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

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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)

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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

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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

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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

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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

seen in patients positive for both 2E11 and one of the two additional markers (UPA or cathepsin D).

The detection of tumor cells in bone marrow is an excellent prognostic marker for the development of metastatic disease. Patients with positive UPA/cathepsin D detection in micrometastatic cells have a worse prognosis and should be considered for a more aggressive adjuvant systemic therapy.

P42 Prognostic significance of mutant p53 in breast cancer patients

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The p53 oncosuppressor gene has a negative influence on cell proliferation. Expression of mutant p53 is frequently seen in breast tumors and has been significantly related with clinical outcome.

At the moment its prognostic role is discussed and this biological parameter is not currently included in decision making about adjuvant therapy in breast cancer.

In order to verify its prognostic role we assessed p53 status in 90 operable breast cancer patients (pts), with a median age of 64 years and a mean follow up period of 31 months. Metastases in axillary nodes were present in 45% of cases; positive estrogen receptor status in 64% and median Ki-67 index was 20.2%. Cut-off level for p53 status was fixed at 5%.

Mutant p53 immunohistochemistry was carried out on formalin-fixed, paraffin embebbed tumor specimens. Surgical and medical adjuvant therapy followed the guidelines of the most important international consensus conferences.

Our data are summarized in the following table and show a significant relationship between p53 status and relapse of disease ($p = 0.01$, Fisher's exact test).

Mutant p53	Relapsed PTS	Disease-free PTS
positive	12	30
negative	4	44

The small number of deaths in our series is not sufficient for statistical analysis but an interesting trend is confirming a negative impact of mutant p53 status on survival.

In our experience mutant p53 status influenced the risk of relapse and, probably, the overall survival. The increasing value of prognostic factors needs a more relevant role in the choice of medical adjuvant therapy of breast cancer.

P43 Evaluation of bone marrow micrometastases as potential surrogate marker for efficacy of adjuvant treatment

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Early occult dissemination of tumor cells to bone marrow (BM) which is detectable in approximately one third of breast cancer patients at the time of diagnosis is assumed a main reason for metastatic relapse. This may provide a rationale for adjuvant treatment. However, currently no concise data are available on the treatment-susceptibility of BM micrometastases. In an ongoing prospective randomized adjuvant trial, node-positive (>4 nodes involved) breast cancer patients were treated with 6 courses of DE (q21d), docetaxel (75 mgm^{-2})-epirubicin (60 mgm^{-2}), *vs* 4 courses of EC (q21d), epirubicin (90 mgm^{-2})-cyclophosphamide (600 mgm^{-2}) followed by 3 courses of CMF (q21d), cyclophosphamide (600 mgm^{-2})-methotrexate (40 mgm^{-2})-fluorouracil (600 mgm^{-2}). Our hypothesis was that clearance of BM from micrometastatic tumor cells is a surrogate marker for treatment efficacy translating into improved disease-free survival. In follow-up BM asapirations, disseminated tumor cells were detected using anti-cytokeratin (CK) monoclonal antibody A45-B/B3. To date, 17 patients have been randomized. Prior to treatment CK^+ cells were detected in 6 of 17 patients (35%). After treatment all previously CK^+ patients treated with DE experienced elimination of CK^+ cells; two of three patients treated with EC/CMF remained CK^+. One, respectively two negative-to-positive transitions were notified in patients treated with DE and EC/CMF. In a total of 53 DE courses (10 patients) and 41 EC/CMF courses (7 patients), side-effects of NCI grades 3 or 4 included hematoxicity (83% *vs* 39%; $P < 0.0001$), alopecia (100% *vs* 63%; $P < 0.0001$), allergic reactions (2% *vs* 0%; P = N.S.). No early cardiotoxicity, fluid retention, febrile neutropenia from chemotherapy as well as no osteomyelitis or hemorraghia from BM aspirations have been observed so far. G-CSF was administered for neutropenia (<500/μl) in 44 DE courses (83%) and 16 EC courses (39%; $P < 0.0001$). In conclusion, our preliminary data suggest that DE might be more effective to eliminate CK^+ cells from BM than EC/CMF. Of course, the low number of patients analysed so far, revealed no significant difference, but an early trend in favor of our hypothesis. Overall, DE was well tolerated in the adjuvant setting, although hematoxicity appeared to be a limitating factor of this combination. Follow-up BM aspirations did not add any notable risk for patients' health. Clinical follow-up will have to demonstrate whether persistence and disappearance of micrometastases are related to treatment-resistance and -susceptibility, respectively.

P44 Age is not a prognostic factor in breast cancer patients with combined losses of heterozygosity (LOH) in BRCA1 and BRCA2 regions

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Aim: Old patients with breast cancer can exhibit a more indolent behavior than young patients. However, this assertion can be changed by alterations in specific genes or chromosomic regions. The cloning of susceptibility genes for breast cancer, BRCA1 and BRCA2 and the study of their chromosomic regions, offer the opportunity of to know their implications like prognostic factors.

Methods: We investigated the presence of LOH in 17q21 and 13q12–13 regions. The molecular findings were correlated with the following pathological parameters: histologic type, tumor size, lymph node metastases, oestrogen receptors, progesterone receptors, histologic grade and peritumoral vessel involvement, in 98 patients with sporadic breast cancer. For the allelic deletion, four microsatellite markers were studied in BRCA1 region (D17S855, D17S856, D17S1323, D17S1327) and two in BRCA2 region (D13S310, D13S260), they were amplified by PCR method and the products were run in 12% nondenaturing polyacrilamide gels and stained with a commercially available silver method.

Results: After analyze the normal tissue of all patients, 49 cases resulted informative for both regions. Fifteen women did not show LOH in any microsatellite marker of both regions; 9 patients displayed LOH at least in one marker of the BRCA1 region; 10 cases in the BRCA2 region and 15 patients exhibited LOH concomitantly in some marker of the two regions. When we compare the pathological parameters between patients with LOH at both regions and those without LOH we observed statistically significant differences in lymph node metastases ($p = 0.0002$), oestrogen receptors ($p = 0.02$), progesterone receptors ($p = 0.003$), histologic grade ($p = 0.02$) and peritumoral vessel involvement ($p = 0.0009$). For to know if old age in the patients offer a protective effect, respect to these poor pathologic characteristics observed in patients with combined losses at two regions, we divided these positive patients in two subgroups, <50 years old and >50 years old, and analyzed the distribution of these parameters in both subgroups. No differences in the distribution of the parameters studied were found.

Conclusions: The data presented in this study suggest that combined losses of the 17q21 and 13q12–13 regions is associated with a poor tumor pathophenotype in patients with breast cancer, and probably with a poor prognosis. Also, these alterations can influence in the similar pathological behavior between patients older and younger than 50 years.

P45 Tumorbiological factors (uPA, PAI-1) as selection criteria for adjuvant chemotherapy in axillary node-negative breast cancer patients

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Morbidity and mortality in breast cancer are caused by the capability of the tumor cells for invasion and metastasis. Tumor-derived proteases are a prerequisite for the dissolution of the tumor surrounding structures enabling the tumor cell to invade and metastasize. Evidence has accumulated that the urokinase-type plasminogen activator (uPA) and its specific inhibitor PAI-1 play a central role in tumor-related proteolysis, invasion and metastasis.

uPA and PAI-1 were quantified (ELISA) in tissue extracts of 316 breast cancer patients. The median follow-up was 77 months (41–108). Optimized cutoff-levels were used for uPA (3 ng/mg protein), PAI-1 (14 ng/mg protein), cathepsin D (45 pmol/mg protein), and S-phase-fraction (7%).

In the multivariate analysis only nodal status (RR = 3.1; $p < 0.0001$), and PAI-1 (RR = 2.5; $p < 0.0001$) were of independent prognostic significance. In *147 node-negative patients* PAI-1 (RR: 3.6; $p < 0.0001$) and uPA (RR: 2.1; $p < 0.049$) were found to be the only independent prognostic factors for disease-free survival (DFS). S-phase, hormone receptors, cathepsin D and tumor size did not add prognostic information in the Cox model. Since uPA and PAI-1 are independent factors, node-negative patients can be grouped further by a combination of these two variables. Node-negative patients with tumors of low content of both uPA *and* PAI-1 have an especially good outlook (93.1% 5-year DFS) in contrast to patients with high content of uPA and/or PAI-1 (67.6% 5-year DFS).

Based on these results a prospective randomized study supported by the "Deutsche Forschungsgemeinschaft" (DFG) was initiated, in which patients