Correlation of HER2 expression by IHC, DNA microarray, and FISH in breast cancer

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Introduction

HER2 overexpression occurs in approximately 15-20% of all breast cancers and is associated with aggressive disease and decreased survival. HER2 status is a predictor of response to trastuzumab and lapatinib. Given the importance of HER2 positive disease, accurate evaluation of HER2 status is essential. The aim of this study is to provide insights into the relationship between HER2 expression by immunohistochemistry (DNA microarray and FISH) in a large cohort of 1202 breast cancer cases.

Materials/Methods

A large cohort of 1202 breast cancer specimens (core needle biopsies or surgical specimens) were analyzed for HER2 by IHC and FISH. DNA microarray and FISH were performed in parallel. HER2 antibody was used for immunohistochemistry, with pathologists using cut-offs (above threshold, below threshold (equivocal), and negative) as described in Table 1A. DNA microarray (DNA, process, fluorescence) was performed on the HER2 (HER2) gene in a possible real gene amplification. Results of gene overexpression were considered to be based on a tissue-specific normal control. Results of "not performed" were considered to be based on normal controls and results considered "not informative" when data obtained from the patient sample or the control sample were not of sufficient high quality to confidently evaluate gene expression.

HER2 by FISH (Pathvysion HER2 DNA Probe Kit, Abbott Laboratories) was performed when IHC results were considered above threshold or below threshold (equivocal), in concordance with ASCO/CAP guidelines for amplification.

All statistical analyses were performed using the IBM SPSS Statistics 17.0 software. Non-parametric analyses (Kruskal-Wallis test) and Pearson’s correlation were utilized for quantitative analyses by group. Pearson correlation coefficients were calculated as the degree of linear relationship between two variables, with a correlation of +1 representing perfect positive linear association and 0 representing no linear association. All variables are trichotomized as follows: above, below, or negative. Pearson correlation coefficients were utilized for quantitative outcomes based on IHC intensity or IHC Quick score, DNA microarray gene ratio, and FISH ratio. Positive Pearson correlation reflects the degree of linear relationship between two variables, with a correlation of +1 denoting a perfect positive linear relationship between variables.

Conclusions

Table 1A: The three tables on the left show the actual and relative distribution of HER2 outcomes by IHC, DNA microarray, and FISH. Two-way comparisons (Table A) provide a statistical indication of the extent of their correlation.

Table 2: The table above provides the median values for gene ratio (Khoury et al., 2010). Our data suggest the following:

Figure 1A - C: The three graphs pictured on the left illustrate qualitative distribution results. In Figure 1A, IHC by ASCO/CAP guidelines for amplification: results were "not performed" is 0.001, whereas IHC by FISH, higher than the ASCO/CAP guidelines and consistent with Shah et al. 2010. Our data did not support such a conclusion.

Figure 2A - D: Figures 2A and 2B provide a visual presentation of the distribution between intensity of staining, Quick score and microarray gene ratio. Graph A demonstrates that intensity of 0 and 1 (positive) are most often associated with IHC negativity (61.2%, n=762) but are also noted samples with HER2 overexpression (38.4%, n=478). The least structured relationship was observed between DNA Microarray and FISH. Pearson correlation coefficients were calculated as the degree of linear relationship between two variables, with a correlation of +1 representing perfect positive linear association and 0 representing no linear association. All variables are trichotomized as follows: above, below, or negative. Pearson correlation coefficients were utilized for quantitative outcomes based on IHC intensity or IHC Quick score, DNA microarray gene ratio, and FISH ratio. Positive Pearson correlation reflects the degree of linear relationship between two variables, with a correlation of +1 denoting a perfect positive linear relationship between variables.

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Based on prior inter-assay agreement, breast cancers with HER2 IHC and HER2 FISH (IHC) results cannot be resolved by DNA microarray. Khan et al. 2011 showed that a higher concordance rate between IHC and FISH could be achieved by expanding the equivocal range to include all cases of staining 2+ intensity. However, our results show that the expanded range to include all those IHC intensities 2+ was in less than 10% of cells did not support such a conclusion.

Our results suggest that an intensity score of 3+ in 10% of cells IHC shows the highest concordance with FISH than the ASCO/CAP guidelines and consistent with Shah et al. 2010. Protein expression – as measured by IHC – is the most robust and cost-effective way to test for HER2 expression when performed per ASCO/CAP guidelines and correlates well with gene amplification by FISH. This also consistent with poor concordance results in Table 3.

References:


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Table 1A: The three tables on the left show the actual and relative distribution of HER2 outcomes by IHC, DNA microarray, and FISH. Two-way comparisons (Table A) provide a statistical indication of the extent of their correlation. The least structured relationship was observed between DNA Microarray and FISH. Pearson correlation coefficients were calculated as the degree of linear relationship between two variables, with a correlation of +1 representing perfect positive linear association and 0 representing no linear association. All variables are trichotomized as follows: above, below, or negative. Pearson correlation coefficients were utilized for quantitative outcomes based on IHC intensity or IHC Quick score, DNA microarray gene ratio, and FISH ratio. Positive Pearson correlation reflects the degree of linear relationship between two variables, with a correlation of +1 denoting a perfect positive linear relationship between variables.