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.

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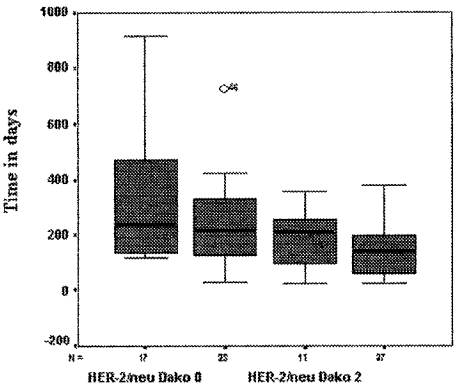
Background: The dogma of clonality between primary solid tumors and their distant metastases is weakened by the evidence of clonal changes in the course of the diseases. The diagnosis of HER-2/neu positivity for selection of stage IV breast cancer patients for trastuzumab therapy is done on the primary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

for changes from HER-2/neu negative primaries to HER-2/neu positive metastases (17.9% of pairs) as compared to reverse changes from HER-2/neu positive primaries to HER-2/neu negative metastases (6.4% of pairs) showed a p-value of p=0.063 in favour of preferential changes from negative primaries to positive metastases, however just missing statistical significance.

Conclusions: Clonal changes between primary breast carcinomas and their distant metastases are more frequent than generally assumed, but do not question the current practice of selecting patients for anti-HER-2/neu targeted therapies. As demonstrated in a previous study on the correlation of the HER-2/neu tissue status and the serum HER-2/neu level at stage IV disease, a simple option to reassure the current HER-2/neu status would be serum testing for HER-2/neu with a level >50 ng/ml (normal < 15 ng/ml) indicating HER-2/neu positive metastatic spread.

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Role of CYP17 and SULT1A1 gene polymorphisms in breast cancer

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The common risk factors for developing breast cancer such as early age at menarche, late first full-term pregnancy, nulliparity, late menopause, family history of breast cancer and socioeconomic status are all a result of cumulative life time exposure to estrogen. Many of the enzymes involved in estrogen metabolism/biosynthesis are polymorphically distributed within human populations. Investigating the distribution of these functionally relevant genetic polymorphisms that alter the bioavailability of steroid hormone among individuals may provide a more direct evidence for estrogen and estrogen metabolites as modifiers of breast cancer susceptibility. The CYP17 gene encode for a CYP P450 C17 alpha enzyme which functions at key branch point in human steroidogenesis. The polymorphic variant to this gene (A2 allele) shows enhanced transcriptional activity due to creation of an Sp-1 promoter motif and may therefore influence breast cancer risk by increasing estrogen hormone level. The SULT1A1 gene encodes for the sulfotransferase ensyme that plays an important role in the inactivation of endogenous estrogens and biotransformation of environmental mammary carcinogens. Sulfotransferases are also found to regulate the metabolism of tamoxifen, a potent antiestrogen and a chemo preventice against breast cancer. Large interindividual variation observed both in the enzyme levels and activity of the sulfotransfereases are mainly due to the genetic polymorphisms of the SULT gene which may ultimately influence the individual susceptibility of breast cancer. The current study evaluated the role of genetic polymorphisms of these estrogen related genes-SULT1A1 and CYP17 in breast cancer susceptibility by a case-control study. In addition the relationship between the estrogen biosynthesizing CYP17 gene polymorphisim and serum estradiol levels were also analyzed. The genotype assay was done by PCR-RFLP assay and serum estradiol levels were measured by ELISA. Our data showed a significant positive association between the CYP17 (OR=2.16; 95%CI=1.23–3.79; p=0.007) and SULT1A1 (OR=1.78, 95%CI=1.09–2.89, p=0.02) gene polymorphisms and breast cancer. Our data also showed evidence for the genetic regulation of serum estradiol levels among premenopausal women with a significant 2.57 fold increase in the serum 17 beta estradiol level for the CYP17 homozygous polymorphic variant genotypes. The results from our study suggests that analysis of functionally relevant polymorphisms in these low penetrance genes would exhibit additive effects on individual susceptibility to breast cancer by influencing lifetime levels and metabolisms of estrogen. We are currently investigating the role of these genes in individual response to tamoxifen. Such a genotype analysis study holds considerable promise for individualizing diagnosis, screening and therapeutic intervention.

165 POSTER

The correlation between serum and normal breast tissue Insulin-like Growth Factor (IGF-I) system components

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IGF-I is an important growth factor and has been associated with increased breast cancer risk in both prospective epidemiological (serum) and experimental studies (tissue). It is suggested that the association between serum IGF-I and breast cancer risk is especially seen in premenopausal women, and in women with a strong family history of breast cancer. In serum, most IGF-I is bound to IGF Binding Protein-3 (IGFBP-3). Both are mainly produced in the liver after stimulation by growth hormone. IGF system components are also locally produced in tissues (e.g. breast tissue), where IGF-I can exert its tumor promoting effect by binding to the IGF type 1 receptor (IGF-IR). In a previous study, we observed a large variation in mRNA expression of IGF-I, IGF-II, IGF-IR, and IGF-IIR in normal breast tissue. We also observed a higher expression of some IGF system components in breast tissue from women with a positive family history than in women without such a history. However, little is known about the relation between serum concentrations and normal breast tissue expression of IGF system components. Therefore, the objective of the present study was to investigate whether the serum concentration of IGF-I is correlated with mRNA and protein expression of different IGF system components in normal breast tissue.

We identified 153 women with a strong family history of breast cancer, who underwent a prophylactic mastectomy at the Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital from 1990 to March 2001. For 29 premenopausal women, suitable snap-frozen normal breast tissue and a serum sample taken within one year before/after mastectomy were available for analysis. Tissue mRNA expression of IGF-I, IGF-II, and IGF-IR was measured by quantitative real-time RT-PCR. Serum IGF-I concentrations were measured by a radioimmunoassay.

In this preliminary series of 29 normal breast tissue samples, no significant correlation between serum IGF-I concentrations and tissue mRNA expression of IGF-I, IGF-II, and IGF-IR was observed [Spearman correlation coefficients (r): IGF-I mRNA: r = 0.02 (p=0.93), IGF-II mRNA: r = −0.01 (p=0.96), IGF-IR mRNA: r = −0.04 (p=0.84)]. As both serum and tissue concentrations of IGF system components within an individual may vary over time (due to e.g. dietary habits, menstrual cycle), we restricted these analyses to 7 women with a serum and tissue sample taken at the same day. In this subset of samples, somewhat stronger correlations were observed with serum IGF-I concentrations (IGF-I mRNA: r = 0.39 (p=0.38), IGF-II mRNA: r = −0.43 (p=0.34), IGF-IR mRNA: r = −0.54 (p=0.22)).

In conclusion, in this preliminary series no correlation between serum IGF-I and normal breast tissue IGF-I, IGF-II, and IGF-IR was observed. This series will be prospectively expanded with both pre- and postmenopausal women with serum and tissue taken at the same day. Immunohistochemistry will be performed to assess tissue protein expression of IGF system components. Additionally, serum and tissue IGF binding proteins will be measured. Results will provide more insight in the relation between serum and tissue IGF system components, and help explain the role of the IGF-system in tumor development and cancer prevention research.

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Insulin-like Growth Factor Binding Protein 3 (IGFBP-3) modifies Epidermal Growth Factor (EGF)-related breast cancer growth depending upon the extracellular matrix (ECM)

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Introduction: IGFBP-3 is the most abundant IGFBP within serum and can modulate cell proliferation. Increasingly, the IGF axis is being implicated in Tamoxifen resistance as well as agents targeting the EGF pathway. Elevated serum IGFBP-3 has been associated with reduced breast cancer risk. Conversely, tumours with high local IGFBP-3 levels have been associated with a poor prognosis, as has tumour production of fibronectin. We have examined the effects of IGFBP-3 on EGF-mediated proliferation in breast epithelial cells, both in the presence and absence of fibronectin.

Methods: Normal breast and breast cancer cell lines were chosen with previously characterised responses to EGF. Cells were dosed with EGF (1 ng/ml and 25 ng/ml), IGFBP-3 (100 ng/ml) and combinations of each together. This was repeated in plastic wells that had been coated with fibronectin (0.25 μg/ml).

Results: In the normal MCF10A cells, EGF and IGFBP-3 each increased cell proliferation on their own (1.7 and 1.4 fold increase, respectively), but together synergistically enhanced cell growth relative to control (3.3 fold increase). When repeated on fibronectin, EGF increased proliferation (2.3 fold), but IGFBP-3 alone reduced proliferation (to 0.78 fold) and blocked the proliferative response to EGF (from 3.3 to 1.38 fold).

In HS578T breast cancer cells, EGF caused an increase in cell proliferation (1.5 fold), IGFBP-3 alone had no effect, but in combination with EGF, markedly inhibited EGF-mediated cell proliferation (from 1.5 to 1.1 fold). This is currently being repeated in HS578T cells on fibronectin-coated plastic. Whilst these cells still proliferate in response to EGF on fibronectin, IGFBP-3's effects change markedly, and it is seen to act as a mitogen in this environment.

Conclusions: IGFBP-3 has differential effects on EGF-mediated proliferation in normal and breast cancer epithelial cells. The results also suggest that the actions of IGFBP-3 may reverse with remodelling of the ECM, potentially explaining the conflicting data regarding the activity