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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

an important part of the trial design. SN biopsies are performed by the combined technique using preoperative lymphoscintigraphy by injection of Technetium-99m nanocolloid, immediate pre-operative injection of Patent Blue dye, and SN retrieval by both discoloration and intra-operative use of a detection probe. A successful learning curve of 30 patients and an approved dummy run protocol are mandatory for participation. During a site visit, prior to participation, original patient files of the learning curve are checked and a SN procedure is witnessed.
Results: As of 25 May 2005, 1795/3485 (52%) patients were included by 23 institutes from Europe and Israel. SN biopsy results demonstrated 34% positive and 64% negative sentinel nodes leading to an overall identification rate of 98%. Last interim quality control analysis revealed one axillary recurrence in the SN negative group resulting in a 5-year axillary recurrence estimate of 1% (95%CI: 0–3%) in the SN negative group. Other preliminary results showed that adjuvant systemic treatment was given to 56% (95%CI: 43–69%) of patients randomised for ALND and to 58% (95%CI: 47–70%) of patients randomised to RT of the axilla.
Conclusions: Accrual status of the AMAROS trial has reached halfway. A strict quality control protocol resulted in a SN identification rate of 98%. Information of the complete axillary lymph node status did not show a difference in distribution of adjuvant systemic treatment between the two treatment arms. Finally, the small number of axillary recurrences developed after a SN negative procedure supports the accuracy of this new promising staging technique in early breast cancer.

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A prospective evaluation of a new technique using aponeurosis padding without vacuum drainage to reduce morbidity in patients undergoing axillary node dissection for localized breast cancer (LBC)

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Objective: Despite the use of sentinel node biopsy, many patients with LBC require axillary lymphadenectomy. Axillary aponeurosis padding appeared to be a valuable alternative technique as it avoids vacuum drainage and its risks. After reporting our first experience with muscular padding [1], we report here the results with axillary aponeurosis padding.
Patients and methods: Aponeurosis padding was prospectively performed in patients with LBC. Level I and II axillary lymphadenectomy was performed through a horizontal skin incision close to the hairline. The aponeurosis was incised at the same level and dissection started underneath. Padding consisted of suturing the edges of the axillary aponeurosis to the underlying muscle with 3 separate stitches, without drainage. A surgeon, a pain clinician and a physiotherapist, respectively evaluated surgical complications (i.e. infection, seroma, ...), pain after surgery and at 6 weeks, mobility of the shoulder and arm.
Results: From 01/2004 to 03/2005, 114 patients were treated. The mean number of excised nodes was 14 (5–36). The mean hospital stay was 2 days. There was no clinical seroma in 91% of the patients and aspiration was required in only 4%. There was no pain at 6 weeks in 71% of the patients. This compares very favourably with a previous cohort of patients operated on by the same team using vacuum drainage: mean hospital stay was 4 days, incidence of seroma and upper arm mobility were similar, and pain at 6 weeks was present for almost 50% of the patients.
Discussion and conclusion: Aponeurosis padding without drainage is easy to learn and effectively reduces morbidity after axillary node dissection in patients with LBC. A longer follow-up is required but this new technique appears to be very promising.

References

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The number of recovered axillary lymph nodes affects lymph node recurrence but not specific survival in node-negative breast cancer

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Background: The number of examined axillary lymph nodes (LN) varies among patients and may also be surgeon-dependent. In case of insufficient LN retrieved upon axillary dissection, it has been often recommended to re-operate. Axillary relapse is known to affect prognosis. Sentinel LN biopsy is increasingly been used, where one of a few LN are examined. The purpose of this study is to examine LN recurrence and specific survival (SS) in node-negative breast cancer patients according to the retrieved number of axillary LN.
Materials and Methods: Between 1973 and 2003, 2461 patients presenting with infiltrating breast adenocarcinoma and registered in our database were examined. They were treated with either conservative surgery (57.9%), or mastectomy (42.1%). All patients had axillary LN dissection and were negative for LN involvement. Radiotherapy was given after conservative surgery, and for T3 or T4 tumours after mastectomy. Some patients with central or inner lesions were given radiotherapy to the internal mammary chain. No axillary radiotherapy was delivered. Hormonal therapy and or chemotherapy was given according to the policy at the time the patient was seen. The mean follow-up was 120 months. The 5- and 10-year Kaplan-Meier rate of LN relapse was studied, as well as the specific survival.
Results: The overall 5- and 10-year LN relapse rate was 1.1% and 1.4%. No LN relapse was observed after 8 years of follow-up, with 1369 patients at risk at that time. In the group of patients with less than 8 axillary LN examined, the 5- and 10-year LN relapse rate were 2.1% and 2.8%. For those with 8 or more LN, the respective values were 0.8% and 1.1% ($p = 0.0046$). For the whole population, the 5- and 10-year SS were 95.5% and 88.3%. Patients with less than 8 axillary LN had SS of 94.9% and 89.8% respectively, versus 95.6% and 88.0% for patients with 8 or more examined LN ($p = 0.58$). Similar results were obtained if patients were classified into more than 2 groups according to the number of retrieved axillary LN.
Conclusions: Even though LN relapse increased by a factor of 2.5 in case fewer than 8 LN were recovered at axillary dissection, the long-term SS was not affected by the number of LN, probably because of the rare occurrence of axillary relapse. These findings do not favour re-operation of the axilla, nor performing axillary radiotherapy in case of insufficient LN examined. In addition, these data tend to comfort the sentinel LN biopsy technique.

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Comparison of peritumoral and periareolar injection of Tc-labeled colloid in sentinel lymphnode biopsy (slnb) in patients with clinically node negative breast cancer

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Background: After it's introduction SLNB gained broad exceptance as a minimal invasive alternative for staging the axilla in breast cancer patients. However the optimal site of injection of blue dye and Tc-labeled colloid remain an issue for debate. The aim of this study was to compare two different injection sites of the radioactive tracer, using a deep (peritumoral) and a superficial (periareolar) technique.
Material and Methods: To this purpose a prospective registration of 525 patients, operated on between 1998 and 2004, was analysed. Group A (284 patients) underwent the SLNB after subareolar injection of the radioactive tracer (60 MBq of technetium-99m nanocolloid). In group B (241 patients) the radioactive tracer was injected peritumoral, the blue dye was injected peritumoral in all patients. The Sentinel Lymph Node (SLN) was identified guided by the preoperative lymphoscintigraphy, the blue lymphatic vessels and hand-held gamma probe. Extra-axillairy SLN's were not harvested.
Results: Patient and tumour characteristics were comparable in both groups. A median of 1.0 SLN's were harvested in group A compared to 2.0 SLN's in group B. In Group A 13 extra-axillairy SLN's were visualised on scintigraphy compared to 21 in group B. In group A 30.6% of all patients had tumour postive SLN's, in group B 39.8% had metastatic SLN's ($p = 0.03$). In group A this concerned micrometastases in 47.7%, in group B 41.7% ($p = 0.42$). In group B a false negative rate of 5.2% was seen (non-SLN harvested was tumour positive while SLN was tumour negative), in group A there were no false negatives. Tumour positive SLN's were hot and blue in 83.4%, hot only in 2.3% and blue only in 13.8% of all metastatic patients in group A, compared to 79.1%, 8.8% and 12.1% respectively in group B.

Metastatic SLN

	Group A (N = 284)	Group B (n = 241)	*P*-value
Total	30.6%	39.8%	**0.03**
Micrometastases	47.7%	41.7%	0.42
False negative rate	0%	5.2%	**0.03**