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Effect of the 2013 ASCO-CAP HER2 Testing Guideline on the Management of IHC/HER2 2+ Invasive Breast Cancer

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Abstract. *Background/Aim: With advances in anti-HER2 treatment and improved prognoses of HER2-positive breast cancer, the American Society of Clinical Oncology and the American Society of Pathologists (ASCO/CAP) have revised the HER2 diagnostic guidelines several times. We examined how to respond clinically to the revisions of the interpretation of the immunohistochemistry (IHC) method. Patients and Methods: We re-evaluated 254 patients diagnosed as HER2 IHC equivocal, who underwent fluorescence in situ hybridization (FISH) before and after the IHC diagnostic criteria update in 2013. Results: Twenty of 131 (15.3%) IHC equivocal cases by the ASCO/CAP 2007 guideline were IHC score 3+ and one of 20 (0.76%) was negative for FISH. Five of 123 (4.1%) IHC equivocal cases by the ASCO/CAP 2013 guideline were negative for IHC as per the 2007 guideline and four were positive for FISH. Conclusion: After revision of the ASCO/CAP 2013 guideline, 3.3% of HER2-negative cases before the revision should have received anti-HER2 treatment.*

Breast cancer is the most common cancer in women and the incidence is still increasing. Among several breast cancer subtypes, the prognosis of human epidermal growth factor receptor 2 (HER2)-positive breast cancer has improved significantly over the last two decades because of advances in anti-HER2 therapeutic agents (1-3). Therefore, HER2 testing needs to be accurate to achieve the most benefit from these treatments and to avoid unnecessary side effects and costs.

The American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) HER2 testing guideline, which is the basis of an accurate HER2 diagnosis to determine which cases should benefit from anti-HER2 treatments, was revised in 2007, 2013, and 2018. In the 2013 revision, the diagnostic criteria for the immunohistochemistry (IHC) method were changed significantly, and in 2018, the *in situ* hybridization (ISH) method was revised in detail. With each new revision, a more detailed ISH test tends to be required (4) and up to four editions of the guidelines have been prepared in Japan by following the revision of the ASCO/CAP guideline, which will be revised again in the future.

HER2 gene amplification indicated by fluorescence *in situ* hybridization (FISH) was previously reported to be more significantly correlated with prognosis than protein expression determined by IHC (5). However, diagnosis is mainly based on the detection of HER2 by IHC, and definitive clinical responses in accordance with the updated HER2 guidelines are unclear. It also means that if the HER2 diagnostic guidelines at the time of recurrence differ from those at the first onset, the indication for anti-HER2 treatment for recurrent patients may be determined based on the old HER2 diagnostic guidelines. The purpose of this study was to examine how to change the correspondence to the HER2 diagnosis at the clinical site as the HER2 diagnostic guidelines update. We reclassified IHC equivocal (score 2+) tumours before and after the 2013 ASCO/CAP revision using the 2007 and 2013 guidelines, respectively. This study demonstrated that the revised ASCO/CAP HER2 diagnostic guideline improved the accuracy of HER2 diagnosis by IHC, but there were cases of overtreatment and cases that did not benefit from HER2 treatment with previous diagnoses.

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Key Words: HER2 diagnosis, IHC/HER2 equivocal, revision of ASCO/CAP guideline.

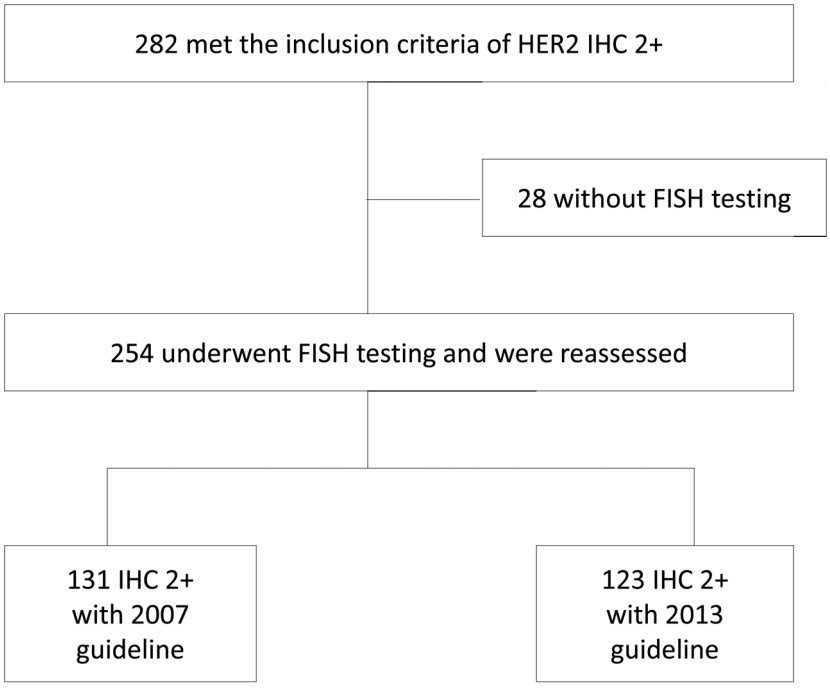

Figure 1. *Study flow chart. A total of 1603 patients with HER2 immunohistochemistry (IHC) score 2+ were recruited, and of 254 patients who underwent fluorescence in situ hybridization (FISH), 131 and 123 were reassessed before and after revision of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline in 2013, respectively.*

Patients and Methods

Patients. This study included all patients who underwent surgery for breast cancer at Kyushu University Hospital from October 2009 to March 2018. To focus on HER2-positive breast cancer, the inclusion criteria were as follows: From the 1,603 patients who underwent surgery for breast cancer, HER2 expression was scored as 2+ in 282 and FISH was performed in 254. Specimens (pre- and post-operative) that had not undergone FISH analysis for any reason, such as ductal carcinoma *in situ* or microinvasion, were excluded (n=28). We finally included 131 patients diagnosed in accordance with the ASCO/CAP 2007 guidelines from October 2009 to April 2015 and 123 patients diagnosed in accordance with the 2013 guidelines from May 2015 to December 2018 (Figure 1).

The study conformed to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Kyushu University Hospital (No. 30-127 and 30-230). Before surgery, participants provided comprehensive written consent stating that the tissue samples from resected specimens may be used for research purposes. All patients had the option to confirm ongoing studies and choose to opt out of consent at any time. The IRB approved this consent procedure.

Immunohistochemistry (IHC). HER2 scores were routinely determined in accordance with the IHC analysis of core needle biopsies or resected specimens by standard IHC staining using rabbit monoclonal antibody 4B5 (Roche Diagnostics, Rotkreuz, Switzerland) and a VENTANA BenchMark ULTRA autostainer (Roche Diagnostics).

They were defined as HER2 equivocal when HER2 IHC staining was scored as 2+ in accordance with the standard ASCO/CAP HER2 diagnostic criteria adopted at that time (6, 7). Specimens were subjected to additional FISH (SRL, Inc., Tokyo, Japan), and only when HER2 amplification was detected, they were defined as positive for HER2. The HER2 diagnosis was defined by the ASCO/CAP guideline at that time.

Evaluation of HER2 diagnosis. Regarding the HER2 status, core needle biopsies or surgical specimens diagnosed as 2+ by the IHC assay before and after revision of the ASCO/CAP 2013 guideline were reconfirmed by another pathologist with abundant experience in HER2 diagnosis and reclassified in accordance with the 2007 and 2013 guidelines. Furthermore, we defined complete and circumferential membrane staining (CMS) that was intense and within ≤10% of tumour cells* as Group 0, membrane staining that was incomplete and intense within >10% as Group 1, CMS that was incomplete and/or weak/moderate within >10% of tumour cells* as Group 2A, and CMS that was complete, intense, and between 10% and 30% of tumour cells* as Group 2B, which was diagnosed as IHC 3+ before the ASCO/CAP 2007 guideline (Figures 2 and 3) (*readily seen using a low-power objective and observed within a homogeneous and contiguous invasive cell population).

Results

Evaluation with the 2013 guideline of IHC score 2+ in accordance with the 2007 guideline. Of these 131 cases classified as IHC score 2+ in accordance with the

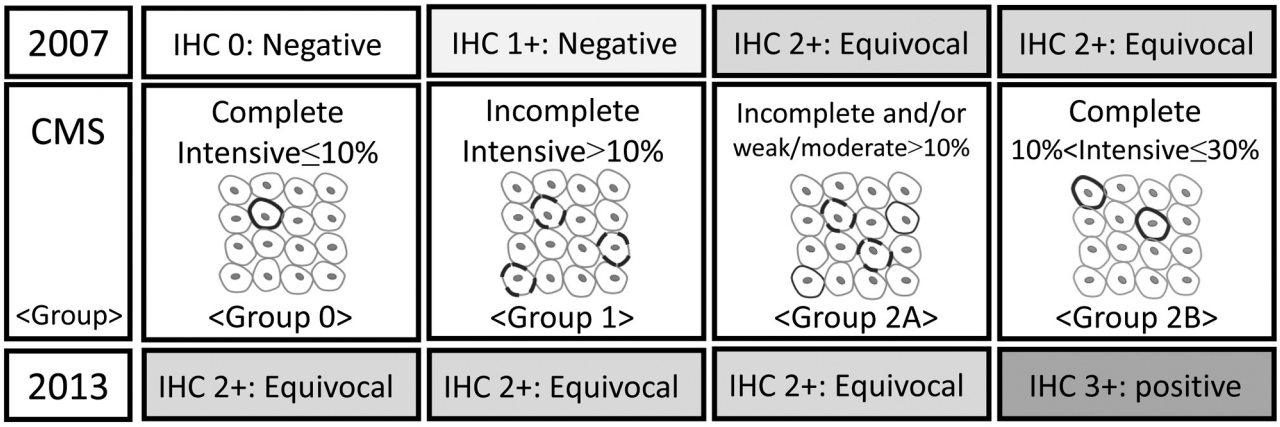

Figure 2. *HER2 IHC diagnostic changes in accordance with the ASCO/CAP guidelines update in 2013. HER2 immunohistochemical (IHC) staining 2+ in accordance with ASCO/CAP 2007 or 2013 guidelines was reclassified into four groups as follows. Group 0 is circumferential complete and intense membrane staining in ≤10%; complete and intense <10%, which is classified as IHC 0 with the 2007 guideline and IHC 2+ with the 2013 guideline. Group 1 is incomplete intense membrane staining >10%; incomplete and intense >10%, which is classified as IHC 1+ with the 2007 guideline and IHC 2+ with the 2013 guideline. Group 2A is incomplete and/or medium/weak membrane staining >10%; incomplete and/or weak >10%, which is classified as IHC 2+ with the 2007 guideline and IHC 2+ with the 2013 guideline. Group 2B is circumferential complete and intense membrane staining in >10%, <30% of tumour cells; complete and intense >10%, which is classified as IHC 2+ with the 2007 guideline and IHC 3+ with the 2013 guideline.*

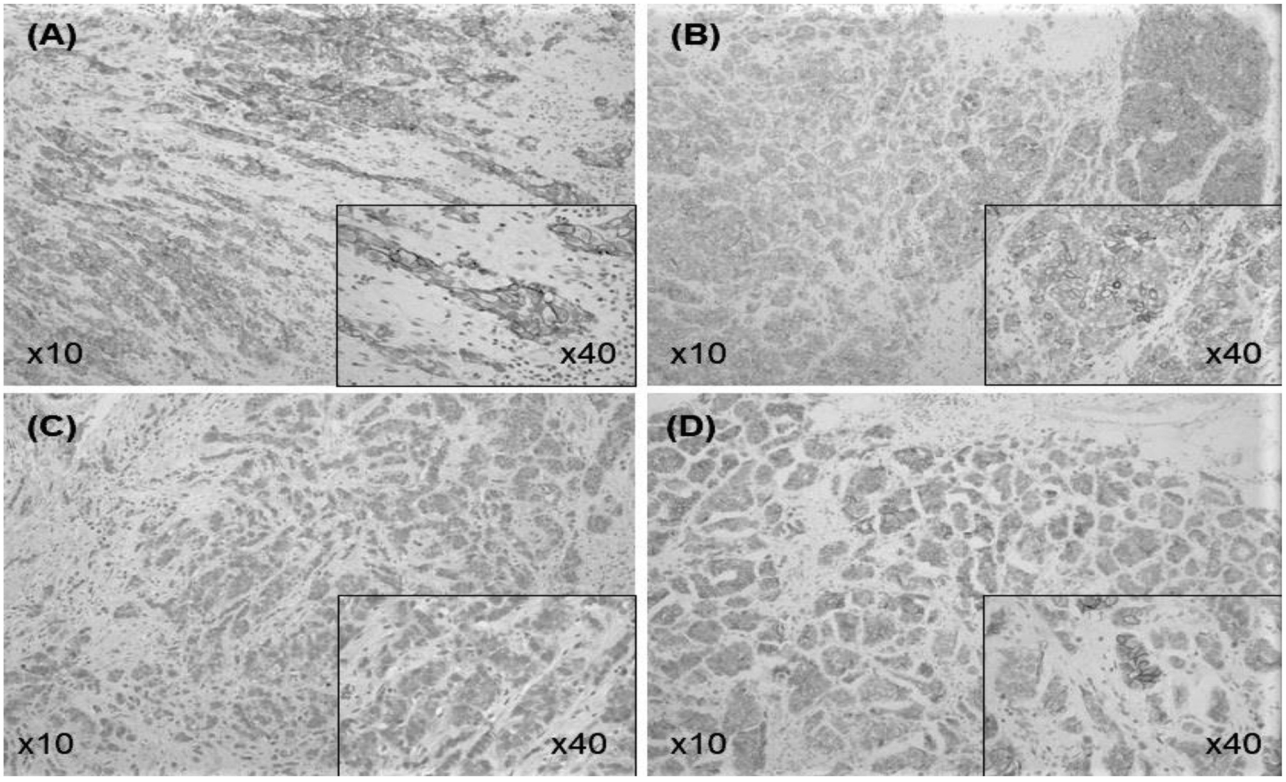

Figure 3. *Representative images of HER2 immunohistochemistry for Group 0 (A), 1 (B), 2A (C), and 2B (D).*

ASCO/CAP 2007 guideline, 20 cases (15.3%) applied to Group 2B, which was "complete and intense >10%", and were IHC score 3+, and one (0.76%) of these cases was negative with HER2 FISH testing (Table I). This case would have received unnecessary anti-HER2 treatment. These results showed that some cases were diagnosed as IHC positive for HER2 in accordance with the ASCO/CAP 2013 and 2018 guidelines although only a few cases were in fact diagnosed as negative by the FISH test.

Evaluation of the 2007 guideline of IHC score 2+ in accordance with the 2013 guideline. Of the 123 cases diagnosed as IHC score 2+ in accordance with the ASCO/CAP 2013 guideline, five cases (4.1%) were Group 0 (n=3) that was "complete and intense <10%" and Group 1 (n=2) that was "incomplete and intense >10%" (Figures 2 and 3, Table II) and classified as IHC score 0 or 1+ in accordance with the 2007 guideline. Surprisingly, four (3.3%) of these cases (Group 0: two of three; Group 1: all) were positive with the FISH test (Table II). Of the patients diagnosed as HER2 IHC 0 or 1+ in accordance with the ASCO/CAP 2007 guideline, a certain number of HER2 FISH-positive patients should have benefited from HER2-targeted treatment.

Compared with HER2 IHC score 2+ patients (50/131, 38.2%) diagnosed in accordance with the ASCO/CAP 2007 guidelines, the positive rate of HER2 FISH testing was lower in those (28/123, 22.8%) diagnosed in accordance with the ASCO/CAP 2013 guideline (Table III).

Discussion

Since the development of trastuzumab, the clinical application of anti-HER2 therapeutics, such as pertuzumab, trastuzumab emtansine, trastuzumab deruxtecan, and neratinib, has been remarkable and the prognosis of HER2-positive breast cancer has improved (2, 8-12). With such progress in anti-HER2 therapeutic agents, new HER2 testing methods, such as next-generation sequencing (NGS) (13, 14) and liquid biopsy (15), are expected to provide a more accurate HER2 diagnosis for patients with HER2-positive breast cancer at all stages (16).

However, IHC and ISH methods are still the most frequently used worldwide because they are time-efficient and cost-effective. Because the ISH method can determine the HER2 gene copy number in the nucleus, it has excellent reproducibility and is more universally used than the IHC method (17-19). Although it has also been reported that gene amplification detected by the FISH method has a stronger correlation with prognosis than the IHC method, IHC diagnosis is still important because the latest anti-HER2 therapeutic agent, trastuzumab deruxtecan, is indicated in accordance with the results of the IHC method (10, 20). In addition, the administration of postoperative treatment based on preoperative chemotherapeutic response-guided strategies established by the KATHERINE study (9), will increase the proportion of anti-HER2 treatments based on preoperative pathological examination of small tissue samples with core needle biopsy. Therefore, more accurate, precise, and rapid HER2 diagnosis by the IHC method will be required by using small samples. Similar to our results, it has been reported that HER2 results were changed by 7.7% and HER2-positive cases were increased after the 2013 revision of the ASCO/CAP guideline (21, 22).

Some previous reports have focused on the ISH test to assess the effect of the guideline revisions (21-25), but we classified and examined the detailed degree of staining by the IHC method. As a result, among some cases categorized as Group 2B, which are "complete and intense >10%, ≤30%, evaluated as IHC 3+ and HER2 positive", one of 20 rare cases was negative with FISH in accordance with the current guideline. This result suggests that overtreatment will be performed for these cases.

Additionally, although not numerous, it cannot be ignored that Group 0 and 2A of "complete and intensity <10% and incomplete and intensity >10%" diagnosed as negative for HER2 by the IHC method with the ASCO/CAP 2007 guideline was found to be positive for HER2 FISH. Therefore, when a case is diagnosed as negative for HER2 in accordance with the ASCO/CAP 2007 guideline, it is essential to re-examine the recurrence site or re-evaluate the initial specimen using the new criteria.

As we have reported previously, among HER2-negative cases in the clinical setting with IHC and ISH methods, there were three cases (2.8%) in which FoundationOne® CDx (NGS) suggested the indication of anti-HER2 treatment (26). All five cases of Groups 0 and 1 were positive for hormone receptor, which suggested that tumour heterogeneity may also be linked to HER2-staining heterogeneity. Both cases categorized as Group 1 were positive with FISH; it has been reported that such cases have intense basolateral membrane staining and invasive micropapillary breast carcinoma (27). Our evaluation by NGS also confirmed amplification of the *HER2* gene in such cases (26). Although the IHC method is still important in the era of genomic medicine, it may be possible to select more accurate anti-HER2 treatment indications by combining HER2 IHC with genomic diagnosis.

There are several limitations to this study. This is a single centre study with a limited number of case validations and the frequency is not rigorous enough for real world data. We are considering this study not including the revised diagnostic criteria of ISH method. It is necessary to design a multicentre study to investigate how revisions of the HER2 diagnostic guidelines affect patient prognosis and how to address them.

Table I. *HER2 FISH testing results of 131 HER2 IHC 2+ cases using the ASCO/CAP 2007 guideline.*

HER2 IHC 2+ with the 2013 guideline	HER2 FISH (%)			
	Positive	Negative	Equivocal	Total
Group 2A	31 (23.7%)	80 (61.1%)	0 (0.0%)	111 (84.7%)
Group 2B	19 (14.5%)	1 (0.8%)	0 (0.0%)	20 (15.3%)
	50 (38.2%)	81 (61.8%)	0 (0.0%)	131 (100%)

Table II. *HER2 FISH testing results of 123 HER2 IHC 2+ cases using the ASCO/CAP 2013 guideline.*

HER2 IHC 2+ with the 2007 guideline	HER2 FISH (%)			
	Positive	Negative	Equivocal	Total
Group 0	2 (1.6%)	1 (0.8%)	0 (0.0%)	3 (2.4%)
Group 1	2 (1.6%)	0 (0.0%)	0 (0.0%)	2 (1.6%)
Group 2A	24 (19.5%)	93 (75.6%)	1 (0.8%)	118 (95.9%)
	28 (22.8%)	94 (76.4%)	1 (0.8%)	123 (100%)

Table III. *Summary of HER2 FISH results of HER2 IHC 2+ cases using the ASCO/CAP 2007 and 2013 guidelines.*

No. of HER2 IHC 2+ (%)	HER2 FISH (%)			
	Positive	Negative	Equivocal	Total
2007 guideline 131/987 (13.3%)	50/131 (38.2%)	81 (61.8%)	0 (0.0%)	131
2013 guideline 123/616 (19.9%)	28/123 (22.8%)	94 (76.4%)	1 (0.8%)	123
				254

In conclusion, in accordance with the ASCO/CAP 2013 guideline, the FISH-positive rate at HER2 IHC 2+ was decreased from 38.2% to 22.8%. However, four of five cases that should be judged as HER2 IHC 1+ with the ASCO/CAP 2007 guideline were positive with HER2 FISH, which suggested anti-HER2 treatment after application of the ASCO/CAP 2013 guideline. We should fully understand that changing the definition of positivity for HER2 determines whether patients with HER2-positive breast cancer can receive a useful anti-HER2 treatment.

Conflicts of Interest

There are no conflicts of interest to disclose regarding this study.

Authors' Contributions

MY and MKubo conceived and designed the study; MY, MKubo, and HY analysed the data; MY and MKubo drafted the manuscript; MY, MKubo, NY, MKai, KZ, KK, AS, SH, HK, MM, YO and MN provided material support and study supervision. All Authors reviewed and approved the final manuscript.

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