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Is ERBB-2 A predictive marker for response to primary chemotherapy for operable breast cancer: A prospective study in a phase II randomized trial of doxorubicin/cyclophosphamide (AC) and doxorubicin/paclitaxel (AT)

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The association between amplification of erbB-2 and poor outcome of patients with N+ primary breast cancer is well known. Our aim was to determine if erbB-2 activation may predict the response to primary chemotherapy.

T2, T3, N0, N1, M0 breast cancers were randomized in AC arm (Doxorubicin 60 mg/m^2, CPM 600 mg/m^2) versus AT (Dox 60 mg/m^2, Taxol 200 mg/m^2) every 3 weeks for 4 cycles, then surgery and radiotherapy. Primary objective was the pathological complete response (pCR); secondary objectives: objective response rate (ORR), disease-free survival, and overall survival, erbB-2 determination was performed at molecular and protein level on AFA-fixed, paraffin embedded biopsies, by competitive quantitative PCR and immunohistochemistery using CB11 monoclonal antibody.

To date, tumor samples of 89 patients were analyzed (64 in AT arm, 25 in AC). Patients characteristics were: T2 (59%) T3 (41%), SBR I 8%, SBR II 39% SBR III 52%, Hormone Receptor (HR) 64% (ER+ and PR+), 19% (ER+ or PR+) 18% (ER− and PR−), median S Phase 4% (range 0.2–23.4%). erbB-2 was overexpressed in 21% and erbB-2 amplified in 29% of informative samples. There was a highly significant concordance between erb-B2 gene amplification status and protein expression (93%, chi2 test $p < 0.001$). In 88 pts evaluable for response, we observed 75% ORR (6 Complete Responses and 60 Partial Responses) and 4 pCR. ORR was correlated with S Phase ($p < 0.001$) and erbB-2 overexpression ($p < 0.02$).

In conclusion, at the time of the analysis, we observed a correlation of erbB-2 expression with ORR.

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Her 2 positivity and ER negativity predicts the complete pathological response of breast cancers treated with primary docetaxel

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Background: Complete pathological resolution of tumour following primary chemotherapy is of considerable prognostic importance in patients with breast cancer. The addition of docetaxel to an anthracycline based primary chemotherapy regimen has been shown to result in significantly improved pathological breast cancer response. The identification of predictors of treatment response will permit cytotoxic regimens to be tailored to individual patient requirements and permit pathological response rates to be improved. Oestrogen receptor (ER) status and tumour grade are known prognostic factors for survival, although their ability to predict the response of breast cancers to chemotherapy is uncertain. Expression of the oncogene her-2 has been shown to predict susceptibility to doxorubicin and resistance to cyclophosphamide. However, the predictive value of her-2 expression in patients who receive docetaxel is unknown.

Aim: To identify any relationship between ER status, tumour grade, her-2 expression and complete pathological response (PathCR) in patients with breast cancer following treatment with docetaxel.

Method: Histopathological parameters were studied on core biopsies taken from 34 patients with breast cancer prior to receiving primary docetaxel. Tumours were typed and graded. Immunohistochemistry was carried out using a standard three-stage avidin biotin peroxidase complex technique. Antigen retrieval employed microwave technology. Specific monoclonal antibodies (with appropriate controls) were used to detect oestrogen receptors and Her-2 oncoprotein. Pathological response was determined from operative specimens.

Results: Univariate and multivariate analysis (UA and MVA) (logistic regression) were used to assess the predictive power of each variable. UA revealed that ER negativity ($p = 0.023$) and Her-2 positivity ($p = 0.023$) distinguished patients with complete pathological response. MVA suggested that her-2 positivity ($p = 0.045$) and ER negativity ($p = 0.010$) independently predict a PathCR. High tumour grade did not predict a PathCR on UA or MVA.

Conclusion: Breast cancers that express Her-2 oncoprotein and/or are oestrogen receptor negative are more likely to achieve a PathCR to primary docetaxel.

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Expression of endothelial and inducible nitric oxide synthase in benign and malignant lesions of the breast

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Background: Nitric oxide synthases (NOS) exists in different human solid tumours and tumour cell lines. Increased NO-concentration, catalysed by NO-synthases (NOS) might be cytotoxic and can promote apoptosis. Expression of endothelial (e-) and induced (i-) NOS was examined in various breast tissues.

Methods: An immunohistochemical staining with a monoclonal antibody (Ab) against e-NOS and a polyclonal Ab against i-NOS was performed on paraffin-embedded tissue of 41 benign, 7 in-situ and 54 invasive breast lesions. Functionality was confirmed indirectly by detection of dinitrosyl-iron complexes (DNC) using electron-spin-resonance (ESR)-spectroscopy.

Results: e-NOS expression was found in 5% of the benign and in 57% of in-situ and 61% of invasive lesions. One fibroadenoma and one proliferative mastopathia stained positive for e-NOS. No benign lesion was positive for i-NOS but 73% of in-situ and 61% of invasive cancers showed staining of endothelial and epithelial tumour cells. Some regions in the tumours showed no staining whereas especially capillaries embedded in lymphocytic stroma showed a positive reaction. ESR-spectrum of 5 invasive carcinomas was axial symmetric to standard DNC representing approximately 50 nM DNC. e-NOS positive tumours appeared more often in younger patients, were more frequently highly or moderately differentiated, more often invasive ductal subtypes, and showed a lower proliferation rate. e-NOS and i-NOS positive tumours were more likely to be node negative. Both, i- and e-NOS showed a strong co-expression.

Conclusions: NOS are predominantly detected in in-situ and invasive but rarely in benign breast lesions. NOS are more frequently found in low malignant invasive carcinomas.

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Immuncytochemical detection of cytokeratin (CK)-positive micro-metastases in lymph nodes (LN) and bone marrow (BM) from node-negativ breast cancer patients with stage I–II disease

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Purpose: Since the detection of BM micrometastases at the time of first diagnosis has been described as independent predictor of poor prognosis in node-negative breast cancer patients, we investigated in the present study whether hematogenous tumor cell dissemination is paralleled by lymphatic spread of single tumor cells.

Methods: At the time of this analysis, we have screened 599 axillary LNs of level I and 63 BM samples obtaind from 63 node-negative patients. Tumor cells in BM and LN were detected using the anti-CK antibodies A45-B/B3 and 5D3, respectively. To avoid interference with CK$^+$ mesenchymal cells present in LN, we applied double-labeling with anti-vimentin and anti-CD45 antibodies to LNs paraffin sections. Of each single LN, adjacent sections were analysed immuncytochemically and compared to independently screened hematoxylin-eosin (HE) stainings. Median follow-up was 30 months (range, 5–50).

Results: We found tumor cells in 27 (5%) of 599 axillary LNs, which resulted in 22 (35%) of 63 patients with CK$^+$ tumor cells in LNs, whereas all HE stainings revealed no tumor cells. Hematogenously disseminated tumor cells were found in 18 (29%) of 63 patients. Neither LN nor BM micrometastasis correlated with established risk factors for recurrence. In 22 patients with CK$^+$ LNs, 3 (14%) relapses had occurred, while 9 (22%) relapses were observed in 41 patients with CK$^-$ LNs ($P = 0.42$). In 18 patients with CK$^+$ BM, 3 (17%) relapses had occurred, while 9 (20%) relapses were observed in 45 patients with CK$^-$ LNs ($P = 0.76$). A single recurrent patient had CK$^+$ tumor cells in both BM and LNs. Distant