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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.

160 POSTER HIGHLIGHT

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