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Impact of the 2018 ASCO/CAP HER2 guidelines update for HER2 testing by FISH in breast cancer

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ABSTRACT

Recently, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) updated the guidelines on HER2 testing for invasive breast cancer. Little is known about the impact of the guidelines update. We aimed to study the impact of the 2018 ASCO/CAP HER2 testing guidelines update. We compared the HER2 FISH results interpreted by 2013 and 2018 ASCO/CAP guidelines in 331 cases of invasive breast cancers. We also analyzed the pathological features and clinical outcomes of these cases. In comparing to the 2013 ASCO/CAP guidelines, the HER2 negative rate was increased significantly from 62.5% to 75.8%($P < 0.05$), and 13.3% changed from equivocal to negative by the 2018 guidelines. Our findings indicate that the guidelines update significantly increased the rate of negative results. The reclassification of the equivocal results by the 2018 guidelines is the main reason for this change. Patients with HER2 equivocal results were associated with larger tumor size and higher Ki67 index than those with negative results, while clinical outcomes were similar between them.

1. Introduction

Human epidermal growth factor receptor 2 (HER2) is amplified or overexpressed in approximately 12–20% of invasive breast carcinomas and patients with HER2 positive breast cancers are considered eligible for treatment with HER2-targeted therapies [1,2]. Numerous studies have shown that HER2 is an important prognostic factor of breast cancer [3–5]. Herceptin, a humanized monoclonal antibody targeting HER2, has long been shown to significantly improve disease-free survival and progression-free survival in breast cancer patients [6–9]. In recent years, other HER2-targeting drugs such as lapatinib, pertuzumab, and T-DM1 have been approved for breast cancer treatment and also have shown survival benefits [10–12]. Accurate HER2 testing is essential to identify right patients that can benefit from these treatments and reduce unnecessary costs and side effects.

Currently, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are two assays approved by the FDA for HER2 testing. IHC is usually used as an initial testing assay, followed by FISH for samples with equivocal or discordant results [13,14]. In 2007, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) published guidelines on HER2 testing for breast cancer to improve the accuracy of HER2 testing for invasive breast cancer, which were then updated in 2013 [13,15]. According to the 2013 ASCO/CAP guideline recommendations, cases with HER2/chromosome enumeration probe 17(CEP17) ratio ≥2.0, or cases with HER2/CEP17 ratio < 2.0 and the average number of HER2 signals ≥6 were classified as positive; and cases with HER2/CEP17 ratio < 2.0 and the average number of HER2 signals per cell ≥4 and < 6 were classified as equivocal when using a dual-probe FISH assay. Recently, the guidelines were once again updated. The 2018 ASCO/CAP guidelines clearly defined HER2 FISH results of five groups: group1 to group 5 [16]. For cases of group 2–4, the HER2 status diagnosis should base on the combined interpretation of FISH and IHC assays. If the HER2 IHC result is 2+, the FISH result should be recounted by another observer.

However, little is known about the impact of these guidelines changes in breast cancer patients. The aim of this study was to assess the potential impact of the 2018 ASCO/CAP HER2 testing guidelines update for HER2 IHC 2+ cases and characterize the affected group with respect to select pathological features.

2. Materials and methods

2.1. Patients

Between April 2013 and January 2018, a total of 331 consecutive cases of primary invasive breast cancer including surgical resection and

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needle aspiration biopsy confirmed by the Department of Pathology, Sir Run Run Shaw Hospital affiliated to Zhejiang University School of Medicine were selected. At our institution, all invasive breast cancers are routinely tested by IHC, and cases showing equivocal staining by IHC require reflex testing by dual-probe FISH. All of these cases were selected from routine HER2 testing performed in our institution and were HER2 equivocal tested by IHC. The overall IHC 2+ rate was 15.4%(331/2149). Detailed clinicopathological data of these cases were collected, including gender, age of onset, tumor size, lymph node status, hormone receptor status and Ki67 index.

Specimens were handled according to the 2013 ASCO/CAP guidelines, including fixation in 10% neutral buffered formalin occurring within 1 h and total fixation time between 6 and 72 h.

2.2. IHC

IHC was performed on 5 μm sections of routinely processed, formalin-fixed, paraffin-embedded specimen using the antibody 4B5(Roche, Basel, Switzerland). Each case was scored independently by two pathologists. An IHC 2+ result was given when more than 10% tumor cells show weak to moderate complete membrane staining.

2.3. FISH

FISH was performed on formalin-fixed, paraffin-embedded tissue sections using dual-color HER2/CEP17 probes(PathVysion Her2/neu DNA Probe Kit; Abbott). A total of 30 cells of invasive carcinoma with optimal nuclear signals were randomly selected in 2–4 separate fields for evaluation. The FISH result of each case was interpreted by two pathologists according to the 2013 and 2018 ASCO/CAP guidelines respectively. According to the 2018 ASCO/CAP guidelines, FISH results were divided into five groups: group 1(HER2/CEP17 ratio ≥ 2.0; average HER2 copy number ≥ 4.0), group 2 (HER2/CEP17 ratio ≥ 2.0; average HER2 copy number < 4.0), group 3 (HER2/CEP17 ratio < 2.0; average HER2copy number ≥ 6.0), group 4(HER2/CEP17 ratio < 2.0; average HER2 copy number ≥ 4.0 and < 6.0), and group 5(HER2/CEP17 ratio < 2.0; average HER2 copy number < 4) [16]. For cases of group 2, 3 and 4, FISH results were routinely recounted by another observer and remained the same classification as before.

2.4. Follow up

The patients were followed up until death or until the date of last follow-up of June 30, 2018.

2.5. Statistical analysis

Statistical analysis was performed using SPSS software(version 22.0). Descriptive statistics for continuous variables were performed using means or medians and range where appropriate. Categorical variables were summarized using frequency and counts. Tumor size, lymph node status, hormone receptor status and Ki67 index were compared between three groups using Chi-square test. OR value was calculated to compare the differences between two groups. Kaplan-Meier survival curve and log-rank test were used to assess survival differences.

3. Results

3.1. Comparison of FISH results between 2013 and 2018 ASCO/CAP guidelines

A total of 331 cases of primary invasive breast cancer were analyzed. The distribution by 2018 ASCO/CAP guidelines FISH group demonstrated that 71(21.5%) cases were in group 1, none was in group 2, 9(2.7%) in group 3, 44(13.3%) in group 4 and 207(62.5%) in group 5 (Table 1). According to the 2013 ASCO/CAP guidelines, 80 (24.2%) patients were interpreted as HER2 positive, 207(62.5%) were negative, and 44(13.3%) were equivocal. By using 2018 ASCO/CAP guidelines to interpret the FISH results, 80(24.2%) cases were interpreted as positive, 251(75.8%) were negative, and 44(13.3%) changed from equivocal to negative (Table 2). Cases of group 1, 3 and 5 remained the same HER2 FISH classification using the 2013 and 2018 guidelines. In comparing to the results according to the 2013 ASCO/CAP guidelines, the positive rate was the same, the negative rate was significantly increased from 62.5% to 75.8% (P < 0.05). All of these changed cases were from group 4 (Fig. 1).

Table 1
Distribution of FISH results based on the 2018 ASCO/CAP guidelines.

group	Description of FISH category	No.(%)
1	Ratio ≥ 2, HER2 average ≥ 4	71(21.5)
2	Ratio ≥ 2, HER2 average < 4	0(0)
3	Ratio < 2, HER2 average ≥ 6	9(2.7)
4	Ratio < 2, HER2 average ≥ 4 and < 6	44(13.3)
5	Ratio < 2, HER2 < 4	207(62.5)
total		331(100.0)

Table 2
Comparison of FISH results between 2013 and 2018 ASCO/CAP guidelines.

		2018 guidelines,No.(%)		
		positive	negtive	total
2013 guidelines	positive	80(24.2)	0(0)	80(24.2)
	equivocal	0(0)	44(13.3)	44(13.3)
	negative	0(0)	207(62.5)	207(62.5)
	total	80(24.2)	251(75.8)	331(100)

3.2. Patients' pathological characteristics between groups

All of the 331 cases had detailed clinicopathological data for review. Two patients were male. Table 3 summarizes patient clinicopathological characteristics of each group, including age of onset, tumor size, lymph node status, hormone receptor status and Ki67 index. We compared these characteristics of cases in group 1, 4 and 5. Group 5 is interpreted as HER2 negative by both 2013 and 2018 ASCO/CAP guidelines as a negative control group. Only 9 patients were in group 3 and none was in group 2, which were excluded from the analysis. Tumor size and lymph node status were not significantly different across the three groups (P = 0.083, P = 0.906). Hormone receptor status and Ki67 index had significant difference among them (P = 0.002, P < 0.001). Lymph node status and hormone receptor status were not significantly different between group 4 and group 5, OR = 1.156(95%CI:0.063,2.217) and 1.311(95%CI:0.431,3.989). Meanwhile, tumor size and Ki67 index of patients in group 4 were significantly higher than those in group 5, OR = 2.032(95% CI:1.049,3.938) and 3.817(95%CI:1.692,8.609) (Table 4).

3.3. Survival difference between groups

Because HER2 amplification is a known adverse prognostic marker, we compared clinical outcomes of patients between group 4 and group 5, to determine whether patients in group 4 had similar prognosis as expected for the HER2 negative group. None of the patients in group 4 and group 5 received Herceptin therapy. No patients were lost to follow-up, and totally 8 patients died during the follow-up period, 1 in group 4 and 5 in group 5. The median follow-up interval was 33 months (range: 6–76 months). Using log-rank test to compare overall survival difference, clinical outcomes of these 44 patients did not differ from outcomes of patients in group 5, P = 0.867(Fig. 2).

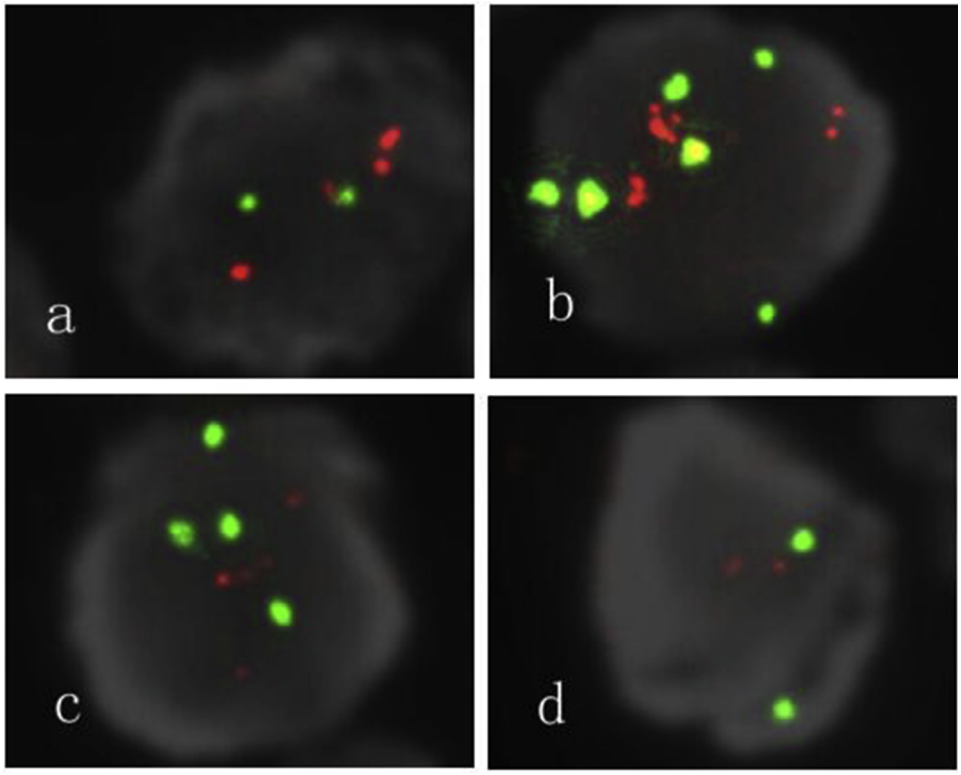

Fig. 1. Examples of HER2 FISH results according to the 2018 ASCO/CAP guidelines.(a) group 1:HER2/CEP17 ratio ≥ 2.0; average HER2 copy number ≥ 4.0.(b)group 3:HER2/CEP17 ratio < 2.0; average HER2copy number ≥ 6.0. (c) group 4:HER2/CEP17 ratio < 2.0; average HER2 copy number ≥ 4.0 and < 6.0.(d)group 5:HER2/CEP17 ratio < 2.0; average HER2 copy number < 4.Red,HER2;green,CEP17.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3
Clinicopathological characteristics of patients according to the 2018 ASCO/CAP guidelines groups.

	group 1	group 3	group 4	group 5
Number of patients	71	9	44	207
Mean age onset(years)	54.5	57	57.5	55
Tumor size(%)				
T1	36(51)	8(89)	18(41)	121(58)
T2	29(41)	1(11)	23(52)	80(39)
T3 and above	6(8)	0(0)	3(7)	6(3)
Nodal involvement(%)				
No	38(54)	6(67)	22(50)	111(54)
Yes	33(46)	3(33)	22(50)	96(46)
Hormone receptor status(%)				
ER or PR positive	51(72)	8(89)	40(91)	183(88)
ER and PR negative	20(28)	1(11)	4(9)	24(12)
Ki67 index(%)				
< 14%	14(20)	2(22)	8(18)	95(46)
≥14%	57(80)	7(78)	36(82)	112(54)

4. Discussion

Accurate HER2 assessment for patients with invasive breast cancer is crucial to determining which patients may benefit from HER2-targeted therapy. The most recent ASCO/CAP guidelines have again redefined HER2 gene amplification as determined by dual-probe FISH since some studies have reported an increase in number of equivocal cases after the 2013 update [17–19]. Some other studies reported an increase of HER2 positive rate [20]. The current HER2 testing protocol in patients with breast cancer is to assess samples initially with IHC and follow up with FISH for samples with equivocal or discordant results. For this reason, our study focused on cases of IHC 2+. We studied the distribution of FISH results in each group. We also compared the 2013 ASCO/CAP HER2 testing guidelines for breast cancer with the updated 2018 guidelines. The single center retrospective study observed that for IHC 2+ breast cancer patients FISH results of group 5 was the majority, which was consistent with some other studies [21,22]. Michael F.Press et al reported that the proportion of group 2 was 0.4%–0.7% and most group 2 breast cancers had IHC 0/1+ immunostaining [21]. Morgan Ballard et al also demonstrated a low frequency of monosomy cases (group 2), accounting for 1.4%, and only 12.4% of these cases were IHC 3+ [23]. In our study, none of the FISH results met the criteria of group 2. This may due to our small sample size and that all of the breast cancer patients were IHC 2+. Few patients were in group 3, which was consistent with several recent studies [21–23]. And we also found that patients in group 4 accounted for a significant portion, which was probably because our patients were all IHC 2 + .

The 2018 ASCO/CAP HER2 testing guidelines recommend that FISH results of group 2 to group 4 need to be interpreted combined with IHC. If the IHC result is 2+, the FISH result should be recounted by another observer [16]. In our experience, repeat counting rarely shows different results. Several recent studies also supported this point [17,24]. Thus, the majority of FISH results with breast cancer patients in group 2 and group 4 will be finally interpreted as HER2 negative, FISH results in groups 3 will be interpreted as HER2 positive according to the 2018 ASCO/CAP guidelines. It is conceivable that the negative rate of FISH results will increase according to the new guidelines. In comparing to the results according to the 2013 ASCO/CAP guidelines, the positive rate was the same, the negative rate was increased from 62.5% to 75.8%, and 13.3% changed from equivocal to negative. Our data suggested that the increase in HER2 FISH negative rate according to the 2018 ASCO/CAP guidelines was mainly due to the reclassification of cases in group 4.

Bethune GC et al reported that traditional pathological features such as tumor size, tumor grade and nodal involvement of the HER2 equivocal group(group 4) appeared to be intermediate between HER2 positive and HER2 negative tumors [17]. Morgan Ballard et al also supported the concept that the HER2 equivocal cases have features intermediate between HER2 positive and HER2 negative cases [23]. Another study found that cases of group 4 were significantly associated with some worse prognostic factors, such as higher ki67 index, higher

Table 4
Comparison of prognostic factors between groups according to the 2018 ASCO/CAP guidelines.

	P value for comparison of three groups	group 1 versus group 4 Odds ratio(95% CI)	group 1 versus group 5	group 4 versus group 5
Tumor size T1 versus T2 and above	0.083	0.673(0.315, 1.439)	1.368(0.796,2.350)	2.032(1.049,3.938)[b]
Nodal involvement yes versus no	0.906	0.868(0.409,1.844)	1.004(0.585,1.724)	1.156(0.603,2.217)
Hormone receptor status positive versus negative	0.002[a]	0.255(0.081,0.806)[b]	0.334(0.171,0.653)[b]	1.311(0.431,3.989)
Ki67 index ≥14% versus < 14%	< 0.001[a]	0.905(0.345,2.372)	3.453(1.811,6.585)[b]	3.817(1.692,8.609)[b]

CI,confidence interval.
[a] Comparisons between three groups that were statistically significant.
[b] Comparisons between two groups that were statistically significant.

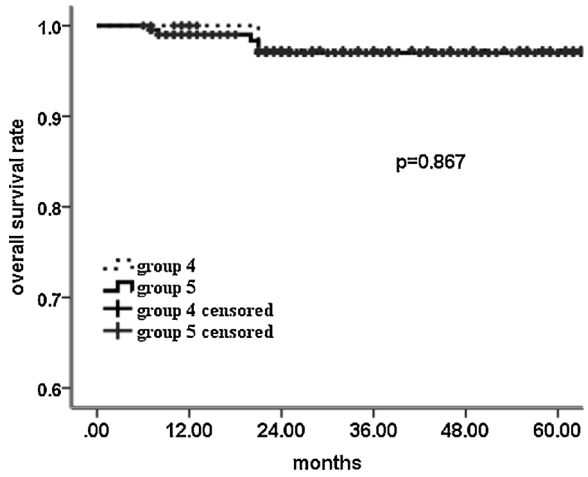

Fig. 2. Kaplan-Meier plots of overall survival.

tumor grade, more frequent lymph node metastasis, and lower hormone receptor expression compared with HER2 negative cases [24]. In our study, tumor size and Ki67 index of cases in group 4 were higher than in group 5, while nodal involvement and hormone receptor status did not have a significant difference. The reason for these differences between studies is unclear, but may be related to small sample size. Larger sample studies are needed to better identify pathological differences between the newly defined HER2 negative group(group 4) and the original HER2 negative group(group 5).

Patients in FISH group 4 were HER2 equivocal according to the 2013 ASCO/CAP guidelines, all of these patients in our studies did not receive Herceptin therapy, nor did the patients in group 5. We compared outcomes between these two groups. Totally 1 patient died in group 4 because of tumor metastasis, 5 patients in group 5 died for the same reason. Survival analysis did not show any difference between these two groups. In trial BCIRG-005/006/007, outcomes of 176 patients in group 4 did not differ from outcomes in group 5, which suggested that patients in group 4 seem to be HER2 not amplified [25]. Although our research sample size and number of events were small, it illustrated the same point. However, further direct randomized controlled trials that compare group 4 patients that receive Herceptin therapy with those who do not may better determine whether group 4 patients can benefit from Herceptin-targeted therapy.

In summary, this study shows that the 2018 new guideline will increase the negative rate of HER2 FISH for IHC 2+ patients. The reclassification of group 4 is the main reason for this change. Patients in group 4 are associated with some worse prognostic factors and seem to have similar outcomes with those in group 5.

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