



PROSPECTIVE STUDY OF HER2 STATUS IN 1386 INVASIVE BREAST CARCINOMAS. THE IMPACT OF THE ASCO/CAP 2007 GUIDELINES.

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Background

The introduction of the ASCO/CAP guidelines in 2007 followed the need to unify methodologies used in the HER2 evaluation and to guarantee the predictive test value in the different laboratories. The aim of this study is to review our experience in the assessment of the HER2 status and evaluate the impact of the application of the guidelines in our results.

Design

A cohort of 1386 primary invasive breast cancer formalin-fixed, paraffin-embedded samples, from 2000 to 2010, were prospectively analyzed by immunohistochemistry (IHC) performed by ABC immunoperoxidase staining, using a mouse monoclonal antibody (CB11) and fluorescent "in situ" hybridization (FISH) with chromosome 17 centromeric probe. In a first phase, the IHC results were evaluated, following the HercepTest scoring and the FISH results following the manufacturer's recommendations (n=710 cases) till the implantation, in a second phase, of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines in January 2007 (n=676), from the onwards the following changes were introduced:

IHC scoring

- Positive, score 3+, defined as uniform, intense membrane staining of >30% of invasive tumor cells, (figure 1A).
- Negative, score 0 (not staining) or 1+ (weak, incomplete membrane staining in any proportion of tumor cells), (figure 1B).
- Equivocal, score 2+, defined as complete membrane staining that is either nonuniform or weak in intensity but with circumferential distribution in at least 10% of tumor cells. Invasive tumors showing intense, complete membrane staining of 30% or fewer are also considered as score 2+.

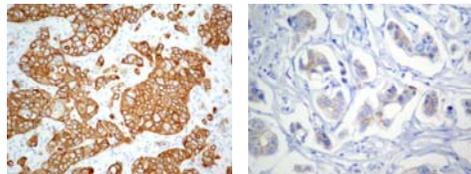


Figure 1. HER2 immunostaining on paraffin-embedded invasive breast cancer showing (A) positive expression (3+) and (B) negative expression (1+) according ASCO/CAP guidelines.

FISH scoring

- Positive, cases showing a HER2/CEP17 ratio of more than 2.2 or average HER2 gene copy number >6.0, for systems without centromeric probe;
- Negative, cases showing a ratio of less than 1.8 or average HER2 gene copy <4.0;
- Equivocal, cases showing a ratio from 1.8 to 2.2 or average HER2 gene copy between 4.0 and 6.0.

The IHC results of both series, obtained before and after the ASCO/CAP guidelines application, were statistically evaluated, using FISH as a gold standard. In addition, a loglineal model was used to study the dependence between the results obtained with the different techniques and the evaluation methodology. The frequency of positive and negative results of both assays was previously analyzed to check their similarity and support the application of a loglineal model.

Results

Figures 2 and 3 illustrates the distribution of cases according to their results with IHC and FISH of both series.

We observed a decrease in the percentage of positive (14,2 vs 6,7%) and negative (62,4 vs 36,5%) cases by IHC, whereas the number of equivocal cases increased considerably (17,2 vs 56,1%). Nevertheless, using FISH as a gold standard there was an evident improvement in the accuracy of this technique (90,9 vs 97,8%) with an increase in its specificity (91,6 vs 97,8%), sensitivity (87,9 vs 97,7%), positive (71,3 vs 89,6%) and negative (97,0 vs 99,6%) predictive values.

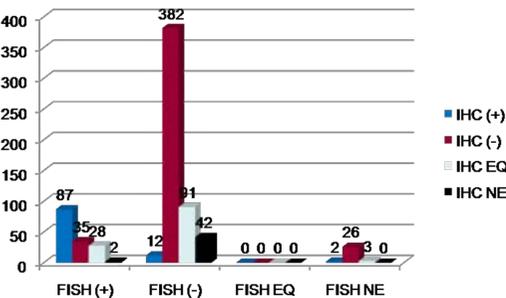


Figure 2. Distribution of cases according to their results with IHC and FISH, obtained on the first phase, previous to the ASCO/CAP application guidelines (n=710). Abbreviations: EQ, equivocal; NE, not evaluable.

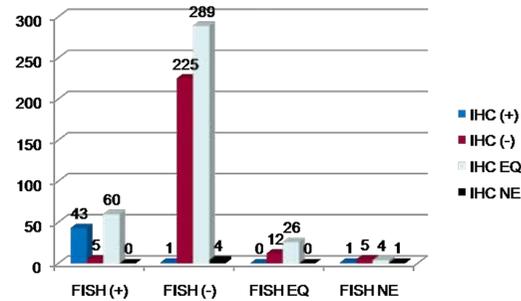


Figure 3. Distribution of cases according to their results with IHC and FISH, obtained on the second phase, following ASCO/CAP guidelines (n=676). Abbreviations: EQ, equivocal; NE, not evaluable.

The newly defined FISH equivocal category represents a 5,6% of cases and causes a decrease in the number of positive cases (21,4 vs 16%). Table 1.

The frequency of positive and negative cases with IHC and FISH was similar when we compared both series, as shown on Figure 4. However, IHC and FISH results were proved to be significantly dependent on the evaluation method by a loglineal model.

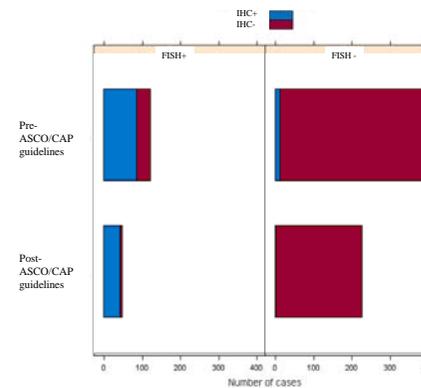


Figure 4. Distribution of cases in the both series, Pre and Post ASCO/CAP application guidelines, in function of their results with IHC and FISH.

IHC vs FISH	2000-2006 Pre-ASCO/CAP Guidelines (n=710)	2007-2010 Post-ASCO/CAP Guidelines (n=676)
IHC positive (%)	14.2	6.7
IHC negative (%)	62.4	36.5
IHC equivocal (%)	17.2	56.1
IHC not evaluable (%)	6.2	0.7
Specificity (%)	91.6	97.8
Sensitivity (%)	87.9	97.7
Positive predictive value (%)	71.3	89.6
Negative predictive value (%)	97.0	99.6
Accuracy (%)	90.9	97.8
FISH positive (%)	21.4	16.0
FISH negative (%)	74.2	76.8

Table 1. Statistical analysis comparing the results obtained, by IHC and FISH, before and after the ASCO/CAP guidelines application.

Conclusions

- In our experience, the recommendations of ASCO/CAP guidelines significantly increased the number of equivocal results; nevertheless improve the exactitude of IHC method.
- The ASCO/CAP guidelines application decreases the number of positive cases by FISH, and therefore, the indications of specific treatment.
- The observed influence of the evaluation method on the IHC and FISH results should be taken into consideration when assessing HER2 status.

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