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Breast Cancer

Indian Data on HER2 Fluorescence In Situ Hybridization in Invasive Breast Cancer with Immunohistochemically Equivocal Results As Per 2018 ASCO/CAP Guidelines

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Abstract


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Introduction Hormonal status and HER2 expression are valuable biomarkers and dictate the management of the patients diagnosed with invasive breast cancer (IBC). It is crucial to identify the patients who truly respond to anti-HER2 targeted therapy. Updated 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines has recommended certain modifications in HER2 interpretation by fluorescence in situ hybridization (FISH) with concomitant immunohistochemistry (IHC).

Objectives We aimed to evaluate HER2 FISH interpretation in IBC with equivocal IHC results as per 2018 ASCO/CAP recommendations and compare FISH results with hormonal receptor status.

Materials and Methods FISH results of 502 cases of IBC with equivocal IHC report between January 2016 to January 2022 were reviewed retrospectively. FISH results were categorized according to ASCO/CAP guidelines 2018 into five respective groups.

Results FISH testing in IHC equivocal cases showed 219 (43.6%) cases were classic amplified (positive) belonged to group 1, 217(43.2%) cases were classic nonamplified (negative) fell into group 5, 39 (7.8%) and 02 (0.4%) patients were in group 2 (negative) and group 3 (positive), and 25 (5.0%) cases were in group 4 (negative). About 52.1 and 49.3% of cases with estrogen receptor and progesterone receptor positivity were reported as HER2 positive. Among 502 cases, 25 equivocal cases according to the 2013 guidelines were redefined as HER2 negative and 02 (0.4%) cases reported positive were classified negative as per updated 2018 guidelines.

Conclusion Revised 2018 guidelines is helpful in accurate identification of HER2 status and in avoiding targeted therapy in unwarranted cases. Updated 2018 guidelines has removed equivocal HER2-FISH category that has eliminated management dilemma in these cases. Only long-term clinical follow-up will establish the validity of the updated guidelines.

Keywords

- ASCO/CAP guidelines 2018
- equivocal
- FISH
- HER2
- invasive breast cancer

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Introduction

Breast cancer is the most common and leading cancer in Indian women.[1] Hormonal status and HER2 expression are mandatory in diagnosed case of invasive breast cancer (IBC) as they dictate the further management and prognosis. The 2007 American Society of Clinical Oncology (ASCO) guidelines has a mandate that HER2 immunohistochemistry (IHC) should be evaluated in every IBC.[2]

The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) introduced guidelines for IHC interpretation of HER2 in 2007, which was revised in 2013 to include maximum number cases that would benefit by anti-HER2 targeted therapy and suggested HER2 testing by fluorescence in situ hybridization (FISH) in cases with HER2 equivocal results on IHC.[3] Following which cases reported as HER2 equivocal by FISH technique was a challenge to manage as definite guidelines were not established.

The ASCO/CAP guidelines was revised in 2018 to limit HER2 equivocal category.[4] HER2 scoring by FISH was divided into five groups. The expert panel recommended that HER2 0, +1, and +2 scoring by IHC with HER2/chromosome enumeration probe 17 (CEP17) ratio lower than 2 and HER2 signals/cell if equal to or more than 4 and less than 6 is now considered as negative.[5] Although few studies have evaluated the impact of 2018 updated ASCO/CAP guidelines, the implications of the same in response to treatment in the clinical practice are still variable. So, it is important to categorize FISH results into various groups especially in equivocal cases on IHC.[6–9]

Updated guidelines recommend upfront HER2 testing by FISH that is much sensitive but it has a longer turnaround time and is not feasible in smaller settings considering the resources and cost. In Indian scenario, common practice is to confirm only HER2 equivocal results reported on IHC by sensitive FISH technique as IHC is definitive when results are either positive or negative.

The purpose of this study was to evaluate IHC equivocal cases and compare the findings with FISH results and also assess how the revised 2018 guidelines affected the final HER2 status.

Materials and Methods

We retrospectively reviewed HER2 FISH results of 502 IBC cases that were reported equivocal on IHC from January 2016 to January 2022. Specimens included core needle biopsies, wide local excision, simple and modified radical mastectomy, biopsies from metastatic sites. and effusion fluids. All cases were evaluated for hormonal receptor and HER2 status by IHC prior to FISH testing. Only equivocal cases reported on IHC and subjected for FISH were included in the study. The FISH results were interpreted based on 2013 and 2018 ASCO/CAP guidelines and change in HER2 status was compared. Institutional review committee approval was obtained.

IHC: Automated IHC was done formalin-fixed paraffin-embedded (FFPE) tissue with appropriate control for estrogen receptor (ER) (clone SP-1, RTU, Ventana, Arizona, USA), progesterone receptor (PR) (clone 1E2, RTU, Ventana, Arizona, USA), and HER2 (clone, 4B5, Ventana, Arizona, USA) was performed on automated slide stainer, Ventana Benchmark XT. The results were interpreted according to the ASCO/CAP 2018 guidelines.

FISH: Technique was performed using Zytovision HER-2dual probe kit (Zytovision, Germany) on interphase invasive tumor nuclei of FFPE. The following probes were used: LSI HER-2/neu (spectrum green) for HER-2 gene locus (17q11) and CEP 17 (spectrum orange) for the α satellite DNA sequence at the centromeric region of chromosome 17.

Paraffin sections of 5 micron thick were transferred onto poly-L-Lysine coated slides and allowed to dry and placed in hot air oven at 90°C for 1 hour, and then placed on slide warmer at 60 to 70°C for 10 minutes. The slides were deparaffinized in xylene at room temperature for 20 minute and rehydrated with downward grading of alcohol from 100%. The washed slides were then transferred into pretreatment solution for 20 minutes at 90°C in water bath. Slides were then placed in humidity chamber at 37°C after adding few drops of pepsin for 15 minutes, and then dehydrated in graded concentration of alcohol up to 100%. Dual-labeled probes of 0.5 mL were added and denatured for 12 minutes at 75°C followed by hybridization for 17 hours at 37°C.

After posthybridization, two washes with wash buffer were performed at 37°C. Slides were then dehydrated in graded concentration of alcohol up to 100%. Ten microliters of DAPI were applied on completely dried slides and coverslip was gently placed. The slides were screened by fluorescent microscope (Olympus, United States) using appropriate filters (DAPI - 4',6-diamidino-2-phenylindole, FITC - Fluorescein isothiocyanate, and SpO - Spectrum orange). Signals were counted in at least 20 cells for both the HER-2/neu gene and chromosome 17 centromere signals under oil immersion. Signal were counted and results were interpreted based on 2018 and 2013 guidelines.

While FISH interpretation, care was taken to ensure only areas with equivocal IHC findings were assessed.

In group 2 to 4 cases with equivocal IHC results, recounting was done in 20 cells by an observer blinded to previous results. Suitable area on the slide was marked by pathologist to ensure adequate tumor and the corresponding equivocal area was studied, and in no case issue of repeat on alternate block was encountered.

Results

The study consisted 502 cases of IBC. Sampled tissues were from the following sites: 458 (91.2%) from the primary site of malignancy, 36 (7.9%) from the metastatic sites (liver, ovary, lung, axillary and clavicular lymph node, cerebellum, bone marrow, pleural biopsy, and ascitic fluid); and 8 (1%) cases were from a recurrent lesion. Two cases from the cytology cell block were done on pleural and ascitic fluid each. Testing was done on specimens obtained by surgical excision ($n = 229$), core needle biopsies ($n = 271$), and cell block ($n = 2$)

Age group of patients with 50 years and below were 240 (47.8%) and above 50 years were 262 (52.2%) cases. Overall

median age was 63.5years (25–82). HER2 expression pattern based on age did not show association with age.

Histomorphologically, majority of the cases (95.8%) were invasive carcinoma (ductal), followed by invasive lobular carcinoma (1.3%). Hormonal and HER2 status along with morphologic type is tabulated (►Table 1).

HER2-FISH results were scored based on both 2013 and 2018 guidelines. With the implementation of 2018 guidelines, the interpretation of HER2 status for a total of 29 (5.7%) cases changed. Findings are discussed below:

1. Cases unchanged according to both 2013 and 2018 guidelines; 258 (51.3%) HER2 positive and 217 (43.2%) HER2 negative cases continued to have the same HER2 status.
2. HER2 equivocal cases as per 2013 guidelines scored HER2-negative, 25 (5.0%) cases scored HER2 equivocal in 2013 scoring schema were HER2 negative. These cases expressed HER2/CEP17 ratio less than 2.0 and average HER2 copy number ≥ 4 and < 6/cell.
3. Two cases grouped in ISH group 2 and reported HER2-negative was diagnosed HER2- positive as per 2013 guidelines.

Following were the overall findings of the 502 cases of IBC when categorized into five groups, based on HER2 FISH interpretation described in 2018 guidelines: 219 (43.6%) cases were classic amplified (positive) belonged to group 1, 217 (43.2%) cases classic nonamplified (negative) fell into group 5, 02 (0.4%) patients were in group 2, 39 (7.8%) cases belonged to group 3 (positive) (►Fig. 1A), and 25(5.0%) cases were in group 4 (negative) (►Fig. 1B).

The hormonal receptor status was analyzed with respect to the HER 2 groups (►Table 2). About 70.5 and 60.8% of cases with ER and PR positivity were reported as HER2-positive. About 29.5 and 39.1% cases with ER and PR negativity showed HER2 positivity, respectively, among total number of HER2-positive cases.

Discussion

The ASCO/CAP guidelines were revised in 2013 with the intention of maximizing the patients who can benefit from anti-HER2 targeted therapy and minimizing false-negative results.[3] With the revision, the equivocal results increased in frequencies and posed a problem in clinical decision making. In that context, the ASCO/CAP guidelines was updated in 2018; the significant modifications in the updated guidelines were refining the interpretation criteria in arriving at the most accurate HER2 status designation (positive or negative) based on concomitant FISH and IHC results. Single institutional study found 502 HER2-IHC equivocal cases subjected to FISH. When HER2 FISH results were compared as per 2013 and 2018 ASCO/CAP guidelines, overall HER2 status was changed in 27 cases (5.4%). 25 equivocal cases were HER2 negative and 02 HER2-positive were interpreted negative (►Table 3).

HER2 amplification (group 1) was seen in 219 (43.6%) cases and HER negative with classic nonamplification (group 5) in 217 (43.2%) cases. Data when compared with other studies is variable, one possible explanation is our study considered only cases with equivocal results on IHC.[6,10] Two (0.4%), 39(7.8%), and 25 (5.0%) cases after HER2 testing by FISH fell under group 2, 3, and 4 category, respectively, which totally accounted for 13.1% cases. As per the 2018 ASCO/CAP focused HER2 update approximately 5% of breast cancer cases are reported to fall into these uncommon categories of groups 2 to 4.[11]

Table 1 Histologic type of breast cancer with hormonal receptor status by IHC and HER2 expression by FISH

Histologic diagnosis	Hormonal status	No of cases	HER2 FISH	
			Positive	Negative
Invasive carcinoma, ductal ($n = 481$)	ER and PR positive	297	143	134
	ER positive, PR negative	54	34	20
	ER negative, PR positive	19	09	10
	ER and PR negative	111	65	46
Invasive lobular carcinoma ($n = 07$)	ER and PR positive	06	03	03
	ER and PR negative	01	00	01
Metaplastic carcinoma ($n = 04$)	ER and PR positive	01	00	01
	ER positive, PR negative	01	01	00
	ER negative, PR positive	01	01	00
	ER and PR negative	01	00	01
Cribriform carcinoma ($n = 02$)	ER and PR positive	02	01	01
Mixed cribriform+ tubular ($n = 02$)	ER and PR positive	02	00	02
Mucinous carcinoma ($n = 02$)	ER and PR positive	02	00	02
Invasive papillary carcinoma ($n = 02$)	ER and PR positive	02	01	01
Apocrine carcinoma ($n = 01$)	ER negative, PR positive	01	00	01
Mucinous cystadenocarcinoma ($n = 01$)	ER and PR negative	01	01	00

Abbreviations: FISH, fluorescence in situ hybridization; ER, estrogen receptor; IHC, immunohistochemistry; PR, progesterone receptor.

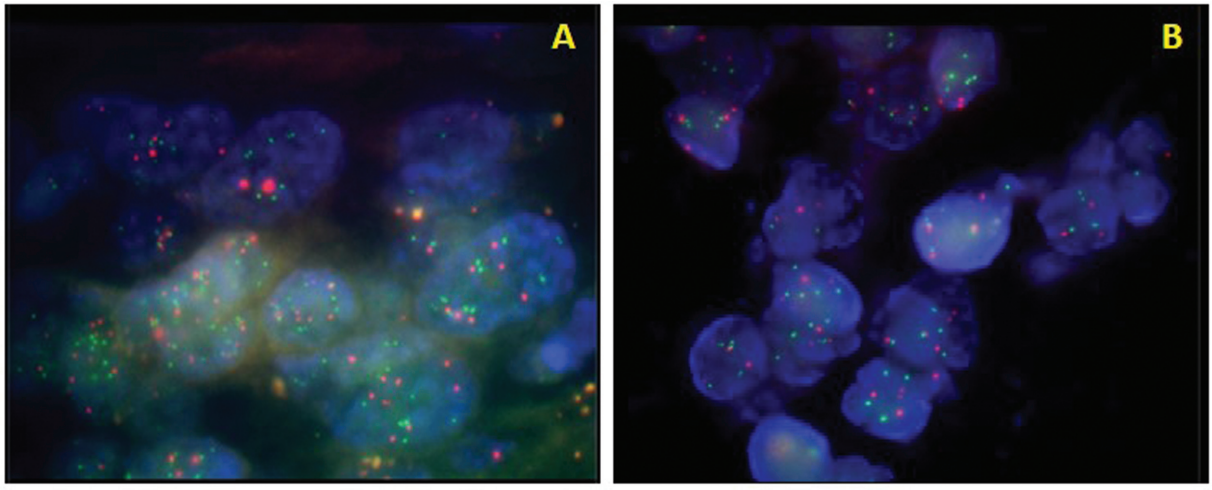

Fig. 1 Representative HER2 fluorescence in situ hybridization; green signal localized to HER-2 gene on chromosome 17, orange signal localized to CEP 17 region. (**A**) Nonclassical amplification—Group 3, reported as HER2 positive with equivocal IHC result, HER2/CEP17 ratio is 1.3 (<2) and average HER2 copy number signal/cell is 6.5. B: Scored equivocal as per 2013 criteria but was negative and fell in group 4 according to 2018 guidelines, HER2/CEP17 ratio: 1.2 and average HER2 copy number 4.5 signal/cell.

Table 2 Distribution of hormonal status expression in different groups as per updated 2018 ASCO/CAP guidelines

Hormonal status	Group 1 ($n=219$)	Group 2 ($n=02$)	Group 3 ($n=39$)	Group 4 ($n=25$)	Group 5 ($n=217$)	Total cases ($n=502$)
ER positive	149 (40.5%)	01(0.2%)	33(9%)	21(5.7%)	163(44.4%)	367
ER negative	70(51.8%)	01(0.7%)	06(4.4%)	4(03%)	54(40%)	135
PR positive	123(37%)	01(0.3%)	34(10.2%)	21(6.3%)	154(46.2%)	333
PR negative	96(56.8%)	01(0.6%)	05(3.0%)	04(2.3%)	63(37.2%)	169

Abbreviations: ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; ER, estrogen receptor; PR, progesterone receptor.

The HER2 status in ISH group 2 is uncommon and only 02 (0.4%) cases were encountered. Other studies have also found very few cases in this group.[6,11,12] These cases were considered as HER2-positive as per 2013 guidelines. Clinical trials with anti-HER2 therapy had no significant effect on patients; hence, to offer definitive diagnosis, such cases should be recounted in at least 20 cells by an observer blinded to the previous results and if the finding is concordant, it should be reported as negative and in discrepant cases are to be resolved after carefully assessing the internal procedure of the FISH testing.[4]

Table 3 Comparison of HER2 FISH results as per 2013 and 2018 ASCO/CAP guidelines

FISH status	2013 guidelines (*n*, %)	2018 guidelines (*n*, %)	Difference (%)
Negative	217 (43.2)	244 (48.6)	+5.4
Equivocal	25 (5.0)	00	−5.0
Positive	260 (51.8)	258 (51.4)	−0.4

Abbreviations: ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; FISH, fluorescence in situ hybridization.

There were 39 (7.8%) cases in ISH group 3; this finding was variable among different studies.[6] A ratio of <2.0 can be attributed to the increase in both HER2 and control centromere signals. A remarkable variability of IHC score for cases in this group was observed across different laboratories.[6,11–14] The positive rate of IHC in this group ranges from 8.3 to 75%; this marked variability was observed across different laboratories.[6] Considering the heterogeneity in HER2-IHC results, 2018 guidelines recommend that cases with concurrent IHC score of 2+/3+ were categorized as HER2 positive.

Twenty-five (5.0%) cases fell in ISH group 4, similar to the finding by Wang et al.[6] 2013 guidelines resulted in increased number of equivocal cases that posed a challenge in management of cases; with revised 2018 guidelines the cases are better stratified as equivocal category does not exist.[15] It is advised not to repeat FISH especially in cases with ISH threshold ratio close to positivity as there is higher likelihood of different result by chance. CEP17 copy number gain is a genetic change commonly observed during dual-probe HER2 ISH for breast cancer, with reported frequency of 3 to 46% in IBC.[16] However, subsequent studies revealed CEP17 copy number gain results from amplification or copy number gain in the centromeric or pericentromeric region and not

polysomy17.[17] 2013 guidelines recommend repeated HER2 testing using alternate probe for CEP17 or other gene in chromosome17 for ISH equivocal cases; however, 2018 ASCO/CAP guidelines does not advocate use of any alternate probe to identify the true polysomy due to limited evidence on its analytical and clinical validity.[4]

With the availability of anti-HER2-targeted agents, accurate assessment of HER2 status is important in identifying the patients who will respond to the therapy and avoid use of drugs in false positive cases. FISH assay has demonstrated a greater accuracy compared with IHC.[5] Current guidelines has simplified and stratified reporting of HER2 status; however, issue of intratumoral HER2 heterogeneity is encountered in certain subsets and is more common in equivocal HER2 protein expression cases. Studies have found cases with HER2 heterogeneity have incomplete response to targeted therapy and decreased disease-free survival. CAP recommends to document the amplification of HER2 in subpopulation if it comprises >10% of tumor cells. It is prudent to know that while assessing for receptor status change upfront by FISH after chemotherapy at metastatic sites, polyploidization could result in false HER2 amplification, and careful evaluation using dual-probe ISH with concomitant IHC review is recommended.[18]

Overall when hormonal receptor status was compared with HER2 amplification, 182 (36.2%) cases were positive for both ER and HER2; these finding are in support with other studies ranging from 8 to 40%.[6] Seventy-seven (15.3%) and 101 (20.1%) cases were amplified (classic and nonclassic types) with negative ER status and PR status, respectively.

Updated 2018 ASCO/CAP guidelines focuses on dual-probe ISH groups (2, 3, and 4) with less common ISH patterns, and significant recommendation is concomitant IHC review for these ISH groups to achieve the most accurate determination of HER2 status. In Indian scenario, the practice is quite different, FISH is not routinely followed as first line test to detect HER2 amplification, and only cases with equivocal expression on IHC are subjected for FISH. The common practice is performing upfront IHC to identify the protein expression; nevertheless, the impact of the updated 2018 guidelines was similar to other studies.[5,6]

Data from our study depict a 5.4% increase in HER2 negativity rates from 217 (43.2%) to 244 (48.6%) cases. Very small fall in positivity rates 02 (0.4%) was noted and these positive cases fell into group 2 category (►Table 3). As per Kong et al study, 27 (5.4%) equivocal cases were changed to HER2 negative as per the 2018 guidelines. Variation in HER2 status reported in other studies is around 5 to 10%.[20] These findings explain the impact of the revised guidelines in identifying more patients with HER2 negative status in whom anti-HER2 targeted agents is avoided. The patients in group 2 and 4 categories are associated with low HER-2 protein expression, and there has been no strong evidence of treatment benefit in these two groups; hence, the updated 2018 guidelines identifies the false positive FISH cases to avoid unwarranted treatment.[21]

Our study presents the frequency and characteristic of different HER2 FISH categories and reports the Indian database on the 2018 ASCO/CAP guidelines. The findings suggest that cases are better stratified in identifying patients who benefit on receiving anti-HER2 therapy. With the 2018 guidelines, FISH results are more definitive in treatment making as the equivocal category is removed. As majority of equivocal cases are categorized as HER2 negative and this explains the increase in negative rates of HER2 by FISH.[22] Only long-term clinical outcome of these patients will determine will the validity of the updated guidelines.

Note

Institutional review was obtained for the study.

Conflict of Interest

None declared.

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