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.

161 POSTER HIGHLIGHT

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.

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163 POSTER HIGHLIGHT

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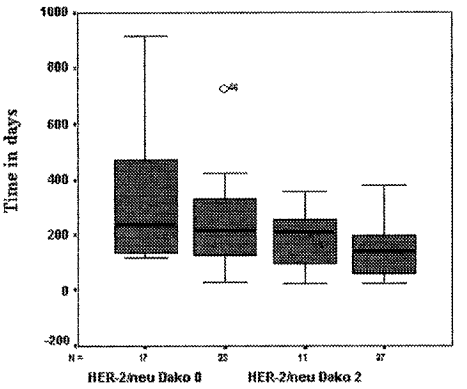
Background: The dogma of clonality between primary solid tumors and their distant metastases is weakened by the evidence of clonal changes in the course of the diseases. The diagnosis of HER-2/neu positivity for selection of stage IV breast cancer patients for trastuzumab therapy is done on the primary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