**82** POSTER HIGHLIGHT

**Discovery of previously undetected micrometastases by mRNA markers in sentinel lymph nodes of breast cancer patients**

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**Background**: In breast cancer, the axillary lymph node status (ALN) remains the most valuable individual prognostic factor for disease course and recurrence. However, 20–30% of node-negative patients will develop a relapse in five to ten years after diagnosis. Based on these shortcomings, we used a sensitive real-time PCR approach including a marker panel of four genes (CK19, p1B, EGP-2, SBEM) for the detection of metastases in sentinel lymph nodes (SN). The PCR method was then compared to the routine analysis of SN, including multiple step sectioning and immunohistochemical staining.

**Material and Methods**: Tumor positive axillary lymph nodes (n=50) of breast cancer patients were selected from the fresh-frozen tissue bank of the Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital (NKI/AvL). As negative controls 48 ALN's were obtained from patients without breast cancer undergoing a preventive breast ablation. First, several candidate marker genes were tested for their specificity by real-time PCR. The marker gene panel selected, was subsequently applied to detect metastases in 19 tumor cell positive SN's and 70 SN's that were free of metastases as determined by standard histological evaluation.

**Results**: Seven negative SN's showed increased marker gene expression, suggesting the presence of (micro) metastases. Four of the seven SN's with an elevated real-time PCR signal proved to contain tumor deposits after review of the slides or further sectioning of the paraffin-embedded material. In three PCR positive SN's however, no tumor cells were found by haematoxylin and eosin staining and immunohistologically analysis. Using the real-time PCR approach we achieve an upstaging of SN's containing metastases of 10% compared to the standard histological analysis.

**Conclusions**: The follow-up times of the seven patients, whose histologically negative SN's showed a positive signal using the real-time PCR approach, are too short to give an indication whether the upstaging of SN's reached by quantitative PCR has a prognostic significance. Recently it was suggested that approximately 18% of the sentinel nodes harbouring micrometastases, might be associated with further nodal non-SN metastases (Cserni *et al.*, 2003). Therewith, our results provide information that could lead to a better management of breast cancer patients by reducing the rate of false-negative sentinel lymph nodes.

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**Factors associated with non-sentinel node involvement in breast cancer**

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After completion axillary dissection, many breast cancer patients with axillary sentinel nodal involvement are found to have regional disease limited to the sentinel nodes. These patients are therefore exposed to the morbidity of axillary clearance without any expected therapeutic benefit.

Sentinel node biopsy was performed either with Patent blue dye or with a combined dye, radiocolloid and gamma-probe-guided method involving peritumoral tracer administration. For a series of 150 consecutive patients with involved axillary sentinel nodes and axillary dissection, factors associated with non-sentinel nodal involvement were analysed. The following data were considered for inclusion in the models: the age, the tumour size, the maximum size of the SN metastasis, the number of SNs recovered, the number of positive SNs, the percentage of involved SNs (the number of positive SNs divided by the number of SNs $\times$ 100), the extracapsular spread of the SN metastasis, the detection of the SN metastasis by HE or immunohistochemistry, the involvement of 1 or more than 1 SN, the pN category of the SN metastasis, the pT category of the tumour, the histological grade of the tumour, the presence of lymphovascular invasion and the histopathological protocol used.

In a multivariate analysis based on logistic regression with the use of fractional polynomials, the following variables were found to be potentially associated with non-sentinel node metastases: the tumour size, the sentinel node metastasis size, the number of examined sentinel nodes, the percentage of involved sentinel nodes, and extracapsular perinodal spread.

Isolated tumour cells and micrometastases in axillary sentinel nodes carry a low risk of non-sentinel node metastasis. The risk of metastasis to further echelon nodes is higher with macrometastases, especially if there is extracapsular growth and the proportion of involved sentinel nodes is high.

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**The sentinel node concept in breast ductal carcinoma in situ patients**

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**Introduction and aims:** Worldwide breast cancer (BC) screening programmes led to an increasing incidence of ductal carcinoma *in situ* (DCIS). Theoretically, DCIS is not associated with node metastases; nevertheless, these metastases have been reported in less than 1% of DCIS patients. The advent of sentinel node (SN) biopsy has increased this rate. The aim of this study was to assess the applicability of the SN technology to DCIS patients and it's impact in the detection of node metastases.

**Patients and methods:** From June 1999 to July 2003 we studied, in two distinct phases, two hundred and seventy six BC patients. Of these, 14 had DCIS (10 pure DCIS and 4 DCIS with micro invasion). The initial, validation, phase included 105 female patients, all of them submitted to level I–II axillary dissection. The second was a phase III randomised trial, comparing SN biopsy to axillary dissection in (sentinel) node negative patients. We used a triple technique (peritumoral $^{99m}$Tc sulphur colloid injection and lymphoscintigraphy, subareolar blue dye injection and hand-held *gamma*-probe). SN were studied with single H–E section. Additionally, for the detection of occult micrometastasis, we performed serial 50 μm sections stained with haematoxylin-eosin (H–E) and cytokeratin immunohistochemistry; non-SN were studied with single H–E section only.

**Results:** The median of age of these 14 DCIS female patients was 51.5 years (range: 34–71). Four patients (28.6%) were submitted to previous surgical diagnostic biopsy (excisional biopsy or large tumorectomy); of the remaining ten, five had palpable lesions and five needed pre-operative hook-wire placement. The median tumour size was 41 mm (range: 5–99); five patients had tumours over 40 mm in diameter. The lymphoscintigraphy showed hot-spots in all patients (100%). The SN identification rate was 100%. There was no false-negative cases in the 8 patients with axillary dissection; three patients of the phase III trial were submitted to total mastectomy and SN biopsy: in these patients, whose follow-up time was 12, 5 and 3 months, there were no axillary node relapses. Ten patients (71.4%) were submitted to total mastectomy. Three patients (21.4%) had internal mammary chain SN biopsy. The initial H–E section was negative in all cases. In one patient with pure DCIS (tumour size: 99 mm) the additional SN sections and the IHC revealed isolated tumour cells (ITC); the other 25 SN did not show metastasis.

**Conclusions:** Sentinel node technology can be successfully applied to DCIS patients. In this small patient series the intensive SN analysis did not bring additional useful information to the tumour staging.

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**Multiple sentinel nodes in breast cancer: how many should be removed?**

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**Background:** During sentinel lymph node(SLN) biopsy for breast cancer, a mean of 1 to 3 SLNs, but a range of 1 to 9 SLNs per patient has been found in the ALMANAC trial. A significant minority of patients have 5 or more SLNs. This paper examines the factors associated with the removal of multiple SLNs and whether there is an optimal threshold number of SLNs that should be removed. In patients with positive SLNs, the nodes were analysed to determine which SLN contained metastases.

**Methods:** In the ALMANAC multicentre trial audit phase, 842 breast cancer patients underwent SLN biopsy, followed by standard axillary treatment. The SLN was identified using the combined technique (blue dye and radioactive colloid). SLNs were ranked in the order they were removed and examined for which node contained the metastasis.

**Results:** During biopsy, a mean of 2.2 (range, 1–9) SLNs were found. Factors associated with identification of multiple SLNs are: younger age; low BMI; tumours in the outer quadrant; SLN visualization of