CME/SAM

HER2 Testing of Multifocal Invasive Breast Carcinoma

How Many Blocks Are Enough?

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ABSTRACT

Objectives: *To determine the diagnostic yield of testing multiple blocks for HER2 in cases of multifocal breast carcinoma.*

Methods: *We identified 246 consecutive cases of multifocal invasive breast carcinoma in which HER2 was tested on more than 1 tumor focus. We performed an audit of all cases with respect to tumor size, grade, and histologic type.*

Results: *HER2 status was concordant between multiple foci in 230 (93.5%) of 246 cases, with the largest focus having the most positive HER2 result in 242 (98.4%) of 246 cases. We did not find a single case in which a smaller focus demonstrated a more positive HER2 status unless this focus was either higher grade or different histologically.*

Conclusions: *Our findings support the evaluation of HER2 on the largest focus, with additional testing on smaller foci having a different histologic type or higher grade.*

Upon completion of this activity you will be able to:
- outline the evidence-based best practices for HER2 testing in multifocal breast carcinoma.
- apply current evidence in decisions concerning biomarker testing in multifocal breast carcinoma.

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Based on phenotypic and clonality studies, multifocal invasive breast carcinoma may arise from intratumoral spread of a single clone or, less commonly, as synchronous independent primary tumors.[1-5] The incidence of multifocal breast carcinoma is rising, in part due to advances in preoperative imaging.[6] The prognostic significance of multifocality is unclear at this time. Although multifocality correlates with a higher disease burden,[7-9] current evidence supports conventional staging according to the largest single invasive focus.[10,11]

The human epidermal growth factor gene (*ERBB2*) on chromosome 17 encodes a receptor tyrosine kinase oncoprotein; both gene and protein are commonly called HER2. When overexpressed in breast cancer, HER2 is of prognostic and predictive significance, warranting routine clinical testing by immunohistochemistry and/or in situ hybridization.[12-14] In multifocal tumors, the HER2 status is most commonly determined by testing the largest (ie, highest stage) invasive tumor focus.[14,15] Whereas hormone receptors tend to show only minor differences between multiple tumor foci,[5,16] the significance of HER2 differences between foci is less certain.

Although the handling of multifocal tumors is not addressed in the current (2007) American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines,[14] the CAP Cancer Protocol recommends testing HER2 in additional smaller foci if they are of different histologic type or grade and reporting these findings as "additional pathologic findings."[15] The evidence supporting the HER2 practice guidelines is conflicting. In a small subset of cases, there may be significant heterogeneity among tumor foci[17,18]; however, testing all tumor foci in every case does not appear to be an efficient approach.[5,16] The purpose of this study is to audit retrospectively the diagnostic yield of testing multiple blocks for HER2. A secondary aim is to assess the effectiveness of the current testing guidelines in the setting of multifocal disease.

Materials and Methods

Case Selection

In accordance with the protocol approved by the research ethics board of Mt Sinai Hospital, we conducted a search for cases of breast carcinoma between 2006 and 2011 in which multiple tumor blocks were tested for HER2. Cases were included if there was a clearly reported new diagnosis of breast carcinoma with multifocal ipsilateral invasive foci and if multiple foci were tested from the same excisional (ie, wire localization, "lumpectomy," or mastectomy) specimen. For this purpose, any ipsilateral foci exceeding 0.1 cm in the greatest dimension separated by normal breast tissue (either by gross or microscopic report) were considered independent foci. All cases reporting more than 1 independent focus were considered to have "multifocal" carcinoma. Core biopsy specimens, microinvasive foci, and blocks from metastases (eg, axillary lymph nodes and other organs) were excluded. Also excluded were cases in which testing was performed on a tumor recurrence or previously treated cancer (eg, neoadjuvant cases). A total of 246 consecutive cases meeting these criteria were identified.

HER2 Testing

The cases selected represented tumors tested from intramural material and referral material; however, all cases were tested for HER2 at the same central reference laboratory using licensed and validated reagents and methods. HER2 interpretation was performed by a group of 3 reference pathologists (M.C.C., J.B.M., and 1 other). Although the actual antibody used for HER2 immunohistochemistry varied during the period investigated (including CB11 [Vector Laboratories, Burlingame, CA], A0485 [DAKO Canada, Burlington, Canada], and SP3 [Epitomics, Burlingame, CA]), for any given case of multifocal tumor, the same method was used for all tumor blocks. Cases that were equivocal by HER2 immunohistochemistry were retested by fluorescence in situ hybridization (FISH) in the same central laboratory (Vysis probes; Abbott Laboratories, Des Plaines, IL).

Data Collection

All pathology reports and HER2 testing results were obtained, including the original report from the referring institution where applicable. The following data elements were recorded for each focus of invasive tumor: size, Nottingham histologic grade, histologic type (and other histologic features if available), hormone receptor status, and HER2 results. When cases were tested by confirmatory FISH, the ratio of HER2 signals to the chromosome 17 centromeric probe (CEP17) was also recorded. Although the ASCO/CAP guidelines[14] consider a HER2/CEP17 ratio between 1.8 and 2.2 "equivocal," earlier trastuzumab trials considered a ratio of more than 2.0 eligible for treatment.[19] In our comparison of HER2 status between foci tested by FISH, a HER2/CEP17 ratio of more than 2.0 was considered "positive," and a ratio of up to 2.0 was considered "negative." Thus, all cases could be classified as "concordant"—all foci having the same HER2 status—or "discordant"—with at least 1 focus being HER2 positive while other foci were HER2 negative. For all discordant cases, H&E-stained slides and corresponding HER2 immunohistochemistry slides were retrieved and reviewed by the authors (G.C.B. and M.C.C.) to confirm the accuracy and completeness of the reported data. Descriptive statistics were used to examine the data.

Results

Features of Multifocal Invasive Breast Carcinomas

A total of 246 cases of ipsilaterally multifocal invasive breast carcinoma were included in the analysis. The main characteristics of these cases are summarized in **Table 1**. Of all cases, 198 (80.5%) had 2 foci, 33 (13.4%) had 3 foci, and 15 (6.1%) had 4 or more foci of invasive tumor. In cases with more than 2 foci, not every focus was necessarily tested for HER2. Our selection criteria identified all cases in which 2 tumor foci were present, and both tumor foci were tested. Depending on referring requests, a varying selection of blocks was tested in cases with 3 or more tumor foci. Overall, 2 blocks were tested in 219 (89.0%) cases, 3 blocks in 20 (8.1%) cases, and 4 or more in 7 (2.8%) cases. In some cases (74/246), testing of the additional focus or foci would be recommended under the CAP Cancer Protocl.[15] The reason for testing in the remaining cases was unknown (see Discussion).

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Table 1
Characteristics, Including HER2 Results, of 246 Multifocal Breast Carcinoma Cases in Which More Than 1 Block Was Tested for HER2[a]

Case Characteristics	Value
Total No. of cases	246 (100.0)
Number of invasive foci	
2	198 (80.5)
3	33 (13.4)
4 or more	15 (6.1)
Size of tumor foci, cm	
Largest focus (of 246 cases)	2.1 ± 1.3
Second-largest focus (of 246 cases)	1.1 ± 0.7
Third-largest focus (of 48 cases)	0.8 ± 0.5
Fourth-largest focus (of 15 cases)	0.6 ± 0.3
Histologic types	
Ductal (no special type), all foci	196 (79.7)
Invasive lobular, all foci	17 (6.9)
Other special type, all foci	4 (1.6)
>1 Foci with different types	29 (11.8)
Focus having the highest grade	
Largest focus	237 (96.3)
Smaller focus	9 (3.7)
Number of blocks tested	
2	219 (89.0)
3	20 (8.1)
4 or more	7 (2.8)
Testing of multiple blocks recommended by current protocol?	
Yes	74 (30.1)
No	172 (69.9)
HER2 results (IHC ± FISH)	
Negative, all foci	196 (79.7)
Positive, all foci	31 (12.6)
Equivocal, all foci	3 (1.2)
Discordant results between blocks	16 (6.5)

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.
[a] Values are presented as number (%) or mean ± SD.

With respect to histologic type, 196 (79.7%) cases were invasive ductal carcinomas (of no special type) at all foci. In 11 of these, there were varying histologic patterns of invasive ductal carcinoma at different foci (eg, solid glandular growth vs mixed glandular or scirrhous features). One of these 11 cases had a discordant HER2 result (see below). In 17 cases, there were multifocal invasive lobular carcinomas at all foci, whereas in 4 cases, the tumor was of another special type at all foci. A total of 29 (11.8%) cases had different histologic types at different foci: 18 cases had both invasive ductal and invasive lobular carcinoma, and 11 cases had both invasive ductal carcinoma and a special-type carcinoma (including tubular, mucinous, metaplastic, medullary, and invasive papillary carcinoma).

A subset of the cases was referred for confirmatory HER2 testing only. As such, hormone receptor status was available for 211 (85.8%) of 246 cases. Of these 211 cases, estrogen receptor (ER) was positive in at least 1 focus in 180 (85.3%), low positive in 4 (1.9%), and negative in 27 (12.8%). Of the 211 cases, progesterone receptor (PR) was positive in 154 (73.0%), low positive in 11 (5.2%), and negative in 46 (21.8%). Overall, ER and PR results were concordant between foci in 205 (97.2%) of 211 cases. Three of the 6 discordant cases also showed discordant HER2 results between tumor foci (see below).

Audit of Discordant Cases

Of all cases, only 16 (6.5%) had discordant HER2 results between blocks tested. The features of these cases are summarized in **Table 2**. Testing of the largest focus yielded the most positive HER2 result in 242 (98.4%) of 246 cases. In 3

Table 2
Audit of 16 Cases of Multifocal Breast Carcinoma in Which HER2 Status Differed Between Multiple Blocks Tested

Discordant Case No. (n = 16)	No. of Tumor Foci Tested	No. of HER2-Positive Foci	HER2 Positive in Largest Focus?	HER2 Positive in Highest-Grade Focus?	Difference in Histologic Type?
1	2	1	Yes	Yes	Yes
2	2	1	Yes	Yes	Yes
3	2	1	Yes	Yes	No
4	2	1	Yes	Yes	No
5	2	1	Yes	Yes	No
6	2	1	Yes	Yes	No
7	2	1	Yes	Yes	No
8	2	1	Yes	ND	Yes
9	2	1	Yes	ND	Yes
10	2	1	Yes	ND	No
11	2	1	Yes	ND	No
12	4	1	Yes	ND	No
13	2	1	No	ND	Yes[a]
14	2	1	No	ND	Yes[a]
15	2	1	No	ND	Yes[a]
16	2	1	ND[b]	ND[b]	No[b]

ND, no difference in tumor size or grade.
[a] Cases 13 to 15 were HER2 positive in the smaller focus, but foci were of different histologic types.
[b] In case 16, both foci had the same size, grade, and histologic features.

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of the remaining 4 discordant cases (cases 13-15), 2 different tumor foci showed different histologic types, with the smaller focus having the most positive HER2 result. The fourth discordant case (case 16) was of 2 tumor foci with the same size, grade, and histologic type and ER/PR status (see also FISH results below). The largest tumor focus did not have the highest grade in all 246 cases (there were 9 HER2-concordant cases in which the smaller tumor had a higher grade). Nevertheless, in our series of all discordant cases with a difference in histologic grade, the tumor focus with the highest grade was also the largest.

Nine of the 16 discordant cases had invasive ductal carcinoma at all foci tested, with 1 of 9 cases showing different morphologic patterns. The remaining 7 of 16 cases had different histologic types at different foci (4 with ductal and lobular carcinomas and 3 with a ductal carcinoma and a special-type carcinoma [1 mucinous and 2 with medullary features]). Three of the 16 cases had a different ER/PR status at each focus (cases 1, 2, and 7), and all 3 cases also had differences in either histologic type or grade between foci.

HER2 Discordance in "Equivocal" Cases

In 3 of the 16 discordant cases, the final HER2 status was equivocal by FISH in at least 1 focus. In case 13, a smaller focus (invasive ductal carcinoma) was unequivocally HER2 positive, whereas the larger focus (invasive lobular carcinoma) was equivocal by immunohistochemistry and retested by FISH with a HER2/CEP17 ratio of 1.8. In the other case (case 12), the largest of 4 foci was equivocal by immunohistochemistry and retested by FISH with a HER2/CEP17 ratio of 2.1, whereas the 3 smaller foci were unequivocally HER2 negative.

One additional case (case 16) showed a discrepancy in which the most positive HER2 result was not in a larger focus or in one with a different grade or histologic type. In fact, this case consisted of 2 foci of the same size (1.2 cm), grade (3/3), and histologic type (no special type) and hormone receptor status. Both foci were tested by FISH, with HER2/CEP17 ratios of 1.8 and 2.3. In our tabulated summary, we considered a FISH ratio of more than 2.0 as HER2 positive.

Discussion

This series of 246 cases shows that in most (93.5%) cases, testing more than 1 block from multifocal invasive breast carcinoma does not improve the diagnostic yield with respect to HER2 determination. The largest focus was representative of the most positive HER2 status in more than 98% of cases. By selecting additional blocks according to size, grade, and histologic type, the tumor focus with the most positive HER2 status would be correctly identified in 100% of our cases. By contrast, our audit shows that the routine testing of multiple (or all) foci for HER2—irrespective of size or other parameters—is relatively common and not without its proponents.[17]

Although not addressed specifically in the 2007 testing guidelines from the ASCO/CAP,[14] the CAP Cancer Protocol[15] recommends determining HER2 status from the largest tumor focus unless other foci have a different histologic type or grade. To our knowledge, our clinical case series is currently the largest to provide direct evidence supporting this practice and supplements prior evidence based on tissue microarrays from multifocal tumors.[5] Although our series was not performed prospectively, cases were selected consecutively and irrespective of the reason for testing. Almost 70% (n = 172) cases in our series were tested for an unknown reason (or as routine)—that is, multiple foci were not selected for testing based on clinicopathologic features. As such, there is a high likelihood that the overall case selection is representative of a range of multifocal tumors.

A modified version of the testing protocol **Table 3** fully accounted for the most positive HER2 result in all cases of our series. Nevertheless, we accept that heterogeneity can be an unpredictable phenomenon and that—rarely—scattered smaller HER2-amplified foci can exist. A recent prospective study of 113 multifocal breast carcinomas of similar histologic type[17] showed that 4 (3.5%) of 113 cases had the most positive HER2 result in a smaller tumor focus. However, the histologic features and grades of the major and minor foci were not reported. Although some authors may contend that any differences between foci support testing of multiple blocks,[17] our series shows that pathologists' judgment plays a role in selecting those cases most likely to show differences in HER2 expression.

It is a frequently held assumption that the "most positive" result determines the "most correct" treatment, even when this is present in much smaller foci. Yet the evidence supporting this assumption remains unclear since major clinical trials of targeted treatment have not taken multifocality or heterogeneity into account.[19]

The strongest clinical evidence to date suggests that small subpopulations showing higher-level HER2 within an otherwise HER2-negative tumor are not of clinical significance.[20,21]

Table 3
Proposed Criteria for Testing of Tumor Foci in Multifocal (Ipsilateral) Invasive Breast Carcinoma[a]

Testing of Multifocal Invasive Breast Carcinoma for HER2	
Largest Focus	**Other Foci**
Test 1 representative block	If same size as largest focus If higher grade If different histologic type

[a]In our series, these criteria identified the focus of the most positive HER2 status in all cases.

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However, a subpopulation with lower-level HER2 within an otherwise HER2-positive tumor may actually have adverse prognostic significance in the setting of chemotherapy treatment.[22] Additional work is needed to determine the prevalence and significance of small HER2-positive subpopulations in multifocal disease, particularly when the tumor is best classified as HER2 negative by classic criteria.

Conclusions

In most multifocal invasive breast carcinomas, a single block from the largest tumor focus is sufficiently representative of HER2 status. Testing of multiple blocks should be performed when there are histologic differences or a higher grade within smaller tumor foci. Our findings support a testing approach similar to that of the 2007 ASCO/CAP HER2 guidelines[14] and CAP Cancer Protocol.[15]

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