metastasis had occurred in one patient with CK+ micrometastases in BM, but not in LN.

Conclusion: With the short follow-up, our analysis remains descriptive at the present time. However, there appears to be no correlation between lymphatic and hematogenous tumor cell spread. This suggests the existence of biologic differences between these two tumor cell populations that might be elucidated by molecular characterization of these cells, which is part of further studies.

283 POSTER DISCUSSION

A real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to detect breast carcinoma cells in peripheral blood

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Background: The detection of occult carcinoma cells in patients with breast cancer has been shown to predict disease recurrence and metastasis. To improve on existing methods of immunocytologic and molecular detection we have developed a sensitive and quantitative assay to detect breast carcinoma cells in blood, using real-time quantitative RT-PCR identifying transcripts of the cytokeratin-19 (CK19) gene.

Methods: The ABI Prism 7700 (Taqman®) technology is based on the cleavage of a probe labelled with both a fluorescent reporter and a quencher dye, by the 5, −3, exonuclease activity of Taq polymerase during strand elongation of PCR. This cleavage results in an increase in reporter emission intensity, which corresponds to the target sequence concentration. The fluorescent signal is measured during the exponential phase of product amplification, which ensures sensitive real-time quantification. The cycle at which the emission intensity rises above baseline is referred to as Ct (threshold cycle). The Ct values decrease linearly with increasing target quantity. To evaluate the sensitivity of the assay, we measured CK19 mRNA concentrations after RT-PCR in MDA-231 and EFM-19 breast cancer cells spiked in the CK19 negative AML14 cell line. Parallel amplification of the b-actin house keeping gene allowed normalisation of the target concentration.

Results: Primers and probe were developed specifically for the detection of CK19 transcripts with this technique using Primer Express software. Amplification was specific for CK19 mRNA, no amplification of pseudogenes was observed. CK19 transcripts were still detectable when 0.5 MDA-231 and 1 EFM-19 cell were diluted in 106 CK19 negative cells. The Ct for 100% positive cells was 19.25 for MDA-231 and 15.5 for EFM-19; for 0.5 MDA-231 cells/106 AML14 cells the Ct was 36.8 and for 1 EFM-19 cells/106 AML14 cells the Ct was 30. The correlation coefficient of the standard curve (target cell quantity versus Ct value) was at least 0.98.

Conclusion: We have developed a sensitive, accurate real-time quantitative RT-PCR with high reproducibility within a wide dynamic range, which permits simultaneous analysis of samples with varying input concentrations. The procedure offers several technical advantages over classic quantitative PCR methods (competitive RT-PCR, Northern blotting) such as decreased likelihood of contamination due to absence of post-PCR manipulations, high sample throughput because of absence of post-PCR processing time (no agarose gel electrophoresis). Analyses using this real time quantitative RT-PCR for CK19 mRNA may prove to have clinical implications in the assessment of circulating tumour cells in peripheral blood, micrometastases in bone marrow or lymph nodes in breast cancer patients. Validation of application of this technique in a clinical population may improve diagnosis and monitoring of metastatic breast cancer.

284 POSTER

HER2 interaction with intermediate filament proteins and the influence on prognosis in breast cancer

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Purpose: The oncoprotein HER2 seems to affect cell adhesion and metastasis via structural proteins. In this context we investigated the influence of intermediate filament proteins (IF) on prognosis in relation to HER2 in breast cancer.

Methods: Paraffin embedded specimens of 80 primary invasive ductal breast carcinomas were analyzed immunhistochemically (APAAP) for the expression of *keratin* (K) *8*, characterizing normal breast epithelia, *K19*, inconstantly expressed in normal breast epithelia, *vimentin*, characterizing mesenchymal epithelia and the oncoprotein *HER2*. The results were compared with disease-free (DFS) and overall survival (OS) over a ten-years-follow-up.

Result: Strong expression of K8 was seen in 27.5% of the tumors and was correlated with excellent prognosis (DFS/OS: $p < 0.004$). K19 showed a stronger expression than K8 (35%), but did not correlate with prognosis. Aberrant expression of vimentin was seen in 21.3% of the tumors and was associated with poor prognosis (DFS: $p < 0.003$/OS: $p < 0.006$). HER2-overexpression was noticed in 35% and was associated with short survival rates (DFS: $p < 0.01$/OS: $p < 0.02$). HER2 overexpression was significantly correlated with expression of K19 ($p < 0.0004$) and vimentin ($p < 0.0005$).

Conclusions: HER2-overexpression is associated with fundamental changes of the IF-pattern in breast carcinoma cells. Beside the loss of the K8 and K18, expression of K19 and aberrant expression of vimentin mark structural alterations especially in HER2 overexpressing cells, which obviously leads to early metastasis and poor prognosis in breast cancer. IF-changes therefore may play an important role in the malignant functioning of HER2.

285 POSTER

C-ERB-B2 expression as a predictor of outcome in a randomized trial comparing adjuvant CMF vs single-agent epirubicin in stage I–II breast cancer (BC) patients

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Purpose: c-erbB-2 abnormalities either gene amplification or protein overexpression are associated with worse prognosis of pts with N+ BC but the relationship for pts N− is weak at best. Some studies, mostly retrospective, have reported that pts whose tumors have amplified erbB-2 genes or overexpress c-erbB-2 protein benefit from anthracyclines-containing regimen.

Methods: We completed a randomized study comparing CMF versus weekly epirubicin (E) in the adjuvant treatment of 348 stage I and II BC pts (Proc ASCO 19976:142a). We evaluate retrospectively the expression of c-erbB-2 and its interaction with the treatment.

Results: At a median follow-up of 5.6 yrs c-erbB-2 expression was measured by IHC in 266 pts (76%) using the monoclonal antibodies CB11. 133 pts were in CMF arm and 133 in E arm. A baseline significant excess of erbB-2 positive tumors was observed in E arm (41% vs 28%; HR = 2.78; 95% CI 1.13–6.88; $p = 0.03$). DFS and OS were calculated by the Kaplan Meier method. Long rank and Cox models were used to compare DFS and OS for c-erbB-2 status regarded both as a continuous or binary variable. A significantly worse OS was observed for c-erbB-2 positive pts ($p = 0.02$). A Cox regression model including menopausal status, T and n° of positive nodes, treatment and interaction term between arm and c-erbB-2 showed that c-erbB-2 overexpression is a predictor of a poorer OS (HR = 2.78; 95% CI 1.13–6.88; $p = 0.03$). No statistically significant difference was observed between the two arms.

Conclusions: The E treatment had no significant impact on the outcome of pts with erbB-2 tumor positive ($p = 0.12$), but in our study E (30 mg/m^2) was administered weekly for 16 wks. The small number of events (39 deaths) prevents from definitive conclusions.

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Predictors of axillary lymph node involvement in breast cancer <20 mm

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Purpose: Because the frequency of metastatic involvement of axillary lymph node (ALN) in breast cancer (BC) <20 mm is low, the necessity of ALN dissection in these patients (pts.) is under discussion today. If it would be possible to predict the ALN status by parameters of the tumor, a lot of pts. could potentially be spared ALN dissection.

Method: The data of 386 pts. with BC and a tumor size (TS) of 3–20 mm and non palpable and non suspicious ALN by ultrasound were studied retrospectively. Potential predictive histopathological parameters, hormone receptor status and newer factors (HER/2-neu, EGF, Cathepsin D, pS2, DNA-index, S-phase, p53, uPA, PAI-1) were examined with regard to their correlation with the ALN status.

Results: The frequency of involved ALN increased from 7.2% (8/111) in pts. with <10 mm, to 26.7% (40/150) in pts. with 11–15 mm and to 36.8% (46/125) in pts with 16–20 mm large tumors ($p = 0.0001$). No ALN involvement was detected in 17 pts. with 3–5 mm large tumors. In summary, increasing TS ($p < 0.002$), higher Grading ($p = 0.03$), detection of tumor cells in bone marrow aspirates ($p = 0.01$), lymph/blood vessel invasion ($p < 0.0001$) and aneuploid tumors with increased S-Phase ($p = 0.03$) were associated with positive ALN. Lymph/blood invasion offered a significant ($p < 0.05$) correlation with ALN status throughout all subgroups of TS, whereas other factors revealed significance only in TS subgroups. Multivariate analysis confirmed an increased risk of ALN involvement in patients with TS 16–20 mm by 6.3 ($p = 0.0001$) and in TS 11–15 mm by 4.5 ($p = 0.0003$) as compared to TS < 10 mm. An additional risk of positive ALN was related to lymph vessel invasion (1.8 fold, $p = 0.03$) and blood vessel invasion (2.6 fold, $p = 0.009$).

Conclusion: The risk of axillary lymph node involvement increased with tumor size and lymph/blood vessel invasion. An axillary lymph node dissection in pts. with breast cancer <5 mm seems no longer justified. In pts. with tumor size <10 mm and no lymph or blood vessel invasion the risk of positive axillary lymph nodes is very low, so that an axillary lymph node dissection in these pts. seems unneces-sary.

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Clinico-pathological characteristics of breast cancer associated with thyroid disease

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Purpose: Breast cancer (BC) is a hormone-dependent neoplasm. There is conflicting evidence of clinical correlation between thyroid disease (TD) and BC. TD is common among women and is often associated with autoimmune disorders. The thyroid insufficiency hypothesis sought to relate BC to TD, in particular hypothyroidism. We performed a retrospective analysis of newly diagnosed BC to determine the prevalence of TD.

Methods: Data of 1,287 primary breast cancer patients (pts) treated at MDACC between 6/93 and 9/98, were analyzed. TD was defined as clinical evidence of impairment of thyroid gland function.

Results: TD was found in 128 pts (9.9%); 120 pts were hypothyroid (9.4%) and 8, were hyperthyroid (0.5%). Median age was 51 yrs (range 22–90) and 56.5 yrs (range 25–90) for the entire group and TD pts, respectively. Clinicopathological features of pts according to thyroid status: euthyroid: ER+ and/or PR+ 741 (64%), ER−/PR−: 242 (21%), stage 0-II 990 (86%), stage III 156 (14%); TD: ER+ and/or PR+ 82 (64%), ER−/PR−: 22 (17%), stage 0-II 120 (94%), stage III 8 (6%).

Conclusions: The prevalence of TD in this sample of BC pts is higher than in the general female population. The distribution of receptor status was similar among euthyroid and TD while a predominance of early BC (stage I–II) was noted in the latter group. The biological significance of this clinical association deserves further investigation. Molecular analysis of other members of the steroid receptor superfamily may provide patho-biological correlates.

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Bcl-2 expression, cell differentiation and survival in primary breast cancer

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Introduction: The prognostic significance of Bcl-2 expression was examined.

Methods: In 100 patients with infiltrating ductal breast carcinomas Bcl-2 expression was examined, using immunohistochemistry on formalin-fixed, paraffin embedded representative tumor samples. Between Bcl-2 positive and Bcl-2 negative tumors, hormonal receptor state, tumor grade, tumor size and survival were compared. The Nothingham modification of the Bloom-Richardson system graded all tumors. A non parametric Mann-Whitney U test was used to compare both groups.

Results: Bcl-2 was expressed in normal adjacent tissue, in the carcinoma in situ component and in 74% of the tumors. Between the Bcl-2 positive and negative groups, there was a significant difference for estrogen and progesteron receptor positivity, cell differentiation, disease free survival and survival. There was no significant difference for tumor size, menopausal state or age. Univariate analysis retained tumorgrade, Bcl-2 expression, age, progesteron receptor state and tumor size as prognostic factors. Tumor grade was shown to be an independent prognostic factor by Cox regression multivariate analysis. Bcl-2 expression was of marginal prognostic significance.

Conclusion: These results suggest that Bcl-2 expression in breast tissue is related with cell differentiation. Loss of differentiation is compatible with a loss of Bcl-2 expression. Bcl-2 expression is a highly significant prognostic factor by univariate analysis.

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Fine needle aspiration is associated with hematogenous dissemination of breast cancer cells as determined by RT-PCR

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Purpose: The influence of fine needle aspiration on breast cancer cell shedding into the peripheral blood was investigated using reverse transcriptase-polymerase chain reaction (RT-PCR) targeted against β-subunit of human chorionic gonadotropin (β-hCG), cytokeratin 19 (CK19) and cytokeratin 20 (CK20) mRNAs.

Methods: This analysis was performed before and after fine needle aspiration in 20 cases with breast cancer and 24 cases with benign breast tumor. 5 ml of peripheral blood was drawn before and within 30 minutes after puncture. Total RNA was extracted from peripheral blood mononuclear (PBMN) cells, β-actin was used to assess the quality of cDNA. 367 bp RT-PCR products for β-hCG were digested with StyI endonuclease to produce 2 fragments (96 bp and 271 bp).

Results: For the benign cases, the pre-FNA samples were all negative for β-hCG and CK20 and 12.5% (3/24) positive for CK19. After aspiration, β-hCG and CK20 remained negative, whereas 3 cases became positive for CK19 in 21 evaluable cases. For the malignant cases, one pre-FNA sample was positive for all three markers and two other samples were positive for CK19. Of the 19 evaluable cases for β-hCG and CK20, 3 cases were converted to a positive result for β-hCG but none was positive for CK20. For CK19, there was positive signal conversion in only one of 17 evaluable cases.

Conclusion: Fine needle aspiration to breast tumor may cause hematogenous dissemination of breast cancer cells. Although CK19 is more sensitive to detect both benign and malignant epithelial cells in the circulation, β-hCG is more specific for breast cancer cells. CK20 is the least sensitive marker for circulating cells.

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Local Recurrence (LR) after Breast Conserving Therapy (BCT); risk factors predicting for subsequent distant metastasis

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Purpose: To study risk factors for subsequent distant metastasis (DM) after LR for patients treated with BCT.

Patients and Methods: From a cohort of 1481 breast carcinomas treated with BCT in the period 1980–1994, we studied 68 pT1-3N0-1 breast tumors which relapsed as first event. Patients who developed LR after or concomitant with DM (defined as diagnosis within 4 months of LR diagnosis) were omitted from analysis. The primary as well as the recurent tumor was studied. In addition to clinical factors (age at BCT and LR, interval, mode of detection, location and treatment of LR) the histology slides of the primary and the recurrent tumor were reviewed. Immunohistochemical staining was performed for the following proteins: bcl-2, cyclin D1, E-cadherin, EGF receptor, ER, PR, Ki-67, c-erbB-2/neu and p53. Statistical analyses were performed using conditional logistic regression.

Results: In univariate analysis none of the factors of the primary tumor was found to be statistically significantly associated with DM risk after LR. Of the recurrent tumor the following factors were found to be risk factors for high DM risk after LR: interval < 2 years (RR 2.38 (1.22–4.76); $p = 0.008$) and high mitotic count (RR 2.51 (1.03–6.15); $p = 0.04$). All patients with non-invasive recurrent tumor were alive at time of analysis. Patients with a LR detected after 2 years with high mitotic count were found to have the same poor prognosis as patients with LRs detected after a short interval.