Utility of Human Epidermal Growth Factor Receptor 2 (HER2) Retesting of Histologic Grade 3 Invasive Breast Carcinomas

Rebecca Gologorsky, MD[1]; Elizabeth Cureton, MD[2]; Veronica Shim, MD, FACS[2]

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ABSTRACT

Background: American Society of Clinical Oncology and College of American Pathologists guidelines recommend repeated evaluation of human epidermal growth factor receptor 2 (HER2) status on surgical specimens from patients with a diagnosis by core-needle biopsy of Grade 3, HER2-negative invasive tumors of the breast. However, there are limited data to support reflexive testing.

Objective: To evaluate the utility of HER2 retesting of histologic Grade 3, HER2-negative invasive breast carcinomas.

Methods: We evaluated 78 patients from Kaiser Permanente East Bay in whom Grade 3, HER2-negative invasive breast carcinoma was diagnosed between 2015 and 2017 by core biopsy, to compare HER2 status on core biopsy vs excisional biopsy specimen. The HER2 status was determined by immunohistochemistry, fluorescent in situ hybridization, or both. All patients were retested for HER2 status on surgical specimen according to the aforementioned guidelines. Recipients of neoadjuvant chemotherapy were excluded.

Results: One of the 78 patients demonstrated negative-to-positive status discordance between core biopsy and surgical specimens and was treated with trastuzumab. One patient was HER2 negative by core biopsy and was HER2 equivocal by immunohistochemical and fluorescent in situ hybridization evaluation of the surgical specimen. Seventy-six patients demonstrated concordant HER2 status between core biopsy and surgical specimens.

Conclusion: The rate of clinically significant HER2 status discordance between core biopsy and surgical specimens in patients with Grade 3 breast carcinoma is low. However, given the dramatically improved survival conferred by trastuzumab therapy, our findings support reflex HER2 testing of surgical specimens for patients with core biopsy-diagnosed HER2-negative breast carcinoma.

INTRODUCTION

Determination of human epidermal growth factor receptor 2 (HER2) status in invasive breast cancers is crucial for prognostication and treatment. Patients with HER2-positive tumors are eligible for targeted therapy with trastuzumab, and trastuzumab-treated patients have improved disease-free survival and overall survival regardless of stage.[1] In 2013, revised guidelines for HER2 testing in breast cancer were published jointly by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP). The purpose of these updates was to improve the accuracy of HER2 testing, thereby enhancing its use as a prognosticator and therapeutic marker.[2] Among these guidelines was a recommendation to repeat HER2 testing on the excised (surgical) biopsy specimen for patients with a diagnosis on core-needle biopsy specimen of Grade 3 invasive carcinoma of the breast. This recommendation was supported by data demonstrating that HER2 expression is associated with more aggressive tumor subtypes: Estrogen receptor (ER)-negative tumors, presence of nodal disease, and high-grade disease. Furthermore, for ER-positive tumors, HER2 status correlates with increased grade. The implication of these findings is an increased false-negative HER2 status in high-grade tumors.[3,4]

The accuracy of biomarker and grade testing on core biopsy vs excisional biopsy specimen has also been evaluated in numerous studies. A high concordance rate, approaching 100%, has been described for ER status, but rates of approximately 85% and 75% have been reported for progesterone-receptor status and histologic malignancy grade, respectively.[5] Reported rates of concordance of HER2 in core vs surgical biopsy specimen have been variable, with false-positive rates as high as 50% in one report,[6,7] whereas others have demonstrated high levels of concordance when excluding specimens with borderline HER2 expression or equivocal results.[5,8,9] The concordance of HER2 status in Grade 3 tumors between core biopsy specimen and surgical excisional biopsy specimen is poorly described.

We evaluated all patients at our institution with a diagnosis of HER2-negative, Grade 3 invasive carcinoma of the breast between 2015 and 2017, and we compared the concordance of HER2 status between core biopsy and surgical specimen to determine the utility of reflex retesting of HER2 status.

METHODS

Patients at Kaiser Permanente East Bay in California were included in the study if they had a diagnosis between 2015 and 2017 on core needle biopsy of HER2-negative invasive carcinoma of the breast, with Grade 3 tumors based on core biopsy specimen or surgical specimen. The population treated at this institution represents the ethnic makeup of the state of California, with ethnic minority groups heavily represented (Table 1). Although these patients are insured and therefore generally have adequate access to health care, the group is clinically diverse, with a typical variety and rate of medical comorbidities such as hypertension, type 2 diabetes, and cardiovascular disease. Exclusion criteria included patients who received neoadjuvant chemotherapy, to avoid receptor status changes secondary to treatment effect, and those who did not undergo reflexive HER2 testing of surgical

Author Affiliations

[1] University of California, San Francisco-East Bay, Oakland, CA

[2] Kaiser Permanente Medical Center, Oakland, CA

Corresponding Author

Veronica Shim, MD, FACS (veronica.shim@kp.org)

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specimens (Figure 1). Patient and tumor characteristics are summarized in Table 1.

The HER2 testing was performed in accordance with ASCO/CAP guidelines via immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH). Specimen testing was performed at the Kaiser Permanente Northern California (KPNC) Regional Laboratory, Richmond, CA. Immunohistochemistry was performed using anti-HER2/neu clone 4B5 rabbit monoclonal antibodies (Ventana Medical Systems, Tucson, AZ) according to the guidelines validated by ASCO and CAP. The IHC was interpreted by board-certified pathologists using ASCO and CAP guidelines. On all equivocal specimens, FISH was performed using the Dako HER2 IQ FISH Kit (NeoGenomics Laboratories Inc, Fresno, CA, a CAP-accredited laboratory), following ASCO and CAP guidelines. All IHC and FISH cases were evaluated by 2 board-certified pathologists. Tumor grade scoring was performed by board-certified surgical pathologists. Two or more pathologists evaluated and reached consensus on each specimen.

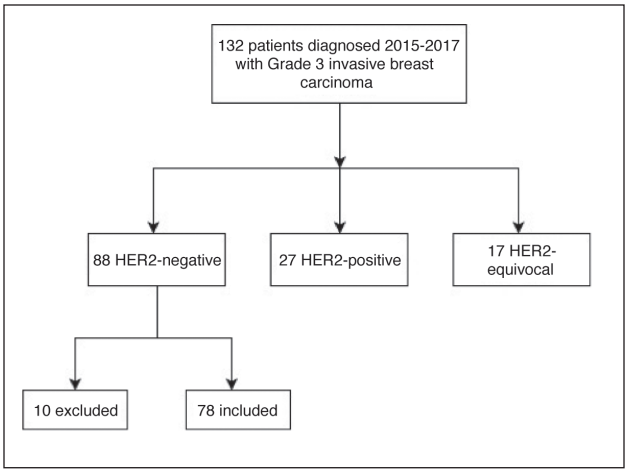

Figure 1. Patient selection.
HER2 = human epidermal growth factor receptor 2.

Table 1. Patient and tumor characteristics of patients with HER2-negative tumors by core biopsy (N=78)[a]

Characteristic	No. (%)
Median age, y (range)	60 (29-85)
Race/ethnicity	
White	31 (40)
Black/African American	27 (35)
Asian/Asian American	11 (14)
Hispanic/Latina	8 (10)
Declined to state	1 (1)
Genetic testing	
Yes	39 (50)
No	39 (50)
Genetic mutation identified	
BRCA1	4
BRCA2	1
CHEK2	1
RAD50/51	2
MUTYH	1
Surgical intervention[b]	
Lumpectomy	48 (62)
Simple mastectomy	15 (19)
Nipple-sparing mastectomy	6 (8)
Modified radical mastectomy	9 (12)
Hormone receptor status	
ER/PR negative	37 (47)
ER/PR positive	24 (31)
ER positive	41 (53)
PR positive	26 (33)
Grade on core biopsy	
1	0 (0)
2	21 (27)
3	57 (73)

Characteristic	No. (%)
Lymphovascular invasion	
Yes	32 (41)
No	38 (49)
Indeterminate	5 (6)
Not reported	3 (4)
T status (TNM staging system)	
T1a	6 (8)
T1b	5 (6)
T1c	16 (21)
T2	42 (54)
T3	7 (9)
T4	2 (3)
N status (TNM staging system)	
N0	50 (64)
N1	14 (18)
N2	7 (9)
N3	5 (6)
NX	2 (2)
Cancer stage	
1A	21 (27)
1B	0 (0)
1C	1 (1)
2A	32 (41)
2B	8 (10)
3A	7 (9)
3B	1 (1)
3C	4 (5)
4	4 (5)

[a] Percentage is expressed as percent of total patients for whom the given data point is available. Percentages may not total to 100% because of rounding.
[b] Two patients underwent bilateral mastectomy, both of whom had a *BRCA1* mutation.
ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PR = progesterone receptor; TNM = tumor, node, and metastases.

Table 2. Histologic characteristics of discordant specimens[a]

Case number	Core biopsy			Surgical biopsy			Treatment	
	IHC	FISH	Interpretation	IHC	FISH	Interpretation	Trastuzumab	Surgery
1	1+	N/A	Negative	2+	2.1	Positive	Yes	NSM
2	1+	N/A	Negative	1-2+	0.9	Equivocal	No	NSM

[a] Specimens with discordant human epidermal growth factor receptor 2 results between core biopsy and excision.
FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; N/A = not available; NSM = nipple-sparing mastectomy.

RESULTS

Two patients were excluded on the basis of receiving neoadjuvant chemotherapy, and 1 patient was excluded because of lack of residual disease in the surgical specimen. The analysis included 78 patients, whose median age at diagnosis was 60 years (range = 29-85 years). Most patients (62%) elected to undergo lumpectomy, and almost one-fifth of patients (19%) underwent simple mastectomy. Half of patients underwent genetic testing (n = 39), 9 (23%) of whom demonstrated at least 1 genetic mutation associated with breast cancer development.

Seventy-six patients (97.5%) demonstrated HER2 status concordance between core biopsy specimen and surgical specimen. Two (2.5%) of the 78 patients demonstrated HER2 status discordance between core biopsy specimen and surgical specimen. One patient (1.3%) was HER2 positive by surgical specimen testing and was ultimately treated with trastuzumab. Interestingly, this patient was also positive for a *BRCA1* mutation and underwent bilateral nipple-sparing mastectomy. The second patient was HER2 equivocal on IHC and FISH testing of the surgical specimen and underwent unilateral nipple-sparing mastectomy. Characteristics, including IHC and FISH results, of the 2 discordant cases are listed in Table 2.

Tumor was upgraded from Grade 2 to Grade 3 in 21 patients (27%), all on the basis of mitotic count. None of the cases demonstrated morphologic heterogeneity.

DISCUSSION

Determination of HER2 status in breast carcinoma is essential for prognostication and treatment planning, and guidelines are continually reviewed by ASCO and CAP to improve the accuracy of testing. The newest ASCO/CAP consensus published in 2013 recommends HER2 testing of all invasive breast carcinomas initially and retesting of all HER2-negative, Grade 3 tumors; core biopsy specimen with equivocal result; surgical specimens containing carcinoma that is morphologically different from the core biopsy specimen; or if the amount of invasive tumor in the core biopsy specimen is small.[2] The recommendation for reflexive HER2 testing of HER2-negative, Grade 3 tumors is based on higher rates of HER2 positivity among high-grade tumors and, therefore, a theoretical higher false-negative rate among these specimens.[10] However, there are few data on the utility of reflex retesting of HER2-negative, Grade 3 carcinomas. Here, we report the results of HER2 retesting for all patients in whom HER2-negative, Grade 3 breast carcinoma was diagnosed between 2015 and 2017. Of 78 patients, 1 showed HER2 amplification on repeat testing of the surgical specimen, translating to a discordance rate of 1%. The core biopsy from this patient was strongly negative by IHC, and the excisional biopsy specimen was HER2 positive by FISH (Table 2). This patient was treated with trastuzumab.

Prendeville et al[6] described 100 patients with HER2-negative breast carcinomas diagnosed by core needle biopsy and Grade 3 disease diagnosed by surgical specimen whose HER2 status was reevaluated on excision. Three of 100 showed a change in HER2 status, 2 of whom were ultimately treated with HER2-directed chemotherapy. Their findings are consistent with those in our cohort, and the conclusion reached by these authors was against reflective testing because of low rates of discordance.

Concordance between core biopsy and surgical specimens with HER2-positive specimens was also examined in this study. The median age for this group of patients was notably lower at 53 years (range = 30-81 years), and this group had a greater percentage of low-stage tumors. Two patients had genetic mutations associated with breast cancer; however, only 26% of the HER2-positive patients underwent testing. Surgical management was comparable between the HER2-negative and HER2-positive patients. Interestingly, of the 6 patients whose surgical specimens were tested (6/27), 1 demonstrated positive-to-negative discordance. This patient received trastuzumab because of initial HER2 positivity.

Given the morbidity and mortality associated with improper HER2 status designation and the significant survival benefit from appropriate administration of HER2-targeted therapy, these data support current ASCO recommendations to retest all patients with HER2-negative, Grade 3 tumors diagnosed on core biopsy.

A greater understanding of factors associated with or influencing HER2 status is necessary to address the utility of tumor marker retesting, as are factors contributing to tumor heterogeneity. Improved understanding of these characteristics would enable a more educated study of tumor marker testing and an ability to determine in which set of patients reflexive testing is most valuable. Additionally, there is a degree of variability among pathologist determinations of grade in breast cancer. The Nottingham Grading System, which is based on tubule formation, nuclear pleomorphism, and mitotic rate, is the most widely adopted protocol in the US for determination of tumor grade. Overall agreement for any grade has been variably reported with $\kappa = 0.4$ to 0.7, and better κ for Grade 1 and Grade 3 tumors (both 0.7).[11,12] Grade concordance between core biopsy and surgical specimens is also variable. Upgrade is seen in approximately 30% to 40% in tumors described as Grade 1 or 2 on core biopsy specimen; however, a change of Grade 1 to Grade 3 is unlikely (approximately 1%), as is a downgrade from Grade 3 (approximately 5%).[12] These data are consistent with the cohort

represented here, which demonstrated a 27% rate of upgrade from Grade 2 to Grade 3, and 0% upgrades from Grade 1 to 3 or downgrades from Grade 3. Improvement in standardization of grading protocol and tissue sample handling has increased interobserver agreement of grade determination.[11]

This study is limited by a small population size and demographic characteristics that are approximately representative of California, which is 1 of 2 states with ethnic minorities making up greater than 50% of the population and significantly higher rates of Asian American and Hispanic Americans vs the remainder of the US. This may affect the generalizability of these findings. Additionally, the cost of repeated testing to patients, hospital systems, and taxpayers is unclear. A cost-benefit analysis would provide necessary objective data on the utility of retesting. A study by Solomon et al[13] addressing a similar issue evaluated the cost-benefit of retesting the HER2 status by FISH for all specimens initially evaluated by IHC and found that 10% of HER2-amplified tumors would have been missed by retesting only IHC-equivocal specimens. The authors concluded there is a cost benefit to retesting all specimens by FISH.[13]

CONCLUSIION

The number of publications evaluating HER2 retesting is limited, and the size of the population described here is small. Further study is needed to more thoroughly validate reflexive HER2 testing guidelines and to elucidate the cause of discordance. ❖

Disclosure Statement

The author(s) have no conflicts of interest to disclose.

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