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Quantitative Impact of the 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) Practice Guideline Update on Human Epidermal Growth Factor Receptor 2 (HER2) Testing in Breast Cancer

A Systematic Analysis

Christina H. Wei, MD; Lino Garcia, MS; Joyce Murata-Collins, PhD; Daniel Schmolze, MD; Sophia Apple, MD

• ***Context.***—**The global impact of the new 2018 American Society of Clinical Oncology/College of American Pathologists human epidermal growth factor receptor 2 (HER2) practice guideline update on the overall HER2 status designation, compared with the prior 2013 iteration, is unknown.**

Objectives.—**To report the quantitative impact of the new guideline on HER2 status distribution.**

Design.—**The analysis comprised a retrospective cohort of patients from the author's institution, combined with other peer-reviewed publications that assessed the impact of the 2018 guideline in relation to the 2013 guideline.**

Results.—**Our study revealed that the new guideline led to an average 9% reclassification rate for the overall HER2 status, with a net gain in overall HER2 negative designation. This is largely due to reclassification of the equivocal (Group 4) groups. Unexpectedly, infrequent but consistent discordance between Group 1/5 and fluorescence in situ hybridization results are observed across studies (1.8%; 73 of 3965 cases where fluorescence in situ hybridization and immunohistochemistry are both reported).**

Conclusions.—**Early clinical recognition of these resultant changes, including emerging issues of tumor heterogeneity, and potential discordance between immunohistochemistry to fluorescence in situ hybridization, is important for accurate clinical assessment of individual HER2 test results.**

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The 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) human epidermal growth factor receptor 2 (HER2) practice guideline updates the interpretive algorithm of HER2 testing in breast cancer, focusing on less common HER2 fluorescence in situ hybridization (FISH) groups 2, 3, and 4.[1] It recommends concurrent review of HER2 protein expression by immunohistochemistry (IHC) to determine the significance of uncommon HER2 FISH group results. The global impact of the new guideline on the overall HER2 status designation, compared with the prior 2013 iteration, is unknown. Herein, we report the quantitative impact of the new guideline on HER2 status distribution.

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From the Departments of Pathology (Wei, Schmolze), Cancer Cytogenetics (Garcia, Murata-Collins), City of Hope National Medical Center, Duarte, California; and the Department of Pathology, Huntington Memorial Medical Center, Pasadena, California (Apple).

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Corresponding author: Daniel Schmolze, MD, Deptartment of Pathology, City of Hope National Medical Center, 1500 E. Duarte Road, Duarte, CA 91010-3000 (dschmolze@coh.org).

METHODS

The study was approved by the institutional review board (institutional review board #19605). It comprised a retrospective cohort of patients from the author's institution spanning 2017 to 2019, combined with other peer-reviewed publications that assessed the impact of the 2018 guideline in relation to the 2013 guideline. Pubmed.gov was searched for publications between January 1, 2018 to March 19, 2020 with search terms "HER2 guideline," and "HER2 FISH breast." To minimize referral and selection biases, and to meet the guideline's best practice recommendation (performing IHC and FISH at the same laboratory), we included studies that performed co-testing and interpretation of FISH and IHC on all breast cancer cases at the same institution.

RESULTS

Five sites (4 studies from PubMed that fulfilled study selection criteria)[2–5] were included. Across all studies, adoption of the 2018 guideline led to an average 7.1% (range, 5.8%–11.5%) reclassification rate for the overall HER2 status, with a net increase in negative HER2 status designation by an average of 8.4% (range, 5.8%–11.5%).

Table 1. Summary on the Quantitative Impact of New Human Epidermal Growth Factor Receptor 2 (*HER2*) Testing Guideline by Study Sites

FISH Group	Study Site	Guangdong General Hospital, Guangzhou, China	University of California, San Diego, San Diego, CA, USA	Ohio State University, Columbus, OH, USA	City of Hope Medical Center, Duarte, CA, USA	Seoul National University Bundang Hospital, Seongnam, Korea
	Sample Size (n)	2233	1542	1350	1593	1348
	Reclassified by 2018 guideline (n)	8.2 (183/2233)	10.7 (165/1542)	6.2 (84/1350)	11.5 (183/1593)	5.8 (78/1348)
	Tumor classified as HER2 positive by 2013 criteria (n)	24.0 (534/2233)	17.4 (269/1542)	13.3 (180/1350)	18.8 (300/1593)	20.9 (282/1348)
	Tumor classified as HER2 positive by 2018 criteria (n)	23.6 (528/2233)	13.2 (203/1542)	12.9 (174/1350)	17.0 (270/1593)	20.4 (275/1348)
	Tumor classified as HER2 negative by 2013 criteria (n)	68.3 (1524/2233)	76.4 (1178/1542)	81.0 (1092/1350)	71.6 (1140/1593)	73.8 (995/1348)
	Tumor classified as HER2 negative by 2018 criteria (n)	76.4 (1705/2233)	86.8 (1339/1542)	87.1 (1176/1350)	83.1 (1323/1593)	79.6 (1073/1348)
Group 2	HER2 positive by 2013 criteria (n)	100 (8/8)	100 (23/23)	100 (4/4)	100 (20/20)	100 (4/4)
	HER2 positive by 2018 criteria (n)	0 (0/8)	0 (0/23)	0 (0/4)	0 (0/20)	0 (0/4)
	HER2 negative by 2018 criteria(n)	100 (8/8)	100 (23/23)	100 (4/4)	100 (20/20)	100 (4/4)
Group 3	Positive by 2013 criteria (n)	100 (14/14)	100 (8/8)	100 (14/14)	100 (17/17)	100 (16/16)
	Positive by 2018 criteria (n)	100 (14/14)	75.0 (6/8)	85.7 (12/14)	41.2 (7/17)	81.2 (13/16)
	Negative by 2018 criteria (n)	0 (0/14)	25.0 (2/8)	14.3 (2/14)	58.8 (10/17)	18.8 (3/16)
Group 4	Positive by 2013 criteria using alternative probes (n)	Not reported	34.0 (49/144)	Not reported	0 (0/141)	Not reported
	Positive by 2018 criteria (n)	1.1 (2/175)	5.6 (8/144)	0 (0/78)	0 (0/153)	5.3 (4/75)
	Negative by 2018 criteria (n)	99 (173/175)	94.4 (136/144)	100 (78/78)	100 (153/153)	94.7 (71/75)

Abbreviation: FISH, fluorescence in situ hybridization.

Fluorescence In Situ Hybridization (FISH) Group definition per 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 guideline: Group 2 = HER2 ratio $\geq$ 2.0 and HER2 $<$ 4.0 signals per cell; Group 3 = HER2 ratio $<$ 2.0 and HER2 $\geq$ 6.0 signals per cell; Group 4 = HER2 ratio $<$ 2.0 and HER2 $\geq$ 4.0 and $<$ 6.0 signals per cell.

The net gain in HER2-negative status was largely attributable to the elimination of the equivocal category (Group 4); using 2018 criteria with concurrent IHC testing, this group was mostly reclassified as HER2 negative (Tables 1 and 2; 97.1% of Group 4 tumors are classified as "negative" by 2018 criteria). In 1 study where the Group 4 tumor was classified as "positive" by an alternative probe method in accordance with the 2013 guideline, 45 of 49 (92%) alternative probe-positive cases are reclassified as negative by the 2018 criteria. The cohort from our institution showed that none of the Group 4 cases tested by alternative probe (0 of 141) was positive during the study period. Most Group 3 cases remain classified as overall *HER2* positive; reclassification to *HER2* negative status occurred in 17 of 69 Group 3 cases (35.2% of combined Group 3 cohort, range 0%–58.8%). Correlation between *HER2* IHC scores and FISH groups revealed that most Group 2 and Group 4 cases have IHC scores of 2 or less (Table 2). Infrequently, discordant HER2 IHC with Group 1 and 5 FISH occurred (1.8%; 73 of 3965 cases where FISH and IHC are both reported; Table 2). A majority of the discordant cases are attributable to Group 1/IHC score 0/1+ cohort (60 of 603; 10% of Group 1 cohort).

DISCUSSION

Our study provides the most comprehensive evidence to date that the new guideline led to an average 7.1% reclassification rate for overall HER2 status, with a net gain in overall HER2-negative designation. This is mostly due to reclassification of Group 4 and Group 2 to an overall "negative" status by their concurrent IHC scores (3+ overexpression is threshold to attain positive status). This observation is in keeping with the Breast Cancer International Research Group study that these FISH groups are associated with low HER2 expression.[6] The 10% IHC/FISH discordance rate in Group 1 is also similar to the 12.5% rate observed in the Breast Cancer International Research Group cohort.[6] This finding is especially relevant in instances where only IHC is used as first-line HER2 testing, which could lead to undercalling of HER2 status. Furthermore, it also raises the possibility of existing discordant scenarios in other FISH groups, particularly Group 3. A subset of Group 3 is associated with HER2 overexpression and worse disease-free survival than the comparator Group 5.[6,7] With IHC now playing a substantial role in the 2018 HER2 algorithm and the net increase in HER2-negative designation, the possibility of IHC/FISH discordance and other trade-offs associated with using IHC only as the tiebreaker for borderline cases warrants attention. While the concordance rate between HER2 IHC and FISH is cited as high as 95% by the ASCO/CAP guideline,[8] the methodologic differences between these 2 testing approaches (in situ hybridization—which uses molecular probes, and immunohistochemistry—which tests for protein expression) need to be acknowledged. Optimization of analytic conditions for HER2 immunohistochemistry (laboratory validation and quality assurance, avoid specimen de-calcification, avoid under and over fixation, minimize ischemic time) is paramount to minimize false negatives by IHC. Despite best efforts, differences in analytic sensitivity between the Food and Drug Administration–approved kit and laboratory developed HER2 IHC tests have been reported.[9] In this study, 5 of 22 HER2 FISH positive (Group 1) breast tumors were found to be negative by the Food and Drug Administration–approved IHC kit. However, they were all positive by the laboratory developed HER2 IHC

Table 2. Summary on Systematic Analysis of Overall Human Epidermal Growth Factor Receptor 2 (HER2) Status and Immunohistochemistry Score Distribution

Groups (n[a])	*HER2* FISH Results	*HER2* IHC Level	Case Number	2013 Guideline Interpretation			2018 Guideline Interpretation		*HER2* Status Reclassified by 2018 Guideline?
				Positive	Negative	Equivocal	Positive	Negative	
Group 1 (n = 603)	*HER2*:CEP ratio ≥ 2.0, avg *HER2* copy number ≥ 4.0 per cell	0/1+	60	Positive	-	-	Positive	-	No
		2+	130	Positive	-	-	Positive	-	No
		3+	413	Positive	-	-	Positive	-	No
Group 2 (n = 59)	*HER2*:CEP ratio ≥ 2.0, avg *HER2* copy number < 4.0 per cell	0/1+	33	Positive	-	-	-	Negative	Yes
		2+	26	Positive	-	-	-	Negative	Yes
		3+	0	-	-	-	-	-	-
Group 3 (n = 69)	*HER2*:CEP ratio < 2.0, avg *HER2* copy number ≥ 6.0 per cell	0/1+	17	Positive	-	-	-	Negative	Yes
		2+	40	Positive	-	-	Positive	-	No
		3+	12	Positive	-	-	Positive	-	No
Group 4 (n = 625)	*HER2*:CEP ratio < 2.0, avg *HER2* copy number ≥ 4.0 and < 6.0 per cell	0/1+	220	-	-	Equivocal	-	Negative	Yes
		2+	387	-	-	Equivocal	-	Negative	Yes
		3+	18	Positive	-	-	Positive	-	No
Group 5 (n = 3362)	*HER2*:CEP ratio < 2.0, avg *HER2* copy number < 4.0 per cell	0/1+	2759	-	Negative	-	-	Negative	No
		2+	590	-	Negative	-	-	Negative	No
		3+	13	Positive	-	-	Positive	-	No

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; -, no observation recorded.

[a] Sample size reflects the total number of cases reported with their HER2 IHC result. Two studies (Liu et al[3]; Woo et al[5]) did not provide the full IHC results for their cohort.

test. The clinical implication is significant, because these patients may be amenable to HER2-targeted therapy, but may be deprived of it if HER2 IHC were to be used as the only first-line test. In 1 study that examined HER2 IHC/FISH discordant cases and their response to anti-HER2 neoadjuvant therapy, 2 of 4 (50%) of IHC1+/Group 1 patients achieved complete pathologic response following neoadjuvant therapy.[10] Pathologic complete response was achieved by 1 of 1 IHC1+/Group 2 patient (100%).[10] On balance, while the occurrence of discordant FISH/IHC is infrequent, knowing this possibility may be helpful in situations where clinical suspicion for discordant result is raised. This problem may be resolved by retesting using different tissue blocks. In a retrospective cohort series reported by Memorial Sloan Kettering Cancer Center, additional FISH testing, either on a different block(s) from the same specimen or from an alternative specimen, led to a change in FISH status in 63% (64 of 101) patients who initially had FISH Group 4/ IHC 2+ double equivocal results.[11]

The study has a few limitations. First, the evidence is derived from aggregated retrospective, single-institution data. However, the outcome variables are similar across all included studies, lending credence to our study's generalizability. Second, the issue of tumor heterogeneity is gaining increased recognition, with possible therapeutic implication. The included retrospective studies did not include this information. As we move forward with the 2018 guideline, early clinical recognition of these resultant changes, and the emerging issues of tumor heterogeneity and IHC/FISH discordance, is important for accurate clinical assessment of individual HER2 test results.

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