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Realtime quantification of HER2/neu gene amplification by polymerase chain reaction (PCR)

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Introduction: The tyrosine growth factor receptor HER2/neu is frequently overexpressed in breast cancer and other solid tumors, mostly due to gene amplification. This gene amplification/overexpression is currently detected by fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). We have evaluated a PCR method (Light Cycler HER2/neu Test, ROCHE; for research use) to quantify HER2/neu gene copies in several breast cancer samples.

Methods: DNA was extracted from formalin-fixed tissue in triplicates from 45 cases with an IHC-score of 0 or 1+, from five cases with an IHC-score of 2+ but non-amplified by FISH and from eight cases with amplification (IHC-score; 2+ or 3+). PCR was performed with the LightCycler Her2/neu test, which uses a reference gene that is also localized on chromosome 17 and therefore serves as a control for polysomy. A positive result is defined by a ratio HER2/reference >2. In order to minimize the diluting effect on the signal by non-tumor/non-amplified intraductal tumor cells, dissections were performed by scratching only invasive tumor areas from the slides in five representative cases.

Results: All fifty negative/non-amplified cases gave a negative PCR result. 5/8 amplified samples were positive by PCR when extracting DNA from the entire section, three were negative. After tumor dissection, the ratio of HER2/reference was generally increased in the positive cases resulting in a 100% concordance of PCR to the FISH results.

Conclusion: These preliminary results indicate that quantitative PCR may be a valid and sensitive alternative to determine HER2 positivity. By virtue of the rapid performance, a high level of reproducibility, fully objective results and moderate costs it might be particularly suited as a first line screening tool for HER2 in breast cancer.

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Minimal sentinel node procedure for staging early breast cancer

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Purpose: Sentinel lymph node dissection (SLND) has been recently evaluated as a new staging technique for early breast cancer instead of routine axillary lymphadenectomy. To further minimize its surgical approach, the feasibility of eradication of the primary lesion along with its sentinel lymph node (SLN) under regional anesthesia was evaluated.

Methods: A selected population of 76 operable breast cancer-suspected patients with no clinically palpable lymph nodes, were enrolled into the present study. Intra- and peri-lesional administration of a radiotracer was performed. Lymphoscintigraphy (LSG) was carried out to confirm the drainage pathway and to locate the SLN. The day after a nervous block of omolateral intercostal nerves followed by the surgical procedure with an hand held gamma-detecting probe was performed. When the primary lesion was diagnosed as invasive carcinoma (by frozen section), the SLN and the rest of axilla (non-SLNs) were eradicated. The status of the SLN and non-SLNs were compared.

Out of 76 cases of breast lesions, 45 invasive carcinomas staged as pT1 (2 pT1a; 11 pT1b; 32 pT1c) were identified; in the remaining 31 cases, 24 resulted to be DCIS and 7 fibroadenomas.

Results: The primary breast lesion was located and excised in all case (identification rate 100%). LSG positively identified SLN in 40/45 (89%) carcinoma patients; in 5 patients lymphatic drainage was not shown. In 38 cases, an average of 1.5 SLN and 14 non-SLNs per patient were eradicated and pathologically analyzed; the remaining 2 patients showed SLNs in the internal mammary chain and, therefore, were not excised. Routine haematoxylin-eosin pathological examination of the SLNs accurately predicted the status of the non-SLNs in the rest of axilla (accuracy 84%). Twenty-nine percent of the patients showed metastatic disease in the lymph nodes examined. Of all patients with affected nodes, 55% had cancer cells only in the SLN. No false negatives (skip metastasis) were seen.

No immediate or long-term complications (pleural lesion, intravascular injection, etc.) due to the anesthesia were shown.

Conclusion: Our data proved the utility of SLND in staging early breast cancer. Regional anesthesia provided a good management and better quality of life of our patients. This time-saving procedure allowed a completeness of the surgical plan, minimizing costs and recovery time without modifying its effectiveness.

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Evaluation of HER-2 protein expression in primary breast cancer (PBC) by immunohistochemistry (IHC): An interlaboratory study assessing the reproducibility of HER-2 testing

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Purpose: To assess the degree of interlaboratory agreement when HER-2 is evaluated by IHC on archival PBC samples.

Methods: IHC for HER-2 was performed on the same archival tissue sections from 394 invasive PBC in two different laboratories (JBI and IMT). Both labs used the primary antibody NCL-CB11; however, different methods of immunostaining (antigen retrieval procedure and manual processing or no antigen retrieval and autostainer processing) as well as different scoring systems were used. Fluorescence in situ hybridization (FISH) which is considered as the gold standard for HER-2 status determination was performed using the PathVysion kit (Vysis, Downers Grove, IL) and compared to the IHC results.

Results: 48 of 394 analyzed tumors (12.2%) were scored as HER-2 positive in JBI laboratory, and 109 (27.7%) in IMT laboratory where antigen retrieval was performed. FISH performed in 248 samples revealed HER-2 gene amplification in 55/248 (22.2%).

Comparison of HER-2 status by FISH vs. by IHC in 248 cases of invasive breast carcinomas

IHC at JBI	IHC at IMT	FISH (n = 248)			Total
		Negative (%)	Moderately amplified* (%)	Strongly amplified** (%)	
–	–	169 (88)	3 (23)	1 (2)	173
–	+	21 (11)	10 (77)	20 (48)	51
+	+	3 (1)	0 (0)	21 (50)	24
Total	193	13	42	248	

*Amplification ratio is 2 < HER-2/CEP17 ≤ 5; **amplification ratio is HER-2/CEP17 > 5.

Conclusion: Based on our data, it must be concluded that HER-2 evaluation by IHC is not a reproducible technique if there is no standardization of the procedure.

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Preoperative chemotherapy for operable breast cancer: role of pathological features in predicting clinical and pathological response

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Purpose: Preoperative chemotherapy (PreCT) is investigational. Identification of a subset of patients more likely to benefit from this treatment might improve therapeutic results. We have therefore studied pathological factors as predictors for outcome before and after PreCT in patients with large operable (T2-T3) breast cancer.

Methods: Analyses were performed on histopathological specimens from 147 breast cancer patients, investigating variables thought to have predictive relevance including: percent of staining for ER (absent, low 1-9, positive 10+); PgR (absent, low 1-9, positive 10+), Ki-67 (< 20, > 20), p53, bcl-2, p27, p21 (< 1, 1-10, 11-25, 26-50, > 50), and overexpression of c-erbB-2 (absent, +1, +2, +3). Logistic regression analysis was used to assess the relative influence of these factors on objective and pathological remissions. Thirty-eight patients were treated with Adriamycin 60 mg/m2 i.v. plus cyclophosphamide 600 mg/m2 i.v. on day 1 q 3 wks (AC regimen). Thirty-three patients received 5-fluorouracil 350 mg/m2 proceeded by folinic acid 100 mg/m2, both i.v. on days 1,2,3, and vinorelbine 20 mg/m2 days 1 and 3

q 3 wks (FLN regimen). Seventy-six patients received 5-fluorouracil 200 mg/m2/die as intravenous continuous infusion, vinorelbine 20 mg i.v. as total dose on days1 and 3, plus cisplatin 60mg/m2 i.v. on day 1 q 3 wks (ViFuP regimen).

Results: The overall response rate (partial and complete remissions) according to radiological and clinical evaluation was 65% (95% C.I: 57%-72%.) In both the univariate and multivariate analyses the most important predictive factor was the baseline absence of expression of PgR ($p<0.01$), high baseline Ki-67 ($p<0.01$) and decrease of p53 positive cells ($p<0.01$). Conversely no significant effect according to other histological features was observed.

Conclusion: Our results indicated that baseline PgR and Ki-67 expression as well as changes during therapy of p53, should be considered in further studies on preoperative chemotherapy.

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Effect of elevated serum carboxyterminal telopeptide (ICTP) on survival in breast cancer patients with and without bone metastases

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Purpose: Bone is made of Type I collagen fibrils that are crosslinked e.g. by pyridinium cross-links. Bone resorption releases the cross-links as free and peptide bound (telopeptide) fragments. There are 2 assays available for the carboxy-terminal telopeptide fragments, ICTP and Crosslaps. ICTP elevations have also been seen in non-bony disease and found to be prognostic in ovarian cancer patients. We evaluated the prognostic value of serum ICTP levels in patients without bone metastasis (BM-) and with bone metastasis (BM+).

Methods: ICTP levels were quantified in serum using an ICTP RIA produced by Orion Diagnostica (Espoo, Finland) and distributed in USA by Diasorin, Inc. (Stillwater, Minn). This was a retrospective study and used baseline serum that was obtained from 253 patients with metastatic breast cancer. These patients had participated in a double-blind randomized study of second-line hormonal therapy with a second-generation aromatase inhibitor (Fadrozole) vs. Megace.

Results: The mean followup for survivors was 868 days (range 264 - 1466). Normal serum ICTP level from a published report of 202 healthy postmenopausal women was 3.0 + 1.6 ug/L (mean + SD). The serum ICTP cutoff of 6.2 ug/L was established using the mean + 2 SD. Using this cutoff, 62/181 patients (34%) in the BM+ group had elevated serum ICTP levels as compared to 6/72 (8%) in the BM- group. Mean serum ICTP for the BM+ group [6 ug/L (1.7 - 24.6)] was significantly higher compared to the BM- group [4.2 ug/L (1.6 - 16.1)]($p= 0.0002$). Overall survival was significantly worse in patients who had elevated baseline serum ICTP levels in both the BM- ($p=0.02$) and BM+ groups ($p<0.0001$).

Conclusion: In summary, an elevated serum ICTP level predicts for decreased survival in breast cancer patients with and without clinically-detectable bone metastasis.

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KI67 and p53 are not predictive of tumor response or survival with trastuzumab-based therapy in metastatic breast cancer patients (pts) with her2/neu-overexpression

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From 2/99 to 3/01, 53 pts with metastatic breast cancer overexpressing HER2/neu were treated with trastuzumab alone (25 pts, group A) or in combination with chemotherapy (28 pts, group B). 3+ -positive HER2/neu overexpression was determined with immunohistochemical staining using antibodies (Ab) 3b5 or A0485. In group B, 19 pts received docetaxel, 5 pts paclitaxel, 2 pts vinorelbine, and 2 pts mitomycin/5-fluorouracil. Pts have been treated either as first-line therapy (17 pts), or as second-line (26 pts) or as third line (10 pts). Using immunohistochemistry staining on primary tumor sections, 23 of 40 pts (58%) showed equal or more than 35% KI67 positive cells (Ab MIB-1) and 17 pts showed equal or more than 50% p53 positive cells (43%, Ab DO7). KI67 and p53 expression in tumor cells were well balanced between groups A and B. To evaluate the predictive value of KI67 and p53 for response, chi-square tests were performed. The predictive value for survival was determined by univariate analysis (log-rank test). The overall response rate (ORR = CR + PR) and the clinical benefit rate (CBR = CR + PR + SD > 24 weeks) were 36% and 60% (group A: 48% and 76%; group B: 25% and 46%), respectively. After a median follow-up of 35 weeks (w) (range, 3 -93 w) the median progression-free survival (PFS) and overall survival (OS) were 25+ w (range, 2-93 w) and 35+ w (range, 3 -93 w) so far. Pts with high or low levels of KI67 or p53 positive tumor cells showed no difference in response rates (ORR: pKI67=0.84, pp53=0.21; CBR: pKI67=0.69, pp53=0.55) and survival rates (PFS: pKI67=0.72, pp53=0.79; OS: pKI67=0.58, pp53=0.56).

In conclusion the proportion of KI67 and p53 positive tumor cells on primary tumor sections might not predict response or survival for a trastuzumab-containing therapy in pts with HER2/neu overexpressing metastatic breast cancer.

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Upstaging of breast cancer patients with PT1 tumours by detection of micrometastasis in sentinel lymph node biopsy (SLND)

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Purpose: Determination of axillary lymph node status is an integral part for patients with early breast cancer and the most important prognostic factor. 15-20% of patients with tumour-free axillary lymph nodes in conventionel H&E staining show recurrences and distant metastasis within 10 years. Serial sectioning and immunohistochemistry (IHC) of the sentinel lymph nodes (SLN) allow more accurate information about micrometastasis (≤2 mm)

Methods: Between September 1997 and April 2001 114 patients with pT1 breast carcinoma were prospectively enrolled and underwent SLND with tumour resection. SLN were detected using blue dye and/or 99mTc labeled colloidal tracer. Completion axillary lymphadenectomy of level I and II was performed in case of macrometastasis in the SLN. Sentinel nodes were examined by serial sectioning and IHC (Lu-5/CK22).

Results: SLN were identified in 108 (95%) of 114 patients. 37 (34%) patients with a pT1 tumour had a SLN metastasis, 71 (66%) being tumour-free. In 14 (38%) cases SLN contained micrometastasis, diagnosed by serial sectioning with H&E staining and by IHC in 7 patients each. 23 (62%) patients had macrometastasis. 12 of 14 micrometastasis and 22 of 23 macrometastasis in SLN were found in patients with pT1c tumours.

Conclusion: In our previous study we reported an incidence of axillary lymph node metastasis in pT1 tumours of 25% without SLN (n=185). Serial sectioning and IHC of SLN lead to an upstaging of one third in this group. Further studies are necessary to assess a potential benefit of ALND in this subgroup of breast cancer patients.

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Sentinel lymph node biopsy and axillary dissection in breast cancer: results and initial experience of a changed technique with subareolar intradermal blue dye injection only

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Introduction: Our study tries to assess the value of intraoperative efficacy to identify the sentinel lymph node in clinical management of breast cancer. We hypothesized that the axillary lymphatic drainage pattern in predicting the pattern of metastatic spread from primary breast carcinoma is independent from a particular breast quadrant and it might be useful to examine our hypothesis in using subareolar intradermal blue dye injection only.

Methods: Between 5/99 and 12/2000 we investigated 86 consecutive patients (85 women, 1 men) using 2 ml of Lymphazurin 1%, which was injected intra- and subdermal into the subareolar area, immediately before definitive surgical treatment of primary breast carcinoma without massage of the breast. The average age of the patients was 59 years (range37-83).In these 86 patients we performed peritumoral blue dye injection in 13 cases, intradermal subareolar injection in the remaining 73 cases. Out of these we found verification of breast cancer in 60 cases, in which complete axillary lymphadenectomy was done and 13 cases with DCIS and/or no completed axillary dissection.