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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