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Disseminated Tumor Cells in the Bone Marrow (DTC-BM) and biological factors of 265 primary breast carcinomas

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Introduction: The prognostic significance of Disseminated Tumor Cells in the Bone Marrow (DTC-BM) of breast cancer patients was demonstrated in many studies. Yet, it is not clear which of the primary tumors' biological factors are responsible for hematogenous dissemination. We therefore examined the expression/amplification of HER2, Topoisomerase IIa (TOP IIa), proliferation marker KI 67, and tumor suppressor gene p53 on "Tissue Micro Arrays" (TMA) of 265 primary breast carcinomas from pts. with known BM-Status.
Methods: BM aspiration and analysis was performed according to a standardized protocol with cytospin preparation and immunocytochemical staining for cytokeratin (CK) as a marker of epithelial cells. TMAs of the primary carcinomas were examined by immunohistochemistry (IHC) for HER2, Top IIa, KI 67 and p53. Additionally, HER2 amplification was examined by fluorescence in situ hybridisation (FISH). IHC Evaluation was done semiquantitatively (HER2) or by percentage of positively stained cells.
Results: HER2 IHC (2+/3+) was positive in 35/167 (21%) cases, FISH in 39/160 (=24.3%). Positive staining for Top IIa was seen in 163/181 (med. 10%), for KI 67 in 52/184 (med. 5%) and for p53 in 106/174 cases (med. 5%). 68/265 pts. (25.7%) showed DTC-BM with a median of 2/2 $\times$ 106 cells (1–1500). BM positivity was not correlated to any of the examined factors. HER2 IHC correlated with FISH ($p < 0.001$), hemangiosis ($p = 0.01$), Top IIa ($p = 0.06$), KI 67 ($p = 0.031$), and p53 ($p < 0.001$), Top IIa significantly with KI 67 and p53, and also KI 67 with p53 ($p = 0.004$). After a median follow-up of 60.5 months (7–255), the presence of DTC-BM showed prognostic relevance for Overall survival ($p = 0.032$), whereas HER2 correlated with disease free ($p = 0.05$) and distant disease free survival ($p = 0.04$).
Discussion: The congruence of the examined tumor biological factors' expression rates indicates a causal line of suppressor-, proliferation- and mitosis markers and growth factor receptors. Hematogenous tumor cell spread seems to be an independent processs. The examination of those factors on DTC-BM themselves is the aim of ongoing research.

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Changes in methylation status of cancer related genes during ductal breast carcinoma progression

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CpG island hypermethylation is emerging as one of the main mechanisms for inactivation of cancer related genes.
We have used Quantitative Methylation Sensitive PCR (Q-MSP) to identify changes in DNA methylation during breast cancer progression. Pre-invasive (Atypical Ductal Hyperplasia and/or Ductal Carcinoma in situ) and matched invasive ductal carcinomas were analyzed for changes in methylation status of eight genes involved in breast carcinogenesis.
The highest frequencies of methylation were found for APC (76% of the lesions), CDH1 (48%), ESR1 (52%) and TIMP3 (68%), whereas levels of methylation ranging between 1% and 18% were detected for THBS1, TMS1, GSTP1, and b-catenin. An increase in the number of methylated genes was found during tumor progression from Atypical Ductal Hyperplasia to invasive ductal carcinomas but no changes were seen in lymph node metastases. We found a marked variability in methylation levels between different subjects, however when paired lesions from the same subject were analyzed a marked increase in methylation levels was found for genes CDH1 (Mean$\pm$SE ADH 0.91$\pm$0.91; DCIS 3.73$\pm$1.66; IDC 17.43$\pm$8.43) and ESR1 (ADH 3.90$\pm$ 3.31; DCIS 12.62 $\pm$5.25; IDC 35.59$\pm$3.53). The analysis of methylation levels in metastatic lymph nodes did not show substantial changes for any of the genes tested. However in two cases histopathological normal lymph nodes displayed low levels of methylation, which might be related to the presence of micrometastases. Although preliminary, our results suggest that the determination of methylation levels may represent a useful marker for breast cancer progression.
To confirm these promising results we are going to analyze additional matched pre-invasive and invasive lesion as well as long term follow up lymph node negative cases with different clinical outcome. The gain of information coming from our research may lead to an improvement in early cancer detection and in the overall management of breast cancer patients.

Poster presentations (Mon, 31 Oct)
Breast cancer – early disease

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Micrometastasis in sentinel (SLN) and non-sentinel lymph nodes of breast cancer: an update including clinico-pathologic impact and survival

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Introduction: Axillary lymph node status is the most important prognostic factor in breast cancer patients. Nevertheless, the clinical and survival relevance of micrometastases in local lymph nodes remains uncertain. The aims of this retrospective study are to assess the rate of positive axillary clearance when the SLN biopsy contains micrometastatic disease and to evaluate the survival impact of local lymph nodes with micrometastases.
Materials and methods: From 1997 to 2005 2.132 consecutive patients (pts) underwent breast surgery with SLN biopsy or axillary dissection. Of these, 80 pts had lymph node micrometastases assessed with haematoxylin and eosin or cytocheratine staining and defined according to the current TNM classification. Lymph node micrometastases were found after axillary dissection in 22 pts and after SLN biopsy in 58 cases; 52 (65%) were micrometastases >0.2–1 mm, 21 (26%) measured 1–2 mm and 6 were isolated tumor cells; location of micrometastases were lymph node sinus in 38 and parenchima in 42 cases.
Patients characteristic were: median age: 53 (range 34–78), 72 (90%) had conservative surgery and 8 pts had mastectomy; 73 pts (91%) had ductal carcinoma and 10 had multifocal carcinoma (12.5%); T stage was: pT1a/pT1b in 14 pts (17%), pT1c in 51 (63%) and pT2 in 15 (18%); 72/80 pts (90%) had positive Er/Pgr; vascular invasion was present in 29 pts (36%), HER-2/neu overexpression in 14 (17%), grading 3 in 23 (28%). Sixty pts (75%) received adjuvant chemotherapy (45 AC, 11 CMF ev, and 4 FEC); 72 pts (87%) receveid radiotherapy and hormonal therapy.
Results: Of the 58 pts with SLN-biopsy positive for micrometastases, 8 (14%) had further axillary involvement; in 5/8 cases size of micrometastases was >1 mm, in 7/8 cases micrometastases were located in the nodal parenchima. Median follow-up time was 26 months (range 2–151). Three of 80 pts had local relapse, 6 developed metastases, 4 died. Three years DFS was 90.85% and OS 94.72%.
Conclusions: Further axillary involvement was found in 14% of pts with micrometastases in the SLN biopsy supporting complete nodal dissection in all pts. Our preliminary results show that even minimal nodal involvement could correlate to worse prognosis and may require chemotherapeutic treatment.

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EORTC 10981–22023 AMAROS trial: after mapping of the axilla radiotherapy or surgery? Current status

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Background: The EORTC Breast Cancer Group launched the AMAROS trial in February 2001. This phase III randomised non-inferiority trial compares a complete axillary lymph node dissection (ALND) versus radiotherapy (RT) to the axilla in sentinel node (SN) positive patients, whereas SN negative patients are followed for the endpoints of the study as well. The main objective of the trial is to prove equivalent local/regional control for patients with proven axillary lymph node metastasis by SN biopsy with reduced morbidity if treated with axillary RT instead of ALND.
Patients and methods: Eligible are patients with an operable invasive breast cancer of over 5 mm and less than 30 mm, without clinically suspected regional lymph nodes. Surgical and RT quality control constitutes