

# Outcome of HER2 Testing by FISH applying ASCO/CAP 2007 and 2013 guideline in IHC equivocal group of breast cancer: Experience at tertiary cancer care centre

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## Abstract

**Background and Objectives:** HER2 testing guideline of ASCO/CAP for interpretation and reporting has recently been revised. The study is aimed to measure the impact of 2013 CAP guideline on equivocal HER2 test outcome (immunohistochemistry [IHC] 2+) when tested by fluorescent *in situ* hybridization (FISH). The study also aims at finding the frequency of polysomy and monosomy of chromosome 17. **Materials and Methods:** Specimens were collected in Rajiv Gandhi Cancer Institute and Research Centre, New Delhi, India. IHC was performed in every case, and FISH was performed in IHC2+ cases. **Results:** In final analysis includes 557 subjects on the basis of CAP guideline 2007 and CAP guideline 2013. One hundred ninety-two subjects (34.4%) were HER2 amplified according to CAP scoring 2007, and 246 subjects (44%) according to 2013 CAP scoring. **Conclusions:** FISH results were evaluated (IHC2+ interpreted according to CAP 2007 guideline) with both 2007 and 2013 ASCO/CAP scoring criteria, we identified significantly more HER2 positive cases as compared to cases evaluated using the 2007 criteria ( $P < 0.05$ ). We also found that in breast carcinoma, HER2 status in the presence of polysomy 17 may vary with the scoring criteria used. Evaluation of FISH result using 2013 ASCO/CAP criteria means that more patients with breast cancer may be appropriate for targeted treatment with trastuzumab, potentially improving their outcome.

**Key words:** Fluorescent *in situ* hybridization technique, HER2 gene amplification, immunohistochemistry

## Introduction

Breast cancer is the second most common cancer for women in the India.<sup>[1]</sup> Researches have shown that about 20–30% of the breast cancer patients have HER2 amplification or overexpression that is associated with a more aggressive phenotype and decreased survival.<sup>[2]</sup> Fluorescent *in situ* hybridization (FISH) is the gold standard because it was the strategy used during clinical trials and has proven clinical utility. Polysomy 17 indicates the presence of more than two copies of chromosome 17 and is reported in 13–46% breast carcinoma.<sup>[3]</sup> Amplification is by far the most common mechanism generating excess HER2 gene copies, polysomy occurs frequently. This has raised the question of whether increased HER2 protein expression can result not only from gene amplification but also from increased number of chromosome 17. The consequence would then be that patients with polysomy 17 tumors might be candidates for trastuzumab.<sup>[4]</sup>

This study includes 557 breast cancer patients with equivocal HER2 test results by immunohistochemical staining (immunohistochemistry [IHC] 2+) with an aim to measure the impact of changed criteria of ASCO/CAP recommendation 2007 and 2013 as well as frequency of polysomy 17 and deletion of HER2 gene.

## Materials and Methods

### Sample collection and fixation protocol

Five hundred and fifty-seven cases with equivocal results on IHC (2+) from July 2010 to September 2013 were studied. Expression of HER2/neu IHC and FISH was analyzed.

### Fluorescent *in situ* hybridization

FISH analysis was performed using the PathVysion HER2 probe kit (Vysis, USA). All procedures were followed according to the HER2 probe kit instruction manual (Vysis, USA). The

slides were screened under fluorescent microscope (Leica 6000 M, Germany) using appropriate filters.

### Scoring methods

Scoring methods for IHC used according to 2007 and FISH were done according to the ASCO/CAP guidelines 2007 and 2013.

### Statistical analysis

A nonparametric Chi-square statistics test was used for testing associations between variables and  $P < 0.05$  were considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences software (version 22.0; SPSS Inc., Chicago, IL, USA).

## Results

One hundred and ninety-two subjects (34.4%) were HER2 amplified according to CAP scoring 2007. Moreover, on reanalysis according to 2013 CAP scoring criteria, we found 246 subjects (44%) HER2 amplified. We also found 106 cases (19%) polysomy17 which showed 24 (22.6%) cases of HER2 amplification according to CAP 2007 scoring criteria and 36 (33.9%) cases according to 2013 CAP scoring.

## Discussion

Using both 2007 and 2013 ASCO/CAP scoring method on FISH technique, we found 10% of difference between two methods of scoring. Using FISH, we also investigated the frequency of polysomy 17 and its association with HER2 alteration in patients with invasive breast cancer. As polysomy 17 are relatively common in breast carcinoma, it is possible that HER2 FISH results can be misinterpreted. Vanden Bempt *et al.* reported that >40% of breast carcinomas with polysomy 17<sup>[5]</sup> and Pu *et al.* reported 57.1% breast carcinoma with polysomy 17.<sup>[6]</sup> However, in the Indian population, our study

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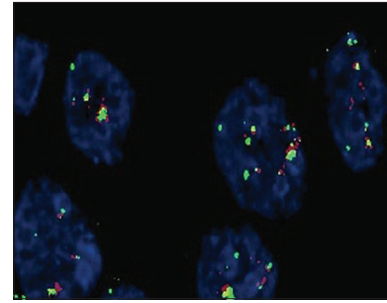
showed 19% breast carcinoma with polysomy 17. When we applied 2013 CAP scoring criteria, we found 14.6% increase in number for HER2 amplification with polysomy 17. As we shown one case in Figure 1, polysomy17 with negative according to CAP 2007 guideline but when we applied 2013 CAP guideline on the same case, the case should be given positive with polysomy. When we re-evaluated the 82 cases that were HER2 nonamplified and polysomy 17 accompanied, we found that 12 (14.6%) cases could be defined as HER2 amplified. These 14.6% patients showed HER2 amplification may be suitable for anti-HER2 targeted therapy. There was a significant increase in the number of HER2 amplified cases, i.e., cases increase from 192 to 246 out of 557 cases. As recent study suggested that the presence of CEP17 alterations could identify a more aggressive subset of breast cancers that are nonresponsive to conventional therapy independently of HER2 amplification status.<sup>[7]</sup> Some researchers believe that polysomy 17 without HER2 amplification do not predict response to lapatinib in metastatic breast cancer.<sup>[7]</sup>

### Conclusion

FISH results were evaluated (IHC2+ interpreted according to CAP 2007 guideline) with both 2007 and 2013 ASCO/CAP scoring criteria, we identified significantly more HER2 positive cases as compared to cases evaluated using the 2007 criteria ( $P < 0.05$ ). Polysomy of CEP17 is a crucial cause of misinterpretation of HER2 FISH result. Using the 2013 ASCO/CAP scoring criteria on IHC2+ cases evaluate significantly higher in a number of HER2 amplification with polysomy of chromosome 17, which shows more patients appropriate for targeted treatment.

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**Figure 1: Polysomy with negative (CAP 2007)**

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### Conflicts of interest

There are no conflicts of interest.

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