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.

287 POSTER

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.

288 POSTER

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.

289 POSTER

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.

290 POSTER

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.

Conclusion: LR after BCT is associated with higher DM risk and poor survival. Especially patients with LRs developed within two years after BCT are at high risk. Late recurrences with high mitotic count have the same poor prognosis as early recurrences. For these patients systemic adjuvant treatment after the occurrence of LR should be considered.

291 POSTER

Quantitation of c-erbB-2 in primary breast cancer allows identification of a further poor prognostic group

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C-erbB-2 amplification or high levels of overexpression are found in about 20% of breast cancers and these patients have a poor outcome. Using a quantitative radioimmunohistochemical method in frozen sections we have previously shown that c-erbB-2 is nearly always overexpressed in primary breast cancers with gene amplification accounting for a population with very high levels of expression.

Here we report on the application of radioimmunohistochemistry to measure the c-erbB-2 protein in a larger set of cases (n ≈ 179) with followup exceeding 5 years. Levels of expression in areas of tumour were expressed relative to normal breast tissue taken from frozen sections of reduction mammoplasties. Disease specific survival was assessed using Kaplan Meier life table analysis and log rank tests.

85% of tumours overexpressed c-erbB-2. 23% had greater than 15 times normal expression. These cases had a significantly poorer survival than the rest of the overexpressors ($p < 0.0001$). 15% of cases had lower c-erbB-2 expression compared to normal breast tissue. These patients also had a poorer prognosis than the non-amplified overexpressors ($p < 0.0001$).

Dividing patients according to the Nottingham prognostic index, those in NPI group 1 with between normal and amplified levels of c-erbB-2 expression had a 5-year survival of 92%. But only 79% of patients in this group with amplified or downregulated c-erbB-2 levels survived 5 years. Similarly, patients in NPI group 2 with between normal and amplified levels of c-erbB-2 expression had a 5-year survival of 66% while those with amplified or downregulated c-erbB-2 levels had a 5-year survival of only 29%. The corresponding figures for NPI group 3 were 29% and 20%.

Quantitative radioimmunohistochemical measurement of c-erbB-2 protein provides additional prognostic information and can identify high risk groups within NPI subgroups which may have treatment implications for these patients.

292 POSTER

Prognostic impact of extended extracapsular component (EEC) in involved lymph nodes (LN) in primary breast cancer (BC)

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The importance of the nodal status of BC patients (pts.) is clear, but rarely are the patterns of LN metastases regarded as prognostic indicator. Extracapsular spread of tumour cells in metastatic LN, called EEC, has been attributed to bad outcome. We prospectively studied the impact of EEC regarding disease-free survival (DFS), distant failure (MFS) and overall survival (OAS).

483 patients with primary, node positive, non metastatic, previously untreated BC underwent surgery including axillary dissection with a median of 16 removed LN (Range 2–46). The axillary LN were extensively examined and regular follow up-examinations carried out.

Mean observation time is 36 months. In 219 cases (45%) EEC was diagnosed. EEC is correlated with the number of LN metastases ($p < 0.001$), lymphangiosis in the primary tumour ($p = 0.002$) and the expression of plasminogenactivator inhibitor PAI-1 ($p = 0.002$). Pts. without EEC had a median of 2 positive LN (1 to 40) and pts. with EEC 5 (1 to 44). Treatment decision for endocrine or cytotoxic therapy was not different in pts. with or without EEC, but pts. with EEC received significantly more often anthracycline-containing regimens ($p < 0.001$) and radiation therapy ($p = 0.006$).

DFS, MFS and OAS after 5 years are 53%, 64% and 78% for pts. without EEC and 37%, 40%, 61% for pts. with EEC respectively. These differences are significant with p-values of 0.007 (DFS), 0.0001 (MFS) and 0.0003 (OAS). The significance disappears in subgroup analysis for LN (1–3 LN, 4–9 LN and 10+ LN), but in multivariate analysis EEC is significant with a RR of 1.6 (1.1–2.3). Pts. with EEC without anthracycline-containing chemotherapy show a worse 5-year MFS and OAS than pts. without EEC (22% vs. 63%, $p = 0.003$ and 50% vs. 76%, $p = 0.002$), while pts. with anthracyclines do not (52% vs. 55% and 60% vs. 67%, p = n. s.).

We conclude, that EEC due to its strong correlation with the number of involved LN might be no independent prognostic marker, but a surrogate for nodal involvement. EEC could perhaps be a predictive marker for bad outcome with chemotherapy without anthracyclines, but this finding has to be confirmed through prospective randomised trials.

293 POSTER

The study of breast cancer metastases to the specific group of the axillary lymph nodes

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Purpose: We have conducted the studies on the metastases to the specific groups of axillary lymph nodes in patients with breast cancer since 1994. The aim of these studies was also to examine the possibility to lessen the lymph-oedema on the side of the operation.

Methods: The studies have been performed on 52 patients with breast cancer of various degrees of clinical advancement T1N0M0, T2N0M0, T2N1M0 and T3N2M0. The lymph nodes located inferior to the axillary vein, externally to the thoraco-dorsalis bundle, and medially to the margin of the muscle latissimus dorsi were included into separate groups. The lymphatic vessels were visualised with blue dye administered before the operation in the region of the tumour and the skin superior to it.

Results: No metastases to this group of the lymph nodes were found. However, it was established that in this triangle there are vessels which drain away the lymph from the upper extremity and the shoulder region, the cutting of which without putting a clip extends the period of lymph-seroma drainage.

Conclusion: The surgical removal of these lymph nodes is by no means an effective treatment, and may increase the frequency of the upper extremity lymph-oedema.

294 PUBLICATION

Overexpression of p27 in relation to p53 and clinicopathologic variables in node-positive breast cancer

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Purpose: The aim of this study was to determine the prognostic implication of p27 in relation to p53 in breast cancer patients with lymph node metastases.

Methods: We retrospectively evaluated 100 breast cancer with positive lymph nodes in the period between 1986 and 1991. Immunohistochemical staining for p27 and p53 was performed on formalin-fixed, paraffin-embedded sections. The results were compared with clinicopathologic variables and outcomes. A nuclear staining over 50% was defined as high expression for p27, and p53 protein expression >10% was defined as positive.

Results: High expression of p27 was shown in 54. There was no correlation between p27 staining and age, menopausal status, nodal metastases, or tumor size, but high p27 correlated to positive estrogen receptor and p53(−). High expression of p27 was significantly associated with longer survival. No differences of survival and disease-free survival were shown between patients with p53(+) and p53(−). A multivariate analysis showed that the independent variables were p27 and lymph node metastases.

Results: The results indicated high expression of p27 was an independent factor associated with good prognosis. Accordingly p27 may be more important than p53 in making therapeutic decisions for patients with lymph node metastases.