Breast Cancer HER2 Equivocal Cases: Is There an Alternative to FISH Testing? A Pilot Study Using Two Different Antibodies Sequentially

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ABSTRACT: **Background:** HER2 is an important prognostic and predictive marker in invasive breast cancer. It is currently assessed by immunohistochemistry for protein over-expression and by fluorescence in situ hybridization for gene amplification. The immunohistochemistry-equivocal cases (2+) are currently retested by FISH to determine eligibility for trastuzumab treatment. Retesting by FISH significantly raises the cost of patient management and sometimes delays treatment. The 4B5 is a new, FDA-approved, rabbit monoclonal antibody for HER2 testing.

Objectives: To examine the reliability of 4B5 IHC HER2 testing in cases found by CB11 IHC to be HER2 status equivocal.

Methods: Twenty-eight invasive breast cancer cases, with an equivocal HER2 status by CB11 IHC, were retested by the 4B5 antibody as well as by FISH analysis. The scoring was performed using the same guidelines as HercepTest and was correlated with the FISH ratio.

Results: Of the original 28 CB11 clone designated equivocal cases, 14 (50%) showed negative HER2 staining using the 4B5 clone (HercepTest score 0 and 1+). Five cases (18%) proved to be positive (HercepTest score 3+) and 9 cases (32%) remained equivocal (HercepTest score 2+). The corresponding FISH ratio results showed that all 4B5 negative cases were negative by FISH testing, with a negative predictive value of 100%; 4 of 5 of the 4B5-positive cases were positive by FISH testing, with a positive predictive value of 80%. One 4B5-positive case was borderline-high (2.2 ratio) by FISH. The correlation between 4B5 IHC and FISH was statistically significant ($P = 0.0013$) by chi-square test.

Conclusions: Sequential testing by 4B5 IHC could greatly reduce the need for FISH testing in cases considered HER2 equivocal by CB11 IHC.

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KEY WORDS: HER2, breast cancer, immunohistochemistry, fluorescence in situ hybridization, rabbit monoclonal antibody

FISH = fluorescence in situ hybridization
IHC = immunohistochemistry
FDA = Food and Drug Administration

The human epidermal growth factor receptor 2 gene *ERBB2* (commonly referred to as *HER2*) is amplified in approximately 18% to 20% of breast cancers [1]. HER2 over-expression is associated with clinical outcomes in patients with breast cancer [2-4]. Perhaps most importantly, several studies have now shown that agents that target HER2 are remarkably effective in both the metastatic and adjuvant settings. Trastuzumab (Herceptin®, Genentech, CA, USA), a humanized monoclonal antibody, improves response rates, time to progression, and survival when used alone or added to chemotherapy in metastatic breast cancer [5,6]. Trastuzumab is also active as a single agent [7,8] and was approved in 1998 by the U.S. Food and Drug Administration for the treatment of metastatic disease.

HER2 proto-oncogene amplification is usually accompanied by protein over-expression. The gene amplification is generally determined by the fluorescent in situ hybridization method and the protein expression is determined by immunohistochemistry. In a typical pathology laboratory, both assays are performed on formalin-fixed paraffin-embedded tissue material. In the FISH method, a labeled probe is added to the denatured patient's DNA, which recognizes the target gene and enumerates fluorescence signals that are counted using a fluorescence microscope to assess HER2 gene amplification. In contrast, immunohistochemistry is performed using monoclonal or polyclonal standardized antibody kits, which generate a colored reaction that is evaluated by bright field microscopy [9]. American Society of Clinical Oncologists/College of American Pathologists recommendations for HER2 testing have clearly defined the categories as positive (immunohistochemistry score 3+), equivocal (immunohistochemistry score 2+) and negative (immunohistochemistry score 0/1+). Among these categories, the equivocal or weakly positive (2+) category creates confusion about trastuzumab treatment; therefore, it requires an additional FISH test, as recommended by American Society of Clinical Oncologists/College of American Pathologists guidelines [10]. A few studies have compared HER2 results by immunohistochemistry and FISH. In these studies, the

greatest concordance rate is observed in immunohistochemistry score 3+ cases in which the majority showed amplification by FISH. In the equivocal 2+ cases, different studies show variable findings with a high rate of false-positive results observed by follow-up negative FISH results. In other studies, the amplification rate of HER2 in the equivocal group was approximately 25% [11], emphasizing the need for additional FISH testing. Retesting equivocal 2+ cases by FISH significantly increases the cost of patient management and delays the final report of the HER2 status. As a result, treatment decisions are also delayed.

The Ventana Medical Systems Inc. PATHWAY anti-HER2/neu (4B5) rabbit monoclonal primary antibody is a new FDA-approved rabbit monoclonal antibody intended for the semi-quantitative detection of HER2 antigen. It was compared to the widely used PATHWAY HER-2 (clone CB11) primary mouse antibody on an independent sample set and was found to provide acceptably concordant results [12].

The objective of our study was to evaluate the expression of HER2 protein expression using the new 4B5 antibody for previously defined (CB11) 2+ equivocal cases, and to correlate the results with the FISH ratio score.

MATERIALS AND METHODS

Twenty-eight invasive breast carcinomas showing equivocal HER2 immunostaining (immunohistochemistry score 2+ by the CB11 clone) were randomly selected from our pathology files from July 2006 to December 2007. The original paraffin blocks were examined for HER2 protein expression, by Ventana Pathway HER-2 (clone 4B5) antibody, using a protocol set by the manufacturer on the Benchmark XT; Ventana Medical System (Tucson, AZ, USA) [12].

HER2 SCORING

The scoring was performed using the same guidelines as HercepTest™, as follows:

- 0 = negative (no staining is observed or membrane staining is observed in < 10% of tumor cells)
- 1+ = negative (a faint/barely perceptible membrane staining is detected in > 10% of the tumor cells)
- 2+ = equivocal (a weak to moderate complete membrane staining is observed in > 10% of the tumor cells)
- 3+ = positive (a strong complete membrane staining is observed in > 10% of the tumor cells).

Corresponding FISH results were obtained from our cytogenetics laboratory. FISH was performed at the time of initial diagnosis using a PathVysion dual color probe (Vysis Inc., Downers Grove, IL, USA). FISH was scored as amplified if the HER2 signals to chromosome 17 centromere signals were greater than 2.2 and non-amplified if the ratio was less than 1.8. Statistical analysis was performed by GraphPad PRISM®4 software, using chi-square testing. Ethical committee approval was granted.

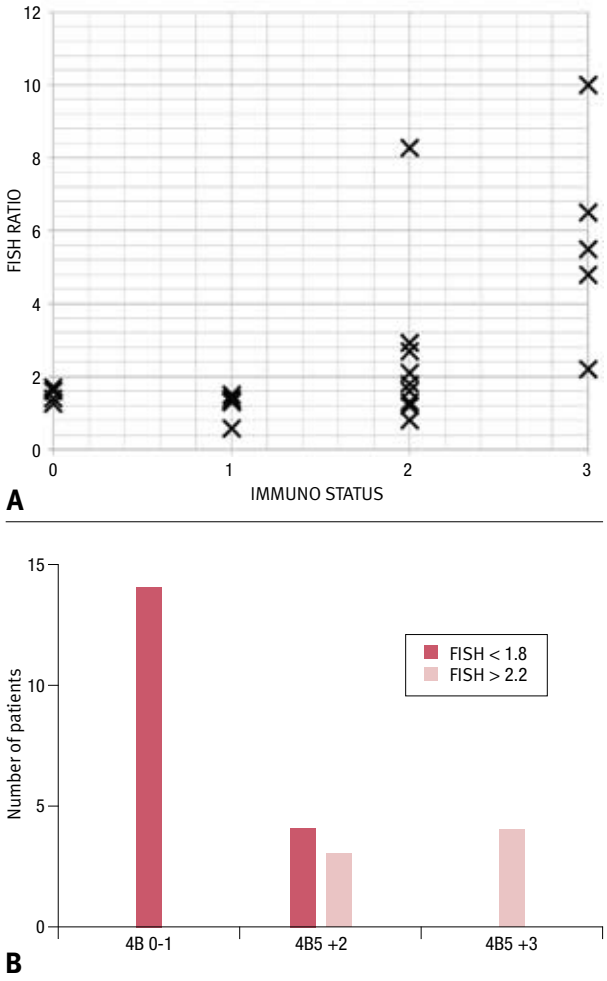
RESULTS

The study results are shown in Table 1 and Figure 1. Of the original 28 CB11 clone designated equivocal cases 14 (50%) showed negative HER2 staining using the 4B5 clone (HercepTest score 0 and 1+). Five cases (18%) proved to be positive (HercepTest score 3+) and 9 cases (32%) remained equivocal (HercepTest score 2+). The corresponding FISH ratio results showed that all 4B5-negative cases were also negative by FISH testing, and that 4 of 5 of the 4B5-positive cases were also positive by FISH testing. One 4B5-positive case was borderline-high (2.2 ratio) by FISH [Figure 1A].

Table 1. 4B5 status and FISH ratio of the study cases

Case no.	4B5 status	FISH ratio
1	0	1.4
2	0	1.4
3	0	1.25
4	0	1.6
5	0	1.6
6	0	1.7
7	0	1.25
8	1	1.33
9	1	1.34
10	1	1.4
11	1	1.32
12	1	1.3
13	1	1.5
14	1	0.58
15	2	0.8
16	2	1.8
17	2	1.2
18	2	1.6
19	2	1.3
20	2	2.7
21	2	8.27
22	2	2.1
23	2	2.93
24	3	5.5
25	3	6.5
26	3	4.8
27	3	2.2
28	3	10

Figure 1. Distribution of FISH results according to the 4B5 immunostatus. **[A]** Numerical FISH results by 4B5 IHC. **[B]** Categorical distribution of FISH results according to 4B5 IHC



Chi-square analysis demonstrated a *P* value of 0.0013 for the association of 4B5 IHC results with the FISH results [Figure 1B]. This finding indicates a statistically significant association between the results of these two tests. However, in order to determine the clinical usefulness of our suggested approach, the predictive value of 4B5 testing has to be taken into account. The positive predictive value of 4B5 +3 results was 80% (4 out of 5). The negative predictive value of a 4B5 negative or +1 result was 100% (14 out of 14 cases).

DISCUSSION

HER2 is over-expressed in 15–20% of invasive breast carcinomas [10]. HER2 expression is an individual prognostic factor for predicting the aggressive behavior of the tumor as well as the benefit from adjuvant therapy. Trastuzumab suppresses HER2 activity, thereby facilitating apoptotic cell death. Clinical trials have shown that the relative risk of recurrence is decreased by 50% when trastuzumab is added to the adjuvant chemotherapy regimen in HER2-positive women. In addition, trastuzumab significantly prolongs survival in HER2-positive metastatic breast cancer patients. Therefore, it is important to accurately assess the HER2 status for patients who may benefit from targeted therapy.

To detect HER2 status the following methods were used – immunohistochemistry to evaluate the level of HER2 protein in invasive carcinomas, and FISH to assess gene amplification. Each method has advantages and disadvantages. The immunohistochemistry method is less expensive, less cumbersome, and has a shorter assay time. However, the results may be affected by the processing and fixation methods. There is variability in the HER2 expression using different monoclonal and polyclonal antibodies, ranging from 26% to 42%. The discrepancies in the published literature regarding increased expression levels of HER2, by different antibodies, are attributed to the possibility of variations in tissue preparation and scoring criteria rather than the nature of these antibodies [11]. The FISH method, on the other hand, is a semiquantitative assay, which is less subjective but expensive [13].

The 4B5 rabbit monoclonal antibody was recently approved by the FDA to assess the HER2 status of invasive breast carcinoma. In a previous study it was compared to the widely used FDA-approved mouse monoclonal antibody CB11 and was found to provide acceptably concordant results. In our study we further analyzed the CB11 determined equivocal 2+ group and demonstrated that 4B5 showed excellent agreement with the FISH method. Although based on a small number of cases, we believe these results indicate the feasibility and potential usefulness of sequential IHC testing by 4B5 following an equivocal CB11 result. Furthermore, only 32% of the previously equivocal cases needed retesting by FISH. These results imply that the sequential testing by CB11 followed by 4B5 antibodies has better segregation capability than using a single antibody. This approach has the potential for significant time and cost savings in the management of breast cancer patients.

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References

1. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987; 235: 177-82.
2. Press M, Pike M, Chazin V, et al. Her-2/neu expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and

association of overexpression with increased risk of recurrent disease. *Cancer Res* 1993; 53: 4960-70.

3. Press MF, Bernstein L, Thomas PA, et al. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. *J Clin Oncol* 1997; 15: 2894-904.
4. Yamauchi H, Stearns V, Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. *J Clin Oncol* 2001; 19: 2334-56.
5. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344: 783-92.
6. Yaal-Hahoshen N, Safra T. Herceptin (trastuzumab): adjuvant and neoadjuvant trials [Review]. *IMAJ Isr Med Assoc J* 2006; 8(6): 416-21.
7. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 1999; 17: 2639-48.
8. Vogel CL, Cobleigh MA, Tripathy D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002; 20: 719-26.
9. Perez EA, Roche PC, Jenkins RB, et al. HER2 testing in patients with breast cancer: poor correlation between weak positivity by immunohistochemistry and gene amplification by fluorescence in situ hybridization. *Mayo Clin Proc* 2002; 77: 148-54.
10. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists Guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; 131: 18-43.
11. Lebeaue A, Deimling D, Kaltz C, et al. HER-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescent in situ hybridization. *J Clin Oncol* 2001; 19: 354-63.
12. Powell WC, Hicks DG, Prescott N, et al. A new rabbit monoclonal antibody (4B5) for the immunohistochemical (IHC) determination of the HER2 status in breast cancer: comparison with CB11, fluorescence in situ hybridization (FISH), and interlaboratory reproducibility. *Appl Immunohistochem Mol Morphol* 2007; 15(1): 94-102.
13. Carlson RW, Moench SJ, Hammond ME, et al. NCCN HER2 testing in breast cancer task force. HER2 testing in breast cancer: NCCN task force report and recommendations. *Natl Compr Canc Netw* 2006; 4 (Suppl 3): S1-22; quiz S23-4.