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PRECLINICAL STUDY

Impact of repeat HER2 testing after initial equivocal HER2 FISH results using 2013 ASCO/CAP guidelines

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Abstract

Purpose The updated 2013 American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 (HER2) testing have made some major changes in HER2 fluorescence in situ hybridization (FISH) interpretation criteria with additional FISH equivocal cases. Repeat HER2 testing is recommended after initial HER2 FISH equivocal results; however, little is known about its impact on final HER2 status. The aim of this study is to investigate whether reflex test clarifies HER2 status, and to characterize clinicopathological features of the newly defined HER2 equivocal group.

Methods A total of 886 consecutive cases of primary invasive breast cancer conducted with dual-probe HER2 FISH testing between November 2013 and December 2015 were reviewed. HER2 immunohistochemistry (IHC) and FISH testing were performed on a different tissue block or a new specimen after initial HER2 FISH equivocal results.

Results Compared to 2007 guideline, 85 (9.6%) cases changed their category by using 2013 guideline. The major change of the 85 cases is that 57 (6.4%) cases in HER2 FISH-negative category changed to equivocal, and the equivocal category cases increased from 36 to 67. HER2 FISH equivocal was significantly associated with HER2 IHC equivocal (2+) and chromosome 17 polysomy ($P < 0.01$). Repeat testing by IHC and FISH clarified HER2 status in 33 and 42% of HER2 equivocal cases, respectively. Overall 32 (48%) initial HER2 equivocal cases stayed HER2 equivocal after repeat FISH and or IHC testing. These tumors were ER/PR+, with high KI-67 index.

Conclusion New guidelines classify more HER2 FISH equivocal cases. Repeat HER2 testing clarifies HER2 status in about 50% of initial HER2 FISH equivocal cases. In addition, HER2 equivocal cases merit further study as there is limited information about prognosis and optimal treatment strategy for this population.

Keywords Breast cancer · HER2 · Immunohistochemistry · Fluorescence in situ hybridization · Equivocal · Chromosome 17 polysomy

Abbreviations

HER2	Human epidermal growth factor 2
IHC	Immunohistochemistry
FISH	Fluorescence in situ hybridization
ASCO	American Society of Clinical Oncology
CAP	College of American Pathologists

Introduction

Human epidermal growth factor 2 (HER2), located on 17q12.21–21.32, is critical for the management of breast cancer [1]. Overexpression of HER2 protein and/or HER2 gene amplification, which is present in 15–20% of breast cancers, is associated with poor prognosis. Anti-HER2

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therapy significantly improves the clinical outcomes of HER2-positive patients [2–4]. Therefore, evaluation of HER2 status becomes more and more critical [5, 6]. Immunohistochemistry (IHC) and in situ hybridization (ISH) assays are used to evaluate the HER2 status, and Fluorescence in situ hybridization (FISH) is most commonly used in situ hybridization technique [7, 8].

The American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) have provided detailed guidelines for conducting and interpreting HER2 testing in clinical practice. ASCO/CAP guidelines for HER2 testing were initially released in 2007 and updated in 2013 [9, 10]. The updated 2013 guidelines have made some major changes on the diagnostic categories for FISH and redefined the FISH equivocal (a dual-probe HER2/CEP17 ratio is <2.0 with average HER2 copy number ≥ 4 and <6/cell). Several studies have shown that the update of the equivocal category led to an increase in FISH equivocal cases compared to those using the 2007 criteria [11–16]. This causes a managerial dilemma for increasing the number of patients diagnosed with HER2 equivocal [9, 10]. The updated guidelines recommend that an equivocal FISH result must prompt reflex testing, including reflex IHC testing, testing on a different tissue block or a new specimen by either IHC or FISH, or FISH using alternative chromosome 17 probes [10]. The overall cost will be increased due to these additional tests for the expanded equivocal cases; however, limited data have demonstrated the impact of these reflex until now [8, 17]. The aim of this study is to investigate the impact of reflex testing on HER2 equivocal cases and to explore the pathological characteristics of HER2 equivocal population.

Materials and methods

Patient population

We retrospectively identified all consecutive cases of primary invasive breast cancer conducted with dual-probe HER2 FISH testing from Guangdong General Hospital (Guangzhou, China) between November 2013 and December 2015. Specimens consisted of core needle biopsy and surgical excisions (partial and full mastectomy specimens). In our institution, IHC was the initial test on all cases and followed by reflex HER2 FISH after 2+ results. Our lab has a history of excellence in participation and performance of the CAP proficiency surveys and National External Quality Assessment Service, United Kingdom (UKNEQAS) as evidence of high standard quality and accuracy of testing. Ethics approval for this study was granted by the Medical Ethics Committee of Guangdong General Hospital, Guangzhou, China.

Fluorescence in situ hybridization (FISH)

The FDA-approved PathVysion HER2 DNA probe kit (Abbott Molecular, Des Plaines, IL, USA) was used in accordance with the manufacturer's recommended protocols, but with minor modifications, which has been described previously [18]. Lymphocytes and normal breast tissue were served as negative control. A known paraffin-embedded primary breast cancer specimen with HER2 amplification was selected as positive control. Analysis of HER2 FISH was performed by one certified pathologist and one certified technologist independently without knowledge of the IHC results. If there was a discrepancy between the two scorers, another pathologist who was usually the most experienced in FISH could rescore the case and generated the final result. HER2 FISH results were interpreted based on 2013 ASCO/CAP guidelines. Chromosome 17 polysomy was defined as $\geq$3CEP17 signals per nucleus. For investigational purposes, a separate result was recorded for each case using the 2007 guidelines.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed on 4-μm-thick formalin-fixed, paraffin-embedded tissue sections by certified laboratory staff using standardized automated methodology (Ventana Medical Systems, Oro Valley, AZ). Standardized immunohistochemical protocols were followed with control slides as appropriate. HER2 IHC was interpreted based on 2013 ASCO/CAP guidelines by two pathologists independently without knowing FISH results. Discrepant cases were concurrently reviewed by these two pathologists using a multiheaded microscope to obtain a consensus score. IHC for Ki67 and P53 was performed using monoclonal rabbit anti-human Ki67 antibody (1:200, MIB-1, DAKO) and P53 (1:500, DO-7; DAKO), respectively. For positivity assessment of the immunostaining for each section, only nuclear staining was regarded as positive. Case was scored as high level expression of Ki67 if over 14% tumor cells were positively stained. 10% of nuclear staining in tumor cells is the cutoff for p53 positivity.

Statistical analyses

Statistical analyses were performed using the SPSS 13.0 statistical software package (SPSS Inc, Chicago, IL). Categorical data were compared using χ^2 test or Fisher's exact test. Statistical significance was assumed if $P < 0.05$.

Result

Increased HER2 FISH equivocal cases using 2013 ASCO/CAP guidelines compared to using 2007 ASCO/CAP guidelines

In total, 886 patients with primary invasive breast cancers were included in this study; some cases were referred from other hospitals for pathological consultation. We reported our final HER2 classification according to the ASCO/CAP 2013 guidelines, and each case was also classified HER2 status using the 2007 criteria for comparison purposes. Of all 886 cases, 801 (90.4%) had the same HER2 classification using the 2007 or 2013 guidelines. Table 1 summarizes the HER2 classification of all cases. Compared to using 2007 guidelines, 85 (9.6%) cases changed their category by using 2013 guideline. Among these 85 cases, the major change is that 57 (6.4%) cases in HER2 FISH-negative category changed to equivocal, followed by 13 cases (1.5%) changed from equivocal to positive, 9 cases (1.0%) changed from equivocal to negative, and 6 cases (0.7%) changed from negative to positive ($P < 0.001$). The equivocal category cases increased from 32 to 67.

HER2 FISH equivocal correlates with HER2 IHC equivocal, chromosome 17 polysomy

Among the HER2 FISH equivocal cases based on 2013 guidelines, 83.6% (56 of 67 cases) showed IHC equivocal results, 11.9% (8 of 67 cases) showed negative IHC results, and three cases had positive IHC results. HER2 FISH equivocal was significantly associated with HER2 IHC equivocal ($P < 0.01$). Interestingly, most of the cases with classification change from negative to equivocal were also IHC equivocal ($P < 0.001$) (Tables 2, 3).

218 of the 886 cases (24.6%) are considered to have chromosome 17 polysomy according to a definition of ≥3.0 CEP17 copies per nucleus. As shown in Table 4, the frequency of chromosome polysomy 17 was significantly higher in the HER2-amplified or equivocal groups than those in the non-amplified group irrespective of criteria used (both $P < 0.001$).

Repeat test help to verify the final HER2 status on the FISH equivocal cases

Because additional cell counts were performed clinically and one technologist and one pathologist have scored the case independently in each HER2 FISH equivocal, and another CEP17 was not available in most laboratory like us, we performed IHC and FISH on a different tissue block or a new specimen for those HER2 FISH equivocal cases to further evaluate HER2 status. Among the 67 HER2 FISH equivocal cases, 46 of them underwent HER2 IHC, with 27 in a different tissue block and 19 in a new specimen (surgical resection). As a result, 3 cases were found HER2 positive (IHC 3+), 12 cases negative (IHC 0/1+), and 31 remained equivocal (IHC 2+). Additional FISH was performed in 48 HER2 FISH equivocal cases, which includes 20 tests in new specimen (surgical resection) and 28 tests in new block. As shown in Tables 5, 6, and 7, these cases were re-categorized as amplified in 8 cases, non-amplified in 12 cases, and equivocal in 28 cases. All the 3 IHC 3+ cases were also amplified by FISH. None of these cases showed HER2 heterogeneity by FISH or IHC.

The clinical-pathologic features of "Deemed Equivocal" cases

Even after performing reflex IHC and new test on new block or specimen, 32 cases ultimately deemed to be equivocal and 29 had complete clinicopathological information available for review. Table 8 summarizes the main clinicopathological characteristic of these "Deemed Equivocal" cases. All patients were female, with an average age of 54, ranging from 21 to 83. Most cases (79.3%) corresponded to invasive ductal carcinomas, no special type, 3 (10.3%) with focal invasive micropapillary carcinoma, 2 (6.9%) with mucinous carcinoma component, and 1 (3.4%) with neuroendocrine feature. Most of them showed intermediate/high histologic grade ($n = 28$, 96.6%), estrogen receptor positive (ER+) concomitant with progesterone receptor positive ($n = 23$, 79.3%), and high Ki67 index ($n = 23$, 79.3%). Tumor of size more than

Table 1 HER2 status of 886 FISH tests classified with 2007 and 2013 ASCO/CAP scoring guidelines

2007 guidelines	2013 guidelines			Total
	Amplified	Equivocal	Non-amplified	
Amplified	202 (22.8%)	0	0	202 (22.8%)
Equivocal	13 (1.5%)	10 (1.1%)	9 (1.0%)	32 (3.6%)
Non-amplified	6 (0.7%)	57 (6.4%)	589 (66.5%)	652 (73.6%)
Total	221 (24.9%)	67 (7.6%)	598 (67.5%)	886

Table 2 HER2 IHC result distribution for FISH equivocal cases according to 2013 guideline

IHC status according to 2013 guideline	*N*	FISH equivocal according to 2013 guideline	*P* value*
0/1+	246	8 (3.3%)	
2+	476	56 (11.8%)	
3+	164	3 (1.8%)	0.000

* χ^2 test

Table 3 HER2 IHC result distribution for cases change from FISH negative according to 2007 guideline to FISH equivocal according to 2013 guideline

IHC status according to 2013 guideline	*N*	2007 negative change to 2013 equivocal	*P* value*
0/1+	246	8 (3.3%)	
2+	476	46 (9.7%)	
3+	164	3 (1.8%)	0.000

* χ^2 test

Table 4 Correlation between Chromosome polysomy 17 and HER2 FISH equivocal

FISH status	FISH according to 2007 guideline		*P* value*	FISH according to 2013 guideline		*P* value*
	N	Chromosome polysomy 17		*N*	Chromosome polysomy 17	
Negative	652	80 (12.3%)		598	43 (7.2%)	
Equivocal	32	7 (21.9%)		67	36 (53.7%)	
Positive	202	130 (64.4%)	0.000	221	138 (62.4%)	0.000

* χ^2 test

Table 5 Summary of HER2 status with repeat test on a different tissue block or a new specimen in 67 HER2 FISH equivocal cases by FISH or IHC

HER2 status	IHC			FISH		
	0/1+	2+	3+	Positive	Equivocal	Negative
N	12 (26.1%)	31 (67.4%)	3 (6.5%)	8 (16.7%)	28 (58.3%)	12 (25.0%)

Table 6 Outcome of repeat test on a different block versus a new specimen in 67 HER2 FISH equivocal cases by IHC

HER2 status	Specimen	
	Different block	New specimen
0/1+	7 (15.2%)	5 (10.9%)
2+	19 (41.3%)	12 (26.1%)
3+	1 (2.2%)	2 (4.3%)

Table 7 Outcome of repeat test on a different block versus a new specimen in 67 HER2 FISH equivocal cases by FISH

HER2 status	Specimen	
	Different block	New specimen
Positive	3 (6.3%)	5 (10.4%)
Equivocal	17 (35.4%)	11 (22.9%)
Negative	8 (16.7%)	4 (8.3%)

2.0 cm, lymph node negative, and P53 expression were seen in more than half of the cases. Chemotherapy plus trastuzumab was administered to 11 patients. Addition of trastuzumab to neoadjuvant chemotherapy was demonstrated to increase overall response rate in breast cancer cases with HER2 equivocal (data in submission).

Table 8 Clinicopathological features of HER2 "Deemed Equivocal" cases

Characteristics	*N*	%
Age (years)		
>50	18	62.1
≤50	11	37.9
Histological type		
IDC, NOS	23	79.3
IDC with focal special type features	6	20.7
Grade		
Low	1	3.4
Intermediate	14	48.3
High	14	48.3
Tumor size (cm)		
>2.0	19	65.5
≤2.0	10	34.5
Nodal status		
N0	17	58.6
N1–3	12	41.4
ER/PR status		
ER+/PR+	23	79.3
ER+/PR−	1	3.4
ER−/PR+	1	3.4
ER−/PR−	4	13.8
Ki67 (%)		
>14	23	79.3
≤14	6	20.7
P53		
Positive	16	55.2
Negative	13	44.8

Discussion

The updated guidelines for HER2 recommended by ASCO/CAP were published in 2013 [10]. One of the significant changes in the updated guidelines was redefining the equivocal criterion with emphasis on HER2/nucleus signal count for interpretation of HER2 FISH results. Several studies have reported that the proportion of HER2 FISH equivocal cases increases substantially after implementation of the new guidelines. Sapino et al. reassessed 957 breast cancers with equivocal (2+) IHC and found that the equivocal FISH cases increased fivefold when using the ASCO/CAP 2013 FISH algorithm (12.3%) compared to those using the ASCO/CAP 2007 ratio criterion (2.4%) [12]. Long et al. reviewed 717 consecutive HER2 FISH results and found that 35 initial negative cases became equivocal when using 2013 guidelines [11]. Espinet et al. reported a consistent trend with 58 new equivocal cases when applying updated guidelines to 622 HER2 FISH results [13]. Singh et al. study showed that 42 initial negative cases became equivocal, resulting in a 2.2% increase of equivocal classification after the use of 2013 guidelines in 836 HER2 FISH results [14]. In our data, both IHC and FISH were used as a primary test. Similar to these reports, we found that 85 cases of the 886 breast cancers changed their categories, among which 57 cases were changed from HER2 FISH negative to equivocal using the 2013 ASCO/CAP guidelines compared to those using 2007 guidelines, representing a 2.1-fold increase in the size of the equivocal category. This further illustrated that the update of equivocal category led to a significant increase in HER2 FISH equivocal cases.

Numerous studies have demonstrated that the concordance rates between IHC and FISH were the highest in tumors scored by IHC as 0/1+ and 3+ and the lowest for 2+ [18, 19]. Interestingly, analysis of the IHC score of these HER2 FISH equivocal cases showed that most of them (56/67) were HER2 2+, and the majority of cases (46/57) changed from initial negative to equivocal were also HER2 2+. It seemed that HER2 FISH equivocal were correlated with IHC equivocal. Since reflex FISH tests were conducted for some IHC 2+ cases in our cohort, which would have a bias towards the correlation, we did a literature review to verify the correlation of HER2 IHC with FISH equivocal. We noted that in the Long's study, most of the HER2 FISH equivocal cases (26/41) were also equivocal 2+ by IHC, and the majority cases (11/18) changed from negative to equivocal were IHC 2+, and they called these cases as "double equivocal" [11]. Bethune's study also showed that the vast majority of cases changed from initial negative (97%, 56/58) to equivocal HER2 was equivocal (2+) by IHC [20]. Collectively, these studies suggested that most HER2 FISH equivocal cases were also IHC equivocal, the so-called "double equivocal."

Our data have illustrated that a significantly higher proportion of cases in the HER2 FISH equivocal group had a chromosome 17 polysomy, suggesting its contribution to the newly defined equivocal group. Chromosome 17 polysomy has previously been reported in series investigating breast cancers. Reported prevalence rates of chromosome 17 polysomy (≥3 CEP17 copies/nucleus) ranged between 3 and 46% [21]. The largest examination of HER2 equivocal breast cancers was HERA (HERceptin Adjuvant) trial, where 69 of 113 new HER2 equivocal cases were chromosome 17 polysomy [22]. Fan et al. reported that 75% of HER2 FISH equivocal cases with 2013 guidelines were chromosome 17 polysomy [23]. In Bethune's study, 77% of their cohort that switched from the HER2 FISH negative to equivocal with new guidelines was also chromosome 17 polysomy [20]. Chromosome 17 polysomy has

also showed its correlation with increased IHC score (2+/3+) in tumors without HER2 amplification [21]. As seen from our data, chromosome 17 polysomy was more frequently detected in tumors with IHC 2+/3+ than IHC 0/1+. In the absence of HER2 amplification, chromosome 17 polysomy has been demonstrated to be correlated with equivocal IHC results (IHC 2+). This may be the explanation for the correlation of HER2 FISH equivocal with IHC 2+. The impact of chromosome 17 polysomy has also been evaluated in several studies but yielded some inconsistent results. Although most studies linked chromosome 17 polysomy with unfavorable clinicopathologic features and poorer prognosis due to several other genes such as BRCA1, TOP2A, TP53 in chromosome 17 implicated in tumor genesis, others have found that chromosome 17 polysomy had no effect on clinicopathologic variables or had more favorable pathologic features [21, 24]. In parallel with these studies, a very recent study by Bethune showed that the pathological features of HER2 FISH equivocal group were intermediate between HER2-negative and HER2-positive tumors [20]. The HER2 FISH equivocal group with chromosome 17 polysomy needs further investigation to evaluate whether there is a subgroup within this cohort that is worth treating with HER2-targeted therapy. However, recent reports suggest that true chromosome 17 polysomy is a rare event in breast cancers and most of the elevated CEP17 signals detected by dual-probe ISH HER2 testing are local gain/amplification in the pericentromeric region of chromosome 17 [25].

Breast cancers with equivocal HER2 scores are particularly problematic for clinical management. The ASCO/CAP algorithm recommends reflex testing to be performed using a different modality for all HER2 equivocal cases. However, there are few studies available for the impact of such additional assays on the ultimate status of HER2 [15, 26, 27]. In a study by Muller which commenced with FISH for HER2, reflex IHC only classifies 29% (5 of 17) of equivocal FISH as positive or negative, but the majority remained equivocal [15]. Regarding the utility of retesting in a separated specimen, Striebel et al. found that 59% of equivocal FISH results based on 2007 guidelines were reassigned as either positive or negative based on evaluation of the surgical resection specimen, albeit 41% (7/17) of equivocal results were not solved [27]. Among the remaining 12 equivocal cases in Muller's study by both IHC and FISH, six equivocal cases had repeat testing in an excisional specimen, and the final results were two negative, two equivocal, and two positive [15]. Here we performed both IHC and FISH on a different tissue block or a new specimen (surgical resection specimen) in a "reflexive" manner for those equivocal FISH cases. We found that new IHC and FISH testing could classify 33% and 42% of cases as negative or positive and the additional 67% and 58% of cases remained equivocal. A very recent study has assessed the use of RARA, SMS, and TP53 as alternative FISH probes and has concluded that using any of these three genes alone, even if used in combination, may not be appropriate as alternative to CEP17 and has little value in daily practice [28]. All equivocal FISH results in our department have been confirmed by counting additional cells or repeating the FISH test. Like most laboratories, we do not have access to alternative chromosome 17 probes, and additional FISH using other reference genes was not performed in our cohort. Collectively, these showed that combining with reflex IHC and new test in a different tissue block or a separate specimen could verify the final HER2 status in more than half of the equivocal FISH cases. However, a nice bit of cases (32/67) ultimately deemed to be equivocal, though without enough tumors present for reflex test accounting for some of the cases.

Analysis of the clinicopathological characteristic of these "Deemed Equivocal" cases displayed that they were predominantly ER/PR+ with higher proliferative index by Ki-67 and were intermediate between HER2-negative and HER2-positive tumors in other pathological factors such as tumor size, grade, and nodal involvement. The findings were concordant with previous reports and showed that "Deemed Equivocal" cases were maybe a unique category of carcinomas and more studies needed to address the questions [16, 20, 29].

Whether patients with HER2 equivocal tumors should receive targeted therapy remains controversial. The 2013 ASCO/CAP guidelines recommended that the patients with an ultimate equivocal HER2 result, even after reflex testing with an alternative assay, should be considered for HER2-targeted therapy and the decision should be left to be made by oncologists [10]. In out cohort, addition of trastuzumab to neoadjuvant chemotherapy was demonstrated to increase overall response rate in 11 patients with HER2 equivocal. These results coincided with the outcomes from the N9831 trial that anti-HER2 therapy may be beneficial to patients with average HER2 copy number >4/cell irrespective of HER2 ratio [30]. Further prospective clinical studies are necessary to better define treatment options and prognosis for patients with cancers in the new "equivocal" category.

In conclusion, a more significant impact of the updated guidelines is seen on the increase in the classification of HER2 FISH equivocal cases. Combining reflex IHC with additional test in a different tissue block or a separate specimen can clarify HER2 status in approximately half of the equivocal FISH cases and has been proved effective in capturing additional patients eligible for anti-HER2 therapy as well as identifying patients with equivocal results who may potentially benefit from anti-HER2 therapy. Likewise, the cases deemed equivocal for HER2 under the 2013 guidelines represent a very different group which

requires more studies to guarantee optimal treatment regimens and clinical outcome.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interests.

References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235(4785):177–182
2. Burstein HJ (2005) The distinctive nature of HER-2-positive breast cancer. N Engl J Med 353:1652–1654
3. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE Jr, Martino S, Mamounas EP, Kaufman PA, Wolmark N (2011) Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 29:3366–3373
4. Gianni L, Dafni U, Gelber RD, Azambuja E, Muehlbauer S, Goldhirsch A, Untch M, Smith I, Baselga J, Jackisch C, Cameron D, Mano M, Pedrini JL, Veronesi A, Mendiola C, Pluzanska A, Semiglazov V, Vrdoljak E, Eckart MJ, Shen Z, Skiadopoulos G, Procter M, Pritchard KI, Piccart-Gebhart MJ, Bell R, Herceptin Adjuvant (HERA) Trial Study Team (2011) Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. Lancet Oncol 12(3):236–244
5. Yaziji H, Goldstein LC, Barry TS, Werling R, Hwang H, Ellis GK, Gralow JR, Livingston RB, Gown AM (2004) HER-2 testing in breast cancer using parallel tissue-based methods. JAMA 291(16):1972–1977
6. Sapino A, Goia M, Recupero D, Marchio' C (2013) Current challenges for HER2 testing in diagnostic pathology: state of the art and controversial issues. Front Oncol 3:129
7. Tubbs RR, Hicks DG, Cook J, Downs-Kelly E, Pettay J, Hartke MB, Hood L, Neelon R, Myles J, Budd GT, Moore HC, Andresen S, Crowe JP (2007) Fluorescence in situ hybridization (FISH) as primary methodology for the assessment of HER2 Status in adenocarcinoma of the breast: a single institution experience. Diagn Mol Pathol 16(4):207–210
8. Lidgren M, Wilking N, Jönsson B, Rehnberg C (2008) Cost effectiveness of HER2 testing and trastuzumab therapy for metastatic breast cancer. Acta Oncol 47(6):1018–1028
9. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF, American Society of Clinical Oncology; College of American Pathologists (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25(1):118–145
10. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF, American Society of Clinical Oncology; College of American Pathologists (2013) Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 31(31):3997–4013
11. Long TH, Lawce H, Durum C, Moore SR, Olson SB, Gatter K, Troxell ML (2015) The new equivocal: changes to HER2 FISH results when applying the 2013 ASCO/CAP guidelines. Am J Clin Pathol 144(2):253–262
12. Sapino A, Maletta F, Verdun di Cantogno L, Macrì L, Botta C, Gugliotta P, Scalzo MS, Annaratone L, Balmativola D, Pietribiasi F, Bernardi P, Arisio R, Viberti L, Guzzetti S, Orlassino R, Ercolani C, Mottolese M, Viale G, Marchiò C (2014) Gene status in HER2 equivocal breast carcinomas: impact of distinct recommendations and contribution of a polymerase chain reaction-based method. Oncologist 19(11):1118–1126
13. Espinet B, Puiggros AM, Corominas JM, Iglesias M, Rodriguez-Rivera M, Melero MC, Albanell J, Tusquets I, Servitja S, Serrano S, Salido M (2015) Increase in equivocal in situ hybridization results in ASCO/CAP guidelines for HER2 testing in invasive breast cancer: comparison with the 2007 criteria (Abstract). Mod Pathol 28:43A
14. Singh K, Tantravahi U, Lomme MM, Pasquariello T, Steinhoff M, Sung CJ (2016) Updated 2013 College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guideline recommendations for human epidermal growth factor receptor 2 (HER2) fluorescent in situ hybridization (FISH) testing increase HER2 positive and HER2 equivocal breast cancer cases; retrospective study of HER2 FISH results of 836 invasive breast cancers. Breast Cancer Res Treat 157(3):405–411
15. Muller KE, Marotti JD, Memoli VA, Wells WA, Tafe LJ (2015) Impact of the 2013 ASCO/CAP HER2 guideline updates at an academic medical center that performs primary HER2 FISH testing: increase in equivocal results and utility of reflex immunohistochemistry. Am J Clin Pathol 144(2):247–252
16. Qian XL, Wen HY, Yang YL, Gu F, Guo XJ, Liu FF, Zhang L, Zhang XM, Fu L (2016) Assessment of dual-probe Her-2 fluorescent in situ hybridization in breast cancer by the 2013 ASCO/CAP guidelines produces more equivocal results than that by the 2007 ASCO/CAP guidelines. Breast Cancer Res Treat 159(1):31–39
17. Tchrakian N, Flanagan L, Harford J, Gannon JM, Quinn CM (2016) New ASCO/CAP guideline recommendations for HER2 testing increase the proportion of reflex in situ hybridization tests and of HER2 positive breast cancers. Virchows Arch 468(2):207–211
18. Liu YH, Xu FP, Rao JY, Zhuang HG, Luo XL, Li L, Luo DL, Zhang F, Xu J (2009) Justification of the change from 10% to 30% for Immunohistochemistry HER-2 scoring criterion in breast cancer. Am J Clin Pathol 132(1):74–79
19. Lim TH, Lim AS, Thike AA, Tien SL, Tan PH (2016) Implications of the Updated 2013 American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations on human epidermal growth factor receptor 2 gene testing using immunohistochemistry and fluorescence in situ hybridization for breast cancer. Arch Pathol Lab Med 140(2):140–147
20. Bethune GC, Veldhuijzen van Zanten D, MacIntosh RF, Rayson D, Younis T, Thompson K, Barnes PJ (2015) Impact of the 2013 American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 (HER2) testing of invasive breast carcinoma: a focus on tumours assessed as 'equivocal' for HER2 gene amplification by fluorescence in situ hybridization. Histopathology 67(6):880–887

21. Hanna WM, Rüschoff J, Bilous M, Coudry RA, Dowsett M, Osamura RY, Penault-Llorca F, van de Vijver M, Viale G (2014) HER2 in situ hybridization in breast cancer: clinical implications of polysomy 17 and genetic heterogeneity. Mod Pathol 27(1):4–18
22. Stoss OC, Scheel A, Nagelmeier I, Schildhaus HU, Henkel T, Viale G, Jasani B, Untch M, Rüschoff J (2015) Impact of updated HER2 testing guidelines in breast cancer–re-evaluation of HERA trial fluorescence in situ hybridization data. Mod Pathol 28(12):1528–1534
23. Fan YS, Casas CE, Peng J, Watkins M, Fan L, Chapman J, Ikpatt OF, Gomez C, Zhao W, Reis IM (2016) HER2 FISH classification of equivocal HER2 IHC breast cancers with use of the 2013 ASCO/CAP practice guideline. Breast Cancer Res Treat 155(3):457–462
24. Ji H, Xuan Q, Nanding A, Zhang H, Zhang Q (2015) The clinicopathologic and prognostic value of altered chromosome 17 centromere copy number in HER2 fish equivocal breast carcinomas. PLoS ONE 10(7):e0132824
25. Koudelakova V, Trojanec R, Vrbkova J, Donevska S, Bouchalova K, Kolar Z, Varanasi L, Hajduch M (2016) Frequency of chromosome 17 polysomy in relation to CEP17 copy number in a large breast cancer cohort. Genes Chromosomes Cancer 55(5):409–417
26. Shah MV, Wiktor AE, Meyer RG, Tenner KS, Ballman KV, Green SJ, Sukov WR, Ketterling RP, Perez EA, Jenkins RB (2016) Change in Pattern of HER2 Fluorescent in Situ Hybridization (FISH) results in breast cancers submitted for Fish testing: experience of a reference laboratory using US Food and Drug Administration Criteria and American Society of Clinical Oncology and College of American Pathologists Guidelines. J Clin Oncol 34:3502–3510
27. Striebel JM, Bhargava R, Horbinski C, Surti U, Dabbs DJ (2008) The equivocally amplified HER2 FISH result on breast core biopsy: indications for further sampling do affect patient management. Am J Clin Pathol 129(3):383–390
28. Jang MH, Kim EJ, Kim HJ, Chung YR, Park SY (2015) Assessment of HER2 status in invasive breast cancers with increased centromere 17 copy number. Breast Cancer Res Treat 153(1):67–77
29. Press MF, Sauter G, Buyse M, Fourmanoir H, Quinaux E, Tsao-Wei DD, Eiermann W, Robert N, Pienkowski T, Crown J, Martin M, Valero V, Mackey JR, Bee V, Ma Y, Villalobos I, Campeau A, Mirlacher M, Lindsay MA, Slamon DJ (2016) HER2 Gene amplification testing by fluorescent in situ hybridization (fish): comparison of the ASCO-College of American Pathologists Guidelines with FISH scores used for enrollment in breast cancer international research group clinical trials. J Clin Oncol 34:3518–3528
30. Perez EA, Reinholz MM, Hillman DW, Tenner KS, Schroeder MJ, Davidson NE, Martino S, Sledge GW, Harris LN, Gralow JR, Dueck AC, Ketterling RP, Ingle JN, Lingle WL, Kaufman PA, Visscher DW, Jenkins RB (2010) HER2 and chromosome 17 effect on patient outcome in the N9831 adjuvant trastuzumab trial. J Clin Oncol 28(28):4307–4315