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Original contribution

Discerning subsets of breast cancer with very low and absent HER2 protein expression☆

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Summary Breast cancers are currently eligible for treatment with anti-HER2 therapies if they exhibit amplification of the gene *ERBB2* and overexpression of its protein product HER2. Recently, breast cancers with low HER2 expression have shown response to novel anti-HER2 antibody–drug conjugates, and the lower end of "low-HER2" tumors has not yet been clinically delineated. The historically binary approach to HER2 scoring will need to evolve and reporting of HER2 status may require refinement to better stratify low-HER2 statuses. We performed a quality review of HER2 immunohistochemical (IHC) scoring of breast carcinomas with low HER2 expression (71 core biopsies and 51 excisions). We also investigated the feasibility of discerning cases with total lack of HER2 expression from those cases with "very low" HER2 expression that did not meet current criteria for a HER2(1+) score. Re-scoring HER2 achieved substantial agreement when performed at 200×, and near-perfect agreement at 400× magnification. Examination under 400× magnification led to recognition of more cases with HER2 expression. Less than 10% of cases showed complete lack of HER2 protein expression by IHC. Cases with "very low" expression were readily identified, and such a category would be feasible to implement in pathologist workflow.

1. Introduction

American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines recommend using immunohistochemistry (IHC) and in-situ hybridization to assess human epidermal growth factor 2 (HER2) in breast cancers [1], as HER2 status plays an important role in clinical management. 15–20% of breast tumors show significant HER2 protein overexpression and amplification of *ERBB2,* the gene encoding HER2, and those are eligible for treatment with targeted anti-HER2 therapies such as trastuzumab [2,3]. HER2 immunohistochemistry is the first step in HER2 assessment. Our approach to HER2 evaluation is currently binary (ie, HER2-positive or HER2-negative) and

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it has been clinically critical to identify HER2-positive tumors with IHC scores of HER2(2+) with gene amplification and HER2(3+). Cases with HER2 IHC scores 0 and 1+ are not currently eligible for anti-HER2 therapies and both are considered "HER2-negative." However, recent studies have demonstrated clinical benefit to patients with primary and metastatic breast cancers exhibiting low HER2 expression, defined as HER2(2+) without gene amplification and HER2(1+), using novel anti-HER2 antibody−drug conjugates [4−6].

In light of promising results for anti-HER2 therapy in low HER2-expressing tumors, it would become clinically important to correctly distinguish HER2(1+) from HER2(0) cases to ensure appropriate management [7]. Although some studies have demonstrated reliable interobserver agreement in HER2 IHC interpretation, multiple others have reported significant interobserver disagreement [8,9]. Discrepancies in distinguishing IHC scores of HER2(0) and HER2(1+) specifically may in part be due to current lack of clinical value in differentiating between these 2 groups [7]. Furthermore, the correlation between the degrees of low HER2 expression by tumor and clinical benefit from newer forms of anti-HER2 therapy remains under investigation. HER2 scoring thresholds at the low end may eventually need to be modified and become more granular. Here, we performed a retrospective study of breast core biopsies containing HER2-negative (scores 0 or 1+) invasive breast carcinomas and their corresponding excisions to perform a quality assurance review of HER2 IHC scoring and to evaluate the feasibility of recategorizing HER2 IHC scores using a modified system for reporting low HER2 expression.

2. Materials and methods

Breast core biopsies diagnosed with invasive breast carcinoma between January 1, 2020, and June 30, 2021 (18-month period), with original HER2 IHC scores of 0 or 1+, and their corresponding subsequent breast excision specimens were identified from the case files of Massachusetts General Hospital, Boston, MA. Hematoxylin and eosin (H&E) stained slides and HER2 immunohistochemical stains (SP3 clone, Ventana/Roche, Oro Valley, AZ) were reviewed. All HER2-stained slides contain an external positive (3+) on-slide control sample. Cases were excluded if the HER2 control was suboptimal, or they contained fewer than 100 invasive cancer cells.

Reflex fluorescence in situ hybridization (FISH) for HER2 was performed in HER2(2+) tumors (PathVysion assay, Abbott Molecular, Abbott Park, IL). H&E sections are reviewed to highlight regions containing invasive carcinoma. The dual-color FISH assay was performed on 4-micron sections of formalin-fixed paraffin-embedded tissue using a probe specific to the chromosome 17q HER2 locus and a copy number control probe recognizing centromere 17. Signal quantitation was used to generate a HER2/centromere 17 ratio and a HER2/cell ratio.

Cases underwent a first round of HER2 IHC rescoring using 10× eyepiece with 20× objective (200× total magnification). HER2 scoring criteria for this study used current published guidelines [1,10]. To provide more refined results, the HER2(0) category was subdivided into 2 groups (Table 1): cases with complete lack of expression (0% tumor cells with any level of membranous staining) were categorized as HER2(absent), and cases with >0% but less than 10% tumor cells with faint membranous staining were categorized as HER2(very low). Cases with HER2 rescores of "absent" and "very low" at 200× were subsequently rescored a second time at 400× total magnification. Re-scoring was by consensus between 2 pathologists (B.B. and A.L.).

Cohen's kappa test was used to analyze agreement between initial and rescored HER2 IHC results.

3. Results

3.1. Core biopsies

During the study period, 284 breast core biopsies containing invasive breast carcinoma were identified, and 99 (34.8%) of these were originally classified as HER2-negative: 32 cases scored 0 (11.2% of all; 32.3% of HER2-negative group), and 67 cases scored 1+ (23.6% of all; 67.6% of HER2-negative group). Thirteen cases were not available for review, and 15 were excluded because of suboptimal HER2 tissue control (Fig. 1). Ultimately, 71

Table 1 HER2 immunohistochemistry scoring criteria.

	HER2 immunohistochemistry score		
Current criteria	0		1+
	No staining or incomplete faint/barely perceptible membranous staining in up to 10% of tumor cells		Incomplete faint/barely perceptible membranous staining in >10% of tumor cells
Study criteria	Absent	Very low	1+
	No staining observed	Incomplete faint/barely perceptible membranous staining in up to 10% of tumor cells	Incomplete faint/barely perceptible membranous staining in >10% of tumor cells

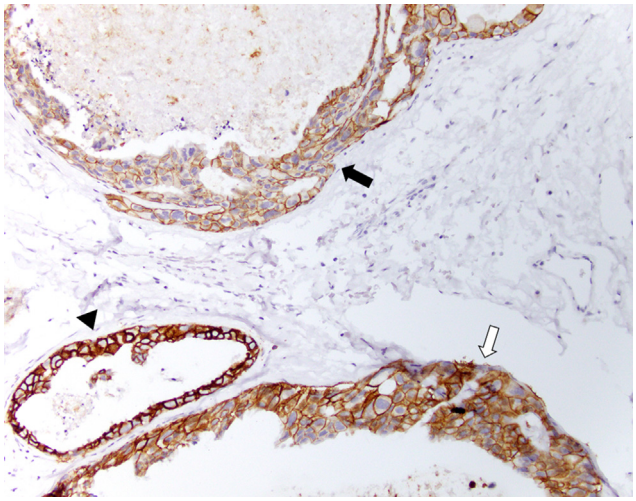

Fig. 1 HER2 immunohistochemistry control quality: This was considered a suboptimal control with heterogeneous staining results. One duct shows appropriate 3+ staining with strong, complete membranous staining (arrowhead). The duct above shows weak to moderate intensity staining in a mostly incomplete membranous pattern, compatible with a 1+ score (filled arrow). The duct below shows a 2+ pattern with moderate intensity membranous staining that is mostly complete (open arrow).

cases were included in the study, with 16 cases originally HER2(0) (22.5% of HER2-negative group) and 55 cases HER2(1+) (77.5%).

The first round of rescoring HER2 at 200× magnification resulted in 7 HER2(absent) cases (9.9% of HER2-negative group), 21 HER2(very low) cases (29.6%), and 43 HER2(1+) cases (60.5%) (Table 2). Second round rescoring at 400× magnification (Fig. 2) resulted in 1 HER2(-absent) case being recategorized as HER2(very low), and 7 HER2(very low) cases being recategorized as HER2(1+), resulting in the following final distribution: 6 cases (8.4%) HER2(absent), 15 (21.2%) HER2(very low), and 50 (40.4%) HER2(1+).

Comparison of rescoring with the initial scoring for biopsies showed a Cohen's kappa value of 0.6176 (substantial agreement) at 200× magnification. The kappa value increased to 0.8184 (almost perfect agreement) after rescoring at 400× magnification (Table 2).

3.2. Excisions

The breast core biopsies with invasive carcinoma in our cohort yielded 61 subsequent breast excision specimens. Seven cases were not available for review, and 3 were excluded because of poor HER2 tissue control quality. Ultimately, 51 excisions were included in our study. Sixteen cases were originally scored HER2(0) (31.3% of all; 37.2% HER2-negative group), 27 cases HER2(1+) (52.9% of all; 62.8% HER2-negative group), and 8 cases HER2(2+) (15.7% of all). All HER2(2+) cases underwent HER2 FISH testing, and none showed amplification of *ERBB2*.

First-round rescoring of the 51 excision specimens at 200× resulted in the following scores: 7 cases HER2(-absent) (13.7% of all; 15.6% HER2-negative group), 18 cases HER2(very low) (35.3% of all; 40.0% HER2-negative group), 20 cases HER2(1+) (39.2% of all,

Table 2 HER2 immunohistochemistry scores in biopsies.

Initial score	**n**	***Re-score***	**n (at 200x)**	**n (at 400x)**
HER2(0)	16 (22.5%)	HER2(absent)	7 (9.9%)	6 (8.4%)
		HER2(very low)	21 (29.6%)	15 (21.2%)
HER2(1+)	55 (77.5%)	HER2(1+)	43 (60.5%)	50 (70.4%)
		Kappa value	0.6176	0.8184

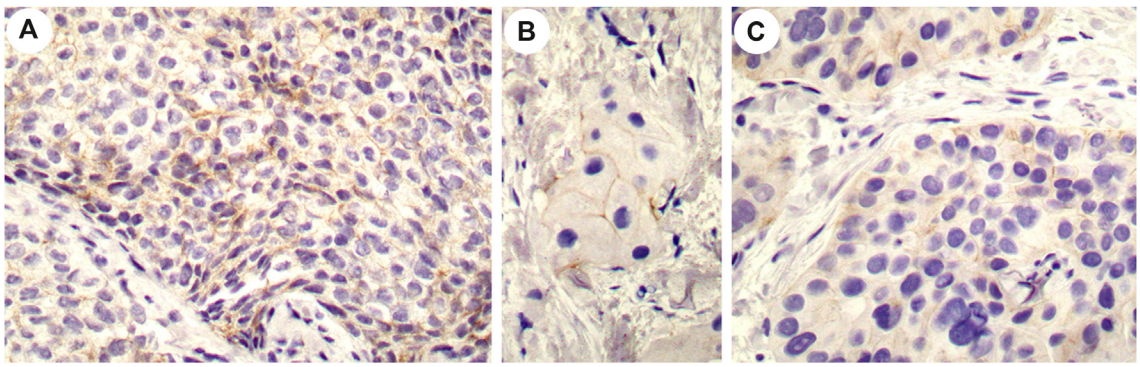

Fig. 2 Representative images of HER2 results by immunohistochemical staining at 400× magnification: A, Breast carcinoma with low HER2 score (ie, 1+ staining). B and C, Breast carcinomas with very low HER2 expression with only rare cells showing faint/weak partial membranous staining, involving fewer than 10% of tumor cells.

Table 3 HER2 Immunohistochemistry scores in excisions.

Initial score	n	*Re-score*	n (at 200x)	n (at 400x)
HER2(0)	16 (31.3%)	HER2(absent)	7 (13.7%)	5 (9.8%)
		HER2(very low)	18 (35.3%)	15 (29.4%)
HER2(1+)	27 (52.9%)	HER2(1+)	20 (39.2%)	25 (49.0%)
HER2(2+)	8 (15.7%)	HER2(2+)	6 (11.7%)	6 (11.7%)
		Weighted Kappa value	0.7154	0.8691

44.4% of HER2-negative group), and 6 cases HER2(2+) (11.7% of all) (Table 3). Second-round rescoring at 400× of HER2(absent) and HER2(very low) resulted in 2 HER2(absent) cases recategorized as HER2(very low), and 5 HER2(very low) recategorized as HER2(1+). The final HER2 score distribution was: 5 cases HER2(absent) (9.8% of all; 11.1% HER2-negative group), 15 cases HER2(very low) (29.4% of all; 33.3% HER2-negative group), 25 cases HER2(1+) (49.0% of all; 55.6% HER2-negative group), and 6 cases HER2(2+) (11.7%).

Comparison of HER2 rescoring and the initial scoring for excisions showed weighted kappa value of 0.7154 (substantial agreement) at 200× magnification, and 0.8691 (near perfect agreement) at 400× magnification.

Clinical data are provided for 77 patients (71 with biopsies and 6 resections whose biopsies were not evaluated in the study). The patients ranged in age from 26 to 90 years (mean 57.6, median 58). All patients except one were women. All were breast primaries, except for 2 recurrences. Other than 3 invasive lobular carcinomas, 1 mucinous carcinoma, and 1 apocrine carcinoma, all were invasive ductal carcinoma, and were of the following grades: grade 1 (n = 16), grade 2 (n = 34), and grade 3 (n = 27). Tumor size and focality were included only for resections: they ranged from 0.2 to 3.3 cm (mean 1.3, median 1.2) and 14 were multifocal cases. Fifty-two were ER+/PR+/HER2-, 17 were ER-/PR-/HER2-, 7 were ER+/PR-/HER2-, and 1 was ER-/PR+/HER2-. Oncotype risk scores were available for 27 and they were distributed as follows: ≤15: 13, 16−25: 7, and ≥26: 7.

4. Discussion

Currently, only HER2 IHC scores of 2+ and 3+ are clinically significant in invasive breast cancer and correspond to potentially significant benefit with targeted anti-HER2 treatment. However, more recent data have shown that patients with HER2(2+) breast cancers without *ERBB2* gene amplification, and those with HER2(1+) expression, conditions considered "low HER2 expression," may also experience advantage when treated with novel anti-HER2 antibody−drug conjugates [4−6]. There has not been a clinical emphasis on accurately distinguishing groups within the "HER2-negative" breast cancer subtype as they have not been eligible for anti-HER2 therapy, and significant discrepancies have been reported in pathologist classification of HER2 IHC scores 0 and 1+ [7,11]. With major therapeutic advances using anti-HER2 antibody−drug conjugates against tumors with low HER2 expression (ie, HER2(2+) without *ERBB2* amplification and HER2(1+)), the binary approach to breast cancer HER2 status used to date (ie, positive or negative) would be insufficient. HER2 protein expression exists along a spectrum, and all breast epithelial cells, neoplastic and benign, contain some level of HER2 cell surface expression [12]. Accurate scoring and reporting of low HER2 expression by tumors is now needed. The use of different criteria for HER2 IHC evaluation in other tumor types (eg, gastric, gynecologic, colorectal) illustrates the heterogeneity in thresholds for HER2 positivity and their clinical significance [13,14]. Providing more detailed reporting of tumor HER2 expression, similar to reporting the percentage of PD-L1 expression by tumors in 5−10% increments, may prove useful as new anti-HER2 therapeutics are investigated and found to provide benefit.

We identified 99 HER2-negative cases in a cohort of 284 invasive breast carcinomas diagnosed on biopsy specimens. Sixty-seven (23.6% of all) were HER2(1+), a frequency similar to those reported by others [1,15,16]. Of the 99 HER2-negative cases, 61 subsequent breast excisions were identified and HER2 scores for all HER2-negative biopsies and their excisions were re-evaluated. Twenty-eight biopsies and 10 excisions were eliminated because of poor control quality, a low number of tumor cells, or slide availability. Published criteria for HER2 IHC scoring were strictly adhered to, and it was important to verify appropriately strong staining of the HER2(3+) on-slide control. In tumors showing at least HER2(2+) by IHC, the control quality may not be as consequential for anti-HER2 therapy eligibility. However, to evaluate cases with "low" and "very low" HER2 expression in our study, assuring good quality HER2(3+) control staining was paramount to maximize test sensitivity. Clinical work-up of a subset of study cases coincided with various peaks of the COVID-19 pandemic when laboratory workflow was significantly disrupted, which may explain suboptimal HER2 control results during this time frame.

We reviewed HER2-negative biopsies and excisions to determine if rescoring with strict adherence to published scoring criteria would alter the original HER2 scores.

Furthermore, we subdivided the current HER2(0) category into 2 categories for this study: HER2(absent) for those with complete lack of HER2 expression and created a HER2(very low) category to capture cases with focal HER2 expression that did not meet the threshold for HER2(1+). Since the exact lower boundary of "low-HER2" expression that could derive benefit from anti-HER2 therapy remains undefined, it may become critical to distinguish between tumors with complete lack of HER2 expression and those with incomplete faint/barely perceptible membranous staining in up to 10% of tumor cells. Both these scenarios currently fall under HER2(0) in the current ASCO/CAP guidelines, but the latter HER2(very low) group may be shown to have some clinical response.

Upon rescoring at 200× magnification using our proposed HER2(absent) and HER2(very low) categories, we identified a significant subset of HER2(0) cases with "very low" levels of HER2 expression: Of the 16 HER2(0) biopsies (22.5% of HER2-negative), only 7 (9.9% of HER2-negative) were rescored as HER2(absent) and similarly, of the 16 HER2(0) excisions (37.2% of HER2-negative), 7 (15.6% of HER2-negative) were rescored as HER2(absent). Use of 400× magnification is recommended in current guidelines to identify faint membranous staining [1] and in this study up-scoring occurred when cases were re-evaluated under 400× magnification, resulting in shifts from HER2(absent) to HER2(very low), and HER2(very low) to HER2(1+). We also noted improved concordance between initial scoring and rescoring when samples were assessed at 400×, as opposed to 200× magnification, further supporting the use of 400× magnification for accurate scoring. Overall, at least one-fifth of breast cancers were HER2(1+) in our study, representing a substantial proportion of HER2-low cases that would be eligible for anti-HER2 therapy. In addition, within the category of cases currently considered HER2(0), there was a large subset with "very low" HER2 expression. Scoring of HER2(very low) cases was quite feasible: once a case was judged as insufficient for meeting the HER2(1+) threshold, it was straightforward to separate those with total lack of staining from those with limited staining.

In our cohort, rescoring of HER2 IHC did not result in major changes in number of HER2(1+) cases (77.5% versus 70.4% in HER2-negative biopsies, and 62.8% versus 55.6% for HER2-negative excisions; Tables 2 and 3). We found that of cases with initial HER2(0) score, 37.5% (n = 6) of biopsies and 31.3% (n = 5) of excisions lacked any staining for HER2 by IHC upon re-scoring. Furthermore, it was straightforward to identify a substantial subset exhibiting "very low" staining in this study. These findings have not been previously reported in the literature. It will be critical for future studies to elucidate the biological relevance of this HER2(very low) group and determine whether such breast cancers may also derive therapeutic benefit from novel anti-HER2 agents. Faint staining and sample heterogeneity may cause challenges in scoring tumors with "very low" HER2 expression; this may be an area in which digital pathology may be helpful if these expression levels are found to be clinically significant.

Since the recent reports of low-HER2 breast cancers responding to novel chemotherapeutics, there have been several studies focusing on the clinicopathologic features and genetic profiles of these tumors, although additional work is needed to clarify the associated pharmacologic implications [17–20]. As our understanding of breast cancer HER2 expression as a spectrum is enriched and therapeutic implications are investigated, clinical guidelines and the pathologist approach to HER2 scoring may evolve. A possible component of reporting based on this study would be to stratify cases with less than 1+ staining into 2 groups: those completely lacking HER2 expression and those with "very low" expression. Further clinicopathological studies are needed to better understand the "very low" end of HER2 expression spectrum and its therapeutic relevance.

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