the presence of B cells in samples of tumor tissue of patients treated with anthracycline and trastuzumab in relation to therapy response.

Patients and methods: Breast cancer patients ($\geqslant$cT2, N0/N1, M0) received neoadjuvant chemotherapy of 4 cycles of epirubicin and cyclophosphamide followed by 4 cycles of paclitaxel (PREPARE trial). Her-2/neu positive tumors were treated with trastuzumab in a three weekly dose regimen (TECHNO trial). Tumor cell content and histology were centrally determined from a HE stained reference slide. RNA was isolated from tissue sections of 10μm thickness by an automated system based on magnetic beads (Siemens Medical Solutions Diagnostics). IGHM was analyzed in an initial group of 56 patients (33 TECHNO/23 PREPARE) by TaqManPCR. IGHM expression was correlated with ESR1, Her-2/neu, TOPO2A and VEGF expression and to histopathological findings in excised tumors.

Results: 10 of the 56 patients included in the preliminary analysis showed a full remission at histopathological evaluation. There was a significant difference of IGHM-RNA expression for those patients with histopathologically complete response to systemic therapy and those with no or partial response (Mann-Whitney-U, $p = 0.047$). Patients with a complete response showed higher levels of IGHM-RNA indicating presence of B-cells. Interestingly, IGHM expression correlated with increased proliferation and vascularization as determined by TOPO2A (Spearman $r = 0.41$; $p = 0.0008$) and VEGFC (Spearman $r = 0.43$; $p = 0.0005$) RNA expression, but not with ESR1 and Her-2/neu status.

Conclusion: In advanced breast cancer, the presence of B cells correlates with proliferation and angiogenetic activitiy. Also, the presence of B cells is increased in tumors that respond to chemotherapy. These data support the hypothesis that the immune response has an influence on the behavior of breast cancer tumors. The analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in breast cancers patients.