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Correlation of chromogenic in-situ hybridisation (CISH) with FISH and IHC for assessment of HER2 gene amplification: an international validation ring study

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In many algorithms for HER2 testing, cases with an immunohistochemistry (IHC) score of 2+ are subsequently tested by FISH. In this study, 76 cases were scored as IHC 2+; for these cases, the concordance between FISH and "external" CISH was also excellent: 100% (7/7 cases) for high level HER2 gene amplification; 96% (44/47 cases) for normal HER2 gene copy number. Of 18 cases with a 2+ IHC score with low level HER2 gene amplification, 11 had a CISH score <5; one tumor a score of 5; two a score of 6 and two a score of >6.

Conclusion: There is high correlation between FISH and CISH for the assessment of HER2 status.

Table 1. Comparison between FISH and "external" CISH. The CISH score is given as the number of spots/tumor cell; for FISH, the ratio of HER2 copies/centromer chromosome 17 copies is given.

FISH\"external" CISH	<5	5	6	>6	no signal	n
Not Ampli <2	91	3		3	3	100
Low Ampli 2–4	11	4	5	15		35
High Ampli >4	1	2	3	70		76
Total	103	9	8	88	3	211

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Breast cancer risk reduction associated with the RAD51 polymorphism among carriers of the BRCA1 5382insC mutation in Poland

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The observed heterogeneity of breast cancer risk among women who carry the same BRCA1 mutation suggests the existence of modifying environmental and genetic factors. The product of the RAD51 gene functions with BRCA1 and BRCA2 in the repair of double-stranded DNA breaks. To establish whether polymorphic variation of RAD51 modifies risk for hereditary breast cancer, we conducted a matched case-control study on 83 pairs of female carriers of the BRCA1 5382insC mutation. Cases consisted of women with breast cancer, and controls were women with the same mutation but who were unaffected. The frequency of the RAD51 135C variant allele was established in cases and controls using RFLP-PCR. The RAD51 135C allele was detected in 37% of unaffected and in 17% of affected BRCA1 carriers. Among 27 discordant matched pairs, the RAD51 135C allele was found in the healthy carrier on 22 occasions and in the affected carrier on only five occasions (odds ratio = 0.23; 95% confidence interval, 0.07–0.62; P=0.0015). This finding suggests that RAD51 is a genetic modifier of breast cancer risk in BRCA1 carriers in the Polish population. It will be of interest to confirm this in other populations as well.

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Evaluation of VEGF expression within breast cancer biopsies & tumour microvasculature assessment by multi-functional dynamic contrast-enhanced MRI

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Background: Vascular endothelial growth factor (VEGF) is the principal angiogenic factor driving neovascularisation within breast cancers. Multi-functional dynamic contrast-enhanced MRI (DCE-MRI) provides a method for assessing & characterising tumour microvasculature. Here we test the possible correlation between tumour vascularity parameters as assessed by DCE-MRI & VEGF expression in breast tumour biopsies.

Materials and Methods: 20 patients with biopsy-proven primary breast cancer (median age 44 years old, range 29–58) were imaged prior to treatment. DCE-MRI was performed using Gd-DTPA as a contrast medium & parametric images were calculated reflecting microvessel permeability (transfer constant [K^{trans}], leakage space [v_e], maximum contrast medium uptake [MaxGd]), perfusion (relative blood volume [rBV], relative blood flow [rBF], mean transit time [MTT]) & oxygenation (T_2*-relaxation rate [R_2*]). Median values for each parameter were derived from whole tumour regions of interest. The expression of VEGF in each diagnostic biopsy specimen was analysed by immunohistochemistry using the anti-VEGF monoclonal antibody JH121 (Neomarker). The intensity of VEGF staining was scored as: negative=0, weak=1, moderate=2, strong=3. The percentage of cells staining was scored as: 0%=0, <5%=1, 5–20%=2, 20–50%=3, >50%=4. A VEGF immunoreactive score was calculated as the product of the intensity of staining & the percentage of cells staining. The association between two parameters was quantified by Spearman's rank correlation coefficient, r_s, & the statistical significance was the 2-tailed P-value for rejecting the hypothesis of zero correlation.

Results: Tumour transfer constant (K^{trans}) & T_2*-relaxation rate (R_2*) correlated significantly with the perfusion parameters rBF (r_s=0.60, p<0.01 & r_s = −0.68, p<0.01 respectively) & rBV (r_s=0.55, p<0.05 & r_s = −0.67, p<0.01 respectively). In addition, correlation was seen between the individual permeability parameters (K^{trans}, v_e & MaxGd) & the individual perfusion parameters rBV & rBF. The median VEGF immunoreactive score was 6 (range 1–12). No correlation was found between tumour VEGF expression & the pre-treatment vascular parameter values as assessed by DCE-MRI (for K^{trans} p=0.45; v_e p=0.93; MaxGd p=0.43; rBVp=0.41; rBF p=0.21; MTTp=-0.63; R_2* p=0.48).

Conclusions: A strong correlation was demonstrated between the DCE-MRI-derived vascular parameters reflecting tumour microvessel permeability, perfusion and oxygenation. No significant correlation was seen between tumour VEGF expression in the pre-treatment breast cancer biopsy specimens & the DCE-MRI-derived vascular parameters. Failure to demonstrate a correlation between the latter may be due to the small patient number within the study or due to the recognised disparity between visible & functional microvasculature. A larger patient cohort is being assessed.

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CISH or FISH? The interlaboratory reproducibility of CISH testing for HER2 and correlation with IHC and FISH results

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Accurate testing for HER2 is essential in order to select patients with breast cancer for Herceptin therapy. The HER2 testing methods most used are immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) to detect protein overexpression and gene amplification respectively. Chromogenic in situ hybridisation (CISH) assesses HER2 gene amplification but uses normal light microscopy thus combining the greater accuracy of FISH in predicting a response to Herceptin therapy with the advantages of immunohistochemistry. Published studies have shown a strong correlation between FISH and CISH. In order to assess the accuracy, ease of use and reproducibility of CISH testing in different pathology laboratory settings, 5 laboratories from 3 different states of Australia took part in a validation study, testing breast cancers whose HER2 status was already known by IHC and FISH.

Material and Methods: Unstained sections from 50 breast cancers numbered 1–50 were each independently tested twice for HER2 using Zymed SPOT-light HER2 DNA probe following the manufacturer's instructions. Each of 5 different laboratories tested 20 cases so that 100 results were available for analysis. None of the laboratories knew the FISH and IHC results for HER2. 31 of the 50 cases had an equivocal and problematic IHC score of 2+ using HercepTest. A correlation was made at the end of the study between HER2 status as assessed by CISH, FISH and IHC and between the different laboratories. Each laboratory also assessed ER status and type and grade of the breast cancer.

Results: 99 results were available for analysis as one slide was found to include insufficient breast cancer cells. The results were expressed as 'HER2 not amplified', 'low level amplification' or 'high level amplification'. All 21 cases with high level gene amplification by FISH showed gene amplification by CISH. 2 of these showed only low level amplification. 1 of 19 (5%) cases showing no gene amplification by FISH showed

low level amplification by CISH. There was good agreement between the two laboratories testing each case, Kappa coefficient 0.67 (95% CI: 0.51–0.84). The main source of disagreement was in the low level amplified CISH cases. All of the 19 cases scoring 3+ (HER2 positive) by IHC showed amplification by CISH in both laboratories. Of the 31 IHC 2+ (equivocal) cases, 9 (29%) and 12 (38%) in the duplicate tests showed gene amplification by CISH.

Conclusions: There is good agreement between CISH and FISH tests for HER2. Pathology laboratories of different types and with no prior experience of using CISH are able to use the technique to assess HER2 gene amplification. The clinical significance of low level gene amplification by CISH needs to be better understood so that this area of disagreement with FISH is further evaluated.

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Abnormalities of erbB oncogene family in breast cancer

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Background: ErbB-2 amplification and/or overexpression in breast cancer is an adverse prognostic factor and predicts response to trastuzumab therapy. It was recently demonstrated that efficacyof trastuzumab may also be influenced by expression of other three members of erbB family. Furthermore, there is a growing body of evidence that tumors with abnormalities of more than one type of erbB receptor are particularly aggressive. Thus, the quantification of all erbB family members is of potential clinical relevance. The aim of this study was to determine in breast cancer samples. Additionally, the relationship between erbB abnormalities and clinical outcomes was investigated.

Material and methods: Study group included 176 consecutive breast cancer patients who underwent primary surgical treatment between 1998 and 2002 in two Polish institutions. Small part of the tumor was taken during surgery, and together with blood samples frozen immediately for further analysis. Gene copy numbers of erbB oncogenes were determined by double differential PCR (ddPCR).

Results: There was a significant correlation between average gene copy numbers (AGCN) of all erbB oncogenes. This correlation was particularly high for erbB-2 and erbB-3, and for erbB-2 and erbB-4 ($p<0.000001$ for both). Amplifications of erbB-1, erbB-2, erbB-3 and erbB-4 (defined as AGCN values >1.6) were detected in 5%, 22%, 11% and 11% of examined cases, respectively. Deletions (defined as AGCN value <0.2) most frequently accompanied erbB-1 amplifications (32% of cases). At least one erbB oncogene abnormality (amplification or deletion) was found in 59% of samples and at least two abnormalities in 29%. Most frequent were co-amplifications of erbB-2 and erbB-3, erbB-2 and erbB-4, and erbB-2, erbB-3 and erbB-4. There was no correlation between AGCN values of particular oncogenes considered separately and major clinical characteristics. However, there was a correlation between co-amplification of erbB-2, erbB-3 and erbB-4 and tumor size and grading.

Conclusions: These early results demonstrated a strong correlation of abnormalities in particular genes of erbB family in breast cancer. Clinical relevance of these findings warrant further studies.

162 POSTER HIGHLIGHT

Detection of Her2/neu gene amplification in breast carcinomas using quantitative real-time PCR. Comparison with immunohistochemical and FISH results

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Background: In Hungary, patients with Her2/neu over-expressing breast carcinoma (++/+++ positive immunohistochemical reaction and positive FISH result) are eligible for Herceptin therapy. Our aim was to evaluate the value and possible role of the cheaper and quicker real-time PCR (RT-PCR) method in everyday practice.

Material and Methods: A total of 213 consecutive breast carcinoma cases were examined. Ready to use CB11 antibody (Novocastra) was used in standard mode to detect Her2/neu oncoprotein overexpression. In cases of ++/+++ positivity FISH was performed using automated technique (Ventana Inform Kit). RT-PCR was performed with the LightCycler-Her2/neu DNA Quantification Kit (Roche) after isolating DNA from paraffin sections. A 112-bp fragment of the Her2/neu gene and a 133-bp fragment of the reference gene were amplified by PCR specific primers.

Results: Eighty-four cases were ++/+++ positive with immunohistochemistry, using the Novocastra evaluating scheme. 129 cases were either completely negative, or + or showed false positive cytoplasmic reaction. FISH was performed in the central laboratory(*) in 87 cases, PCR was performed in 172 cases. In 40 cases both FISH and PCR were done. From this latter group, in 31 cases both methods showed the same results: 15 cases were negative and 16 positive with both methods. In 9 cases FISH and PCR results were discordant: 6 cases were PCR+/FISH-, 3 cases were PCR−/FISH+. The mean amplification ratio in the concordant cases was 5.71, while in the PCR+/FISH− group this ratio was 2.765. In 31 cases the ++/+++ immuno-positivity was correlated with gene amplification as determined with RT-PCR. The mean ratio of the amplification was 6.68. PCR was positive in 12 cases with 0/+/false immuno-reaction. The mean ratio of the gene amplification in these cases was 3.0. It was interesting that 6 of the 8 cytoplasmic (false) immuno-reaction cases, showed gene amplification with PCR.

Conclusion: The key role of Her2/neu in carcinogenesis is well known. This gene and the oncoprotein play important role in many human cancers. However, its significant amplification is not a universal tumor characteristic. Therefore, if PCR is used in breast carcinoma cases for the detection of clinically relevant Her2/neu gene amplification, we suggest the cut-off level to define at least above 2.7. If an optimal calibration may be constructed, we believe that the relatively cheap and quick PCR method could well substitute the labourious FISH technique to define Her2/neu amplification of breast carcinomas.

This work was supported by the following grants: Bio-00014/2001, NKFP-1A/0023/2002, FKFP-0128/2001.

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Concordance of HER-2/neu expression of primary breast carcinomas and their metachronous distant metastases: results of a 10 year retrospective search in two university institutes of pathology

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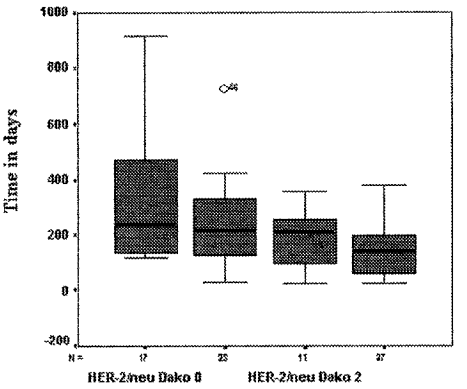
Background: The dogma of clonality between primary solid tumors and their distant metastases is weakened by the evidence of clonal changes in the course of the diseases. The diagnosis of HER-2/neu positivity for selection of stage IV breast cancer patients for trastuzumab therapy is done on the primary tumor. Changes of HER-2/neu expression may lead to a wrong selection of patients for a life-prolonging therapy.

Methods: The archives of 2 university institutes of pathology and reference centers of HER-2/neu diagnostics were searched for pairs of paraffin-embedded tissue blocks of primary breast carcinomas and their metachronous distant metastases. Altogether, 80 pairs dating from 1994–2003 could be identified and stained for HER-2/neu using the method and scoring system of the DAKO HercepTest.



Results: Characteristics of the primary breast cancers were as follows: 73% invasive ductal, T1/T2 tumors 45% and 37%, N1/N2 stage 48% and 42%. Biopsies were distributed as follows: Viscera 9%, bone 10%, soft tissue 78%, rest others. Figure 1 displays the significant ($p=0.017$) prolongation of time to metastatic spread in days with increasing semiquantitative DAKO HER-2/neu scores. A total of 47.7% of the primary lesions were HER-2/neu positive (i.e. DAKO +2 or +3) while 59.0% of the distant metastases showed +2/+3 expression. The concordance between the HER-2/neu expression of the primary tumors and their distant metastases was moderate with a concordance index kappa of 0.52 (0 = weak concordance, 1.0 = strong concordance). The McNemar test