Patients and Methods: Data regarding a series of 255 consecutive women (median age 60 years, range 30-85) with pT1-2 (pT1a=9, 3.5%; pT1b=38, 14.9%; pT1c=107, 42.0%, pT2=101, 39.6%) BC were reviewed, while patients with confirmed pT3-4 BC were excluded. The greatest diameter of the tumor measured by the pathologist (size) ranged from 3 and 48 mm (median 19 mm). There were 71 (27.8%) premenopausal and 184 (72.2%) postmenopausal women. Two groups of patients were considered according to the axillary lymph node status: Group A, 70 (27.5%) cases (pN1), and Group B, 185 (72.5%) cases (pN0). All patients underwent pre-operative CEA and CA 15-3 serum levels measurement, and the removed tissue was routinely processed for the detection of ER, PgR, and MIB1 index.

Results: CEA and CA 15-3 serum levels were above the cut-off (10 ng/mL, and 30 U/L, respectively) in 44 (17.2%) and 75 (29.0%) patients (Group A: 22.9% and 47.0%, Group B: 15.1% and 23.0%, respectively; p=NS). Size (23.9±9.0 vs. 18.2±9.3 mm), ER rate (51.3±37.7 vs. 60.4±30.6), MIB1 index (30.1±26.3 vs. 17.8±21.0), CEA (4.3±4.8 vs. 3.4±2.7 ng/mL) and CA 15-3 (26.8±16.3 vs. 18.2±15.1 U/L) serum levels were significantly different ($p<0.05$) in Groups A and B patients. Overall, a significant correlation between size of the tumor and both CEA (R=0.22, p=0.0003) and CA 15-3 (R=0.57, $p<0.0001$) and between ER rate and MIB1 index (R=-0.59, $p<0.0001$) was found. There was no relationship between age of the patients, size (R=0.08, p=0.20), and ER (R=0.13, p=0.71). Among Group A patients, a significant correlation between number of involved nodes and both CEA (R=0.24, p=0.04) and CA 15-3 (R=0.31, p=0.007) serum levels was found, but there was no relationship (p=NS) with age, ER rate and MIB1 index.

Conclusions: In patients with BC, serum markers CEA and CA 15-3 correlate exclusively with the size of the tumor. On account of their low sensitivity and in lack of relationship with others prognostic factors, preoperative CEA and CA 15-3 serum levels measurements are of little value in patients undergoing curative surgery for primary BC.

420 POSTER

Predictive factors for the status of non-sentinel nodes in breast cancer patients with tumor positive sentinel nodes

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Background: In breast cancer patients with tumor negative sentinel nodes axillary lymph node dissection is omitted and the patient spared from postoperative morbidities. However in patients with tumor positive sentinel nodes, axillary lymph node dissection is routinely performed while a majority of these patients have no tumor involvement in the non-sentinel nodes. The authors tried to identify a subgroup of patients with a tumor positive sentinel node without non-sentinel node tumor involvement.

Methods: In 135 consecutive patients with clinical stage T1-T2 node-negative breast cancer, tumor positive sentinel nodes and axillary lymph node dissection performed, the incidence of non-sentinel node involvement according to tumor and sentinel node related factors was examined.

Results: The size of the sentinel node metastasis, size of primary tumor and number of tumor positive sentinel nodes were the three factors significantly predicting the status of the non-sentinel nodes. The size of the sentinel node metastasis was the strongest predictive factor ($P<0,0001$). In a subgroup of 41 patients with a stage T1 tumor and micrometastatic involvement in the sentinel node only 2 patients (5%) had non-sentinel node involvement.

Conclusion: In patients with small primary tumors is and micrometastatic involvement of the sentinel nodes, the chance of non-sentinel node involvement is small but can not be discarded. Because the clinical relevance of micrometastases in lymph nodes is still unclear it is not advisable to omit axillary lymph node dissection even in these patients.

421 POSTER

Expression of estrogen receptor-β2 and β4 mRNA decreases in breast carcinogenesis

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Background: Since the discovery of estrogen receptor-* (ER*), more than five variants have been identified. There have been many controversial reports on the role of ER* in breast carcinogenesis and cancer progression, and its prognostic implications. The role of the variant forms has not been yet identified.

Materials and Methods: Using reverse transcription polymerase chain reaction (RT-PCR), we examined the expression levels of ER± and ER* variants in 76 paired normal and cancer tissues, 6 paired normal and benign tumor tissues, and 12 metastatic lymph nodes. We compare the densities of RT-PCR products using Tina version 2.10 (Raytest, Germany). Chi-square test and independent t-test were used for the statistical analysis. Differences were considered significant with a p value of less than 5%.

Results: ER± expression was increased in 51 cancer tissues (67.1%) compared to matched normal tissues and decreased only in 9 (11.8%). On the contrary ER* expression was decreased in 42 cancers (55.3%) compared to matched normal tissues and increased only in 4 cancers (5.3%). Among ER* variants, ER*2 was predominant and expressed 100% in both normal and cancer tissues but the level of expression decreased significantly in cancers compared to paired normal tissues. ER*4 was also expressed in both normal and cancer tissues 77.6% and 73.6%, respectively and it decreased significantly in cancer tissues compared to paired normal tissue, too. ER*5 was expressed more frequently in cancer tissue (57.9%) than in normal tissue (31.7%). ER*1 expression was not significantly different between normal and cancer tissues. There was no ER*3 expression in both normal and cancer tissues.

Conclusions: Among ER* variant forms, ER*2 is predominant in both normal and cancerous mammary tissues and ER*4, ER*5, and ER*1 in descending order but ER*3 is not expressed in the mammary tissue. ER± mRNA expression significantly increases but ER* mRNA decreases in the process of breast cancer development and progression. The decrease of ER*2 and ER*4 expression is a dominant phenomenon during the breast carcinogenesis, which suggests that ER*2 and ER*4 may possess a regulatory function of mammary proliferation. Further investigations to verify the roles of ER* variants are mandatory.

422 POSTER

Soluble adhesion molecules and oxidative stress in patients with breast cancer

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Background: Identification of clinically useful prognostic markers and markers of activity could contribute to the improvement of therapy of patients with breast cancer, mainly to the identification of subgroups of patients in higher risk of the formation of metastases and early detection of relapses of the disease. In patients with breast cancer oxidative stress may modify membrane lipids which may then become the target of some autoantibodies. Some receptors (including EGF receptor and apo1/Fas) and adhesion molecules (standard and/or variant CD44 and P-selectin) may detach from the surface of tumor cells and increased levels of their soluble forms may be also identified in sera.

Methods: In our study serum levels of soluble EGF receptor, soluble standard and variant CD44 (CD44s and CD44v6, respectively), soluble P-selectin, soluble apo-1/Fas, advanced oxidation protein products (AOPP), advanced glycation end-products (AGEs), pregnancy associated plasma protein (PAPP-A) and IgG and IgM anticardiolipin antibodies (ACA) were studied in 76 patients (pts) with newly diagnosed, mostly non-metastatic breast cancer (3 pts in stage 0, 37 pts in stage I, 18 pts in stage IIA, 12 pts in stage IIB, 4 pts in stage III and 2 pts in stage IV) and compared with 8 age-matched healthy women.

Results: Patients with breast cancer had significantly higher serum levels of soluble standard form of CD44 (CD44s, 581.5 + 281.1, vs. 406.4 + 48.9 ng/ml, $p < 0.05$), but not soluble variant form, most common on breast cancer cells (CD44v6, 171.4 + 48.4 vs. 160.1 + 48.3 ng/ml, p = n.s.). Serum levels of soluble P-selectin (248.1 + 137.0 vs. 125.5 + 32.0 ng/ml, $p < 0.05$) and serum levels of soluble apo-1/Fas (852.9 + 159.3 vs. 541.5 + 124.5 pg/ml, $p< 0.05$) were also significantly increased in patients with breast cancer. Concerning the markers of oxidative stress patients with breast cancer had higher AOPP (93.6 + 46.8 vs. 68.5 + 23.1 umol/l, $p < 0.05$), but there was no difference in AGEs, PAPP-A and IgM and IgG ACA. We were not able to find any significant difference in serum levels of soluble EGF receptor (3.2 + 3.1 vs. 3.6 + 2.0 ng/ml, p = n.s.). None of measured parameters was able to discriminate the patients with different stages of breast cancer.

Conclusions: Patients with breast cancer (including those in early stages of the disease) may have increased serum levels of some soluble adhesion molecules (sCD44s, sP-selectin), markers of apoptosis (sapo-1/Fas) and oxidative stress (AOPP). Further follow-up should demonstrate the response of these markers to hormonal therapy/chemotherapy and putative prognostic significance of increased levels of these markers in order to improve the current possibilities to monitor the activity of the disease and to predict its

outcome. *Supported by the grant of IGA 6593-3 of the Ministry of Health, Czech Republic*

423 POSTER

Isolated tumor cells in the bone marrow (ITC-BM) of breast cancer patients before and after anthracyclin based therapy - influenced by the HER2neu- and Topoisomerase IIa-expression/amplification of the primary tumor?

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Background: The immunocytochemical detection of ITC-BM of breast cancer patients is an independent prognostic factor in all stages of the disease. Both the expression / amplification of HER2neu and Topoisomerase IIa (TOP IIa), a key enzyme of DNA replication and main target of anthracyclines, in primary breast cancer tissue seem to have predictive value regarding the effectiveness of systemic therapies, what might possibly be expressed by a change of ITC-BM.

Methods: Tumor tissues of 52 pts that were screened for ITC-BM before and after anthracyclin based therapy (CTX) were examined for HER2neu and TOP IIa by IHC and FISH (TOP2A/HER2/CEP17 Multi-color Probe, Vysis). The correlation of these factors and their influence on clinical outcome was analysed retrospectively. Results: By IHC 30% of the tumors showed positive for HER2 (2+/3+), 23% were amplified in FISH analysis (HER2/CEP17 >= 2). TOP IIa overexpression (>20%) was found in 23/48 pts (48%), FISH analysis was pos. in 6/42 pts (14,3%), with co-amplification of HER2 and TOP IIa in 75%. ITC-BM were present in 25% of pts before and 30% after CTX. The detection of ITC-BM before CTX correlated with HER2 (IHC and FISH), but not with TOP IIa. 43 pts had adjuvant, 6 neoadj., 3 palliat. CTX, regimens consisting of EC (6 pts), EC/CMF (18 pts), EC/Docetaxel (26 pts) and others (5 pts). 31 pts (53%) stayed neg for ITC-BM during CTX, 8 (16%) changed from neg to pos, 5 (10%) from pos to neg, and 8 (16%) stayed pos., which either was independent of the HER2- or TOP IIa status. After a median of 46 months (6-127) HER2-IHC (p=0,005), TOP IIa-IHC (p=0,049) and the detection of ITC-BM after completion of CTX (p= 0,047) were sign. prognostic factors for overall survival (log-rank-test), whereas TOP IIa (FISH) neg. pts showed a slight but not significant trend (p=0,09) towards reduced distant disease free survival.

Conclusion: As shown previously, antiproliferative CTX has no or little effect on the elimination of ITC-BM. This seems to be independent of the HER2 or TOP IIa status of the primary tumor. Nevertheless, the detection of ITC-BM after CTX has, like the HER2 status, prognostic relevance for overall survival. For the development of new therapeutic strategies, it would be desirable to examine such factors on ITC directly.

Breast cancer local regional therapy

424 POSTER

Long term results after conservative treatment for invasive breast carcinoma: A 20 year follow-up

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Background: Patients (pts) with invasive breast cancer treated by conservative treatment must be followed for a long time to ensure the efficacy of the procedure in terms of locoregional control. This study was performed to analyze the outcome of a large series of patients submitted to conservative surgery and definitive irradiation, and to evaluate the relationship between locoregional recurrence (LRR), distant metastases (DM) and survival.

Methods: From 1983 to 1998, 547 pts with early breast cancer stage I and II, underwent breast conservative treatment (BCT). Pts with axilary positive nodes received adjuvant chemotherapy, and pts with positive hormonal receptors received hormonotherapy. The following prognostic factors were analyzed for their ability to predict for a distant recurrence: pt age, menopausal status, tumor size, axillary lymph node status, histological grade, experience of LRR and interval from diagnosis to LRR. The outcome of pts after LRR (early≤ 24 months or late >24 months) was documented and factors associated with a favorable survival following recurrence are identified.

Results: With a median f-up time of 78 (3-238) months, 25 pts experienced a LRR as their first site of recurrence and in 58 pts isolated DM were observed. The 20 years overall and cause-specific survival was 51.0%± 17.7 and 84.3%± 4.6 respectively. The 20 years cumulative incidence of LRR was 19%± 12.5 and the annual cumulative incidence of LRR within the first two years was 0.80% and between the 3th and 10th years was 0.48%. Between the 11th and 15th no LRR was observed and one pt experienced a LRR in a different quadrant from the primary tumor 16 years after the diagnosis. The cumulative incidence of DM at 20 years was 17.7 ± 2.6 and the annual cumulative incidence of DM within the first two years was 2.2% and between the 3th and 10th years was 1.3%. From the 12 th year until the end of the study none of the pts present in the study experienced a DM. The 15 years overall survival rate of pts who experienced LRR differed significantly from those pts who never experienced LRR, 72.5%± 6.3 vs 53.9%± 11.7 respectively (p=0.005; RR of death:2.6; 95% CI: 1.3-5.0). The actuarial distant disease free survival (DDFS) of pts who never experienced LRR was significantly higer (86.9%± 1.9) compared to the actuarial DDFS of pts who experienced LRR (62.8% ± 9.8) (p<0.0006; RR of DM: 3.2; 95% CI: 1.5-6.4). The 10 years DM probability in pts who developed an early LRR was 60%± 17.3 and 25%± 10.8 in pts who developed a late LRR (RR: 4; p=0,041; 95% CI:1.05-15.2).The median f-up time for survivor pts after LRR was 44 months.

Conclusions: Long term survival rate was observed among pts who underwent BCT. LRR appears to be a significant predictor of DM and pts who sustain early LRR tend to display a more agressive clinical course

425 POSTER

Interobserver variability of target volume delineation of breast tissue as well as of boost volume in 19 breast cancer patients after lumpectomy and axillary staging

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Purpose: To determine the interobserver variation in delineating the clinical target volume (CTV) of breast tissues as well as of corresponding boost volumes on computed tomography (CT) scans in breast cancer patients treated with breast conserving therapy (BCT).

Material and Methods: Nineteen consecutive breast cancer patients (mean age 53 yrs; 16 T1, 3T2) treated with breast conserving therapy agreed to participate in our study. Palpable glandular breast tissue was marked with a lead wire before CT scanning. Four radiation oncologists and 1 radiologist delineated CTV's. Indices of the corresponding CTV of all 19 patients were compared with one another specifically with respect to the delineation of breast tissue: BRI and to that of the boost volume: BOI. An index of 1.0 implicates that the location as well as the size of the CTV's is exactly the same. We than analyzed where in the breast tissue the magnitude of the observed differences was high or low (medial, lateral, cranial, caudal, outer, inner). With respect to the latter the following variables were also analyzed: i) the volume of the breast tissue; ii) the presence or absence of dense breast tissue; and specifically with respect to the boost: iii) the presence or absence of clips.

Results: The range of the BRI varied between 0.83 and 0.88. Observed differences were between the 5 physicians were largest in the upper-outer quadrant of the breast. The BOI varied between 0.45 and 0.60. Differences were less outspoken in the presence of clips.

Conclusions: Interobserver variation in the delineation of breast target volume on CT scans can be substantial (pending on the presence or absence of dense breast tissue). The differences are large in delineating the CTV of the boost volume. To reduce the interobserver variation, better imaging (including markers) and pathology studies relating glandular breast tissue to imaging may be needed in order to better visualize the true extent of the breast tissue (especially in postmenopausal patients) and the boost volume (especially in the absence of clips).

426 POSTER

Intramammary tumor location (ITL) does not influence prognosis, but the prevalence of axillary lymph node metastases

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The prognostic role of the ITL is discussed controversially (Lohrisch et al.,