Modern Pathology (2008) 21, 1278–1281

www.modernpathology.org

Letters to the Editor

Modern Pathology (2008) **21,** 1278–1280; doi:10.1038/modpathol.2008.131

To the editor: We read with great interest and some concern the paper by Gown *et al*[1] in this issue of *Modern Pathology* regarding their commercial laboratory quality experience with *HER2* testing. Gown *et al* tested 6604 breast cancer patients in the PhenoPath Commercial Lab for *HER2/neu* status. The cases were derived from 100 different hospitals and cancer centers in 29 different states in the form of paraffin block or cut sections of core biopsies, surgical specimens, or metastatic sites. They used the Dako polyclonal antibody A0485 (component of the HercepTest™) and performed FISH assay using the Vysis probe on equivocal cases, as per standard guidelines. The authors describe the quality of the immunohistochemical data obtained using AO485 antibody as an ASR (i.e., without other components of the Hercept™ kit) and the Vysis FISH method, and demonstrate unacceptable lack of correlation between these two methods at a level of 30.6%.

The authors then describe the common occurrence of *HER2* staining of normal breast elements (although the number of cases showing *HER2* staining of the normal breast epithelium was not specifically quantified in their paper). The authors go on to devise a method to 'correct' for this anomalous *HER2* staining by visually 'subtracting' the degree of staining of the normal epithelium from that seen in neoplastic cells. The authors do not mention if they applied the normalized scoring system to biopsies in which no normal epithelium was present. They replace the FDA/Hercept test scoring system (0, 1+, 2+, and 3+) with what they term a normalized scoring system, which they derived by visually subtracting the score, that is intensity of staining of the normal epithelium from the score of the tumor cells. According to the authors, using the normalized scoring system reduced the discordance rate of immunohistochemistry and FISH for the 3+ group from 30.6 to 5.3%. There was no significant change in discordance of the negative tumors having scores of 0 or 1+.

The group concludes that using the normalized scoring system gave them a 94.7% concordance between immunohistochemistry and FISH for negative and positive results. They suggest the introduction of this normalized scoring system and propose that changes should be made to the FDA scoring criteria to meet the new ASCO/CAP mandate for 95% concordance for test validation.

There are several concerns with this proposal. First, Gown and colleagues have vast experience and may well be able to apply consistently the normalized scoring system subtraction method, whereas for many others this approach would introduce a huge variable that will be a step backward in the attempts of our discipline to enhance the quality and consistency of *HER2/neu* testing in breast cancer. Our belief is that the normalized scoring system method described by Gown and colleagues is built on a wrong premise, and although of some academic interest, it should not be adopted for the following reasons:

(1) This approach (normalized scoring system) is in conflict with the recommendations of organizations (public and private), including and not limited to the College of American Pathologists and American Society of Clinical Oncology (CAP/ASCO), which have made concerted efforts to improve the standardization in *HER2/neu* testing using immunohistochemistry. The ASCO/CAP guidelines categorically state that staining of the normal epithelium is one of the exclusion criteria to reject a test result.[2,3]
(2) *HER2* gene is unique in that the most common cause of protein overexpression is due to gene amplification (score ≥ 2); this is rarely, if ever ($<3\%$ of cases), due to the enhanced transcription of a normal *HER2* gene count.[4] Another 1–2% of cases with polyploidy of the tumor may produce enough protein to result in a positive immunohistochemical test that is associated with an ISH score of <2. These cases should not be counted as false positive because they have shown to respond to Herceptin therapy.[5]
(3) Immunohistochemistry is a qualitative test, which in the case of *HER2/neu* is used to produce a semiquantitative result based on technical and clinical validation. The use of the normalized scoring system will introduce another variable for an immunohistochemistry semiquantitative score, which adds to the subjectivity of the test that is basically qualitative by nature.
(4) The ASCO/CAP guidelines[2,3] are the result of an extensive review of the literature that was carefully performed by an expert panel (oncologists and pathologists) bringing their experience to the table. Strict guidelines were proposed, dealing with the pre-analytical, analytical, and post-analytical aspects of *HER2/neu* testing, to enhance the quality of the test and to ensure consistent and accurate results in at least 95% of cases with positive or negative HER2/neu status. This consensus has proven to be of general value and should not be changed without careful consideration.
(5) Immunohistochemistry standardized methodology, if calibrated carefully using cases or cell lines with positive, negative, or borderline status, produces a test with no staining of the

normal breast epithelium.[6,7] This is a pragmatic method that makes it much easier and more straightforward to score the percentage of positive cells and the intensity of staining in the tumor. The 3+ category requires strong complete (chicken-wire) membrane staining in at least 30% of cells. In most positive cases, 90 or 100% of cells are actually positive. The acceptance of 30% cutoff in the ASCO/CAP guidelines is to allow for the loss of the protein that may be associated with fixation.[8]

(6) The authors clearly highlight the problem that labs in North America face with up to 20% discordance between labs.[9–11] The causes of this discordance are manifold. The most important cause may not be the scoring criteria but rather the inadequate appreciation (or disregard) of standardized methodology, coupled with limited participation in external QA programs. As Gown *et al* themselves have indicated in a previous study, strict adherence to scoring criteria and calibrations of the test, plus the use of internal controls and participation in external QA programs can result in excellent immunohistochemical/ISH concordance results of >90%; many labs have achieved that level according to published QA programs.[12,13]

(7) The ASCO/CAP guidelines specifically state that staining of the normal epithelium is a reason to reject a test result. The *HER2* staining of normal breast elements reported by Gown *et al* differs from general experience where laboratories set up the assay in such a way that normal breast epithelial staining is absent or minimal. Under these 'usual' circumstances, the normalized scoring system subtraction is not applicable, as normal staining is not present to an appreciable degree. Thus, cases showing the staining of the normal epithelium should be rejected and the necessary corrective taken.

(8) The use of ASR products in non-expert hands, where validation is difficult, is fraught with a myriad of potential variations stemming from pre-analytic factors that have given rise to the poor immunohistochemical–FISH concordance rates that the authors cite. In a commercial reference, the laboratory environment, such as with Gown *et al*, does face a 'unique' problem in performing immunohistochemical staining for *HER2* on patients subjected to remarkably variable, and entirely unknown fixation protocols. This is an indicated need as intervention to assure that patient specimens have optimized pre-analytic tissue fixation protocols.

(9) The *ad hoc* committee on standardization of immunohistochemistry has recommended the standardization of all aspects of the immunohistochemical method, preferring the use of test platforms (IVD) that have both technical and clinical validation and that are common across multiple laboratories.

Conclusion

For the reasons cited above, we hope that the results reported by Gown and colleagues will not be adopted by pathologists, who may obtain a better outcome by attending closely to all aspects of their testing procedure, including the pre-analytic, analytic, and post-analytic phases, rather than attempting to adopt the normalized scoring system approach, which adds a new level of subjectivity to what already is a difficult assay.

Although the goal of this study is laudable, we believe that the current guidelines for *HER2* scoring are appropriate and that proper intervention to assure quality tissue specimens for *HER2* testing should be the goal of every laboratory.

Wedad M Hanna[1], Elizabeth Hammond[2], Clive R Taylor[3], David J Dabbs[4], Frederique Penault-Llorca[5], Kenneth J Bloom[6], Michael Bilous[7] and Sunil Badve[8]

[1]Department of Anatomic Pathology, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada; [2]Department of Pathology and Internal Medicine, Pathologist Intermountain Healthcare, Pathology and Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA; [3]Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [4]Department of Pathology, Magee-Women's Hospital, UPMC, Pittsburgh, PA, USA; [5]Department of Pathology, Pathology, Centre Jean Perrin, Clermont-Ferrand, France; [6]CLARiENT Inc.; [7]Department of Tissue Pathology, Westmead Hospital, Sydney, NSW, Australia and [8]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Clarian Pathology Laboratory, Indianapolis, IN, USA

E-mail: wedad.hanna@sunnybrook.ca

References

1 Gown AM, Goldstein LC, Kussick SJ, *et al.* High concordance between immunohistochemistry and fluorescence *in situ* hybridization testing for HER2 status in breast cancer requires a normalized IHC scoring system. Mod Pathol 2008; 21:1271–1277 (this issue).

2 Wolff AC, Hammond ME, Schwartz JN, *et al.* ASCO/CAP guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 2007;131:18.

3 Wolff AC, Hammond ME, Schwartz JN, *et al.* ASCO/CAP guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25:118–145.

4 Hofmann M, Stoss O, Gaiser T, *et al.* Central HER2/IHC and FISH analysis in a trastuzumab (Herceptin) phase II monotherapy study: assessment of test sensitivity and impact of chromosome 17 polysomy. J Clin Pathol 2008;61:89–94.

5 Pauletti G, Godolphin W, Press MF, *et al.* Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence *in situ* hybridization. Oncogene 1996;13:63–72.
6 Robertson KW, Reeves JR, Smith G, *et al.* Quantitative estimation of epidermal growth factor receptor and c-erbB-2 in human breast cancer. Cancer Res 1996;56:3823–3830.
7 Nagy P, Jenei A, Kirsch AK, *et al.* Activation-dependent clustering of the erbB2 receptor tyrosine kinase detected by scanning near-field optical microscopy. J Cell Sci 1999;112:1733–1741.
8 Leong AS, Formby M, Haffajee Z, *et al.* Refinement of immunohistologic parameters for HER2/neu scoring validation by FISH and CISH. Appl Immunohistochem Mol Morphol 2006;14:384–389.
9 Roche PC, Suman VJ, Jenkins RB, *et al.* Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. J Natl Cancer Inst 2002;94:855–857.
10 Paik S, Bryant J, Tan-Chiu E, *et al.* Real-world performance of HER2 testing—National Surgical Adjuvant Breast and Bowel Project Experience. J Natl Cancer Inst 2002;94:852–854.
11 Perez EA, Suman VJ, Davidson NE, *et al.* HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol 2006;24:3032–3038.
12 Yaziji H, Goldstein LC, Barry TS, *et al.* HER-2 testing in breast cancer using parallel tissue-based methods. JAMA 2004;291:1972–1977.
13 Vincent-Salomon A, MacGrogan C, Couturier J, *et al.* Calibration of immunohistochemistry for assessment of HER2 in breast cancer: results of the French multicentre GEFPICS study. Histopathology 2003;42:337–347.

Modern Pathology (2008) **21,** 1280–1281; doi:10.1038/modpathol.2008.136

In reply: The ASCO-CAP Guidelines were generated in an attempt to optimize the accuracy of HER2 testing in breast cancer and to address the documented high levels of discordance between HER2 testing reported in the literature. Indeed, the identification of methods that can be employed to ensure the accuracy of HER2 testing was also the motivation of the study of HER2 IHC and FISH concordance that we have reported in this issue of *Modern Pathology*. Furthermore, one of the authors of our study (AG) was present at the *ad hoc* committee meeting that preceded the generation of the published guidelines.[1]

Hanna and co-workers express several 'concerns' about our 'proposal.' Our paper is not, in fact, a 'proposal' of an alternative to, nor is it a rejection of the ASCO-CAP Guidelines. It is, rather, a study documenting a method to improve further upon these guidelines. Our study represents a test of an hypothesis, which is that high levels of concordance of HER2 IHC and FISH results can be obtained, along with the elimination of most false-positive IHC results, through the use of a simple normalization technique. Our data overwhelmingly support this hypothesis.

We take exception to several points raised by Hanna and co-workers:

(1) Hanna and co-workers state that the ASCO-CAP Guidelines 'categorically state that staining of the normal epithelium is one of the exclusion criteria to reject a test result.' In fact, the ASCO-CAP Guidelines state that *strong* staining of the normal epithelium is an exclusion criterion (Table 5 of reference Wolff *et al*[1]). None of our specimens showed strong staining of the normal epithelium; in fact, although not stated in the paper, all staining of the normal epithelium, when present, was at the 1+ level. Indeed, Hanna and co-workers themselves acknowledge in their letter that their 'general experience' is that positive signal on normal breast epithelium is 'absent or minimal,' which would translate to either 0 or 1+, and is precisely the finding in our 6604 cases. Thus, there is nothing unique about the tissues or the level of immunostaining of the normal epithelium we have examined in our study.

(2) Hanna and co-workers state that the ASCO-CAP Guidelines '… ensure consistent and accurate results in at least 95% of cases with positive or negative HER2 status.' In fact, although the guidelines represent a noble effort to improve the accuracy of HER2 testing, they do not represent the product of 'evidence-based' analysis, as there were no data presented to or at the ASCO-CAP Panel that documented this level of concordance. Also unknown by the ASCO-CAP Panel is the contribution of the various elements (preanalytical, analytical, postanalytical) to the significant error rate in HER2 IHC testing. There have been no publications since the promulgation of the guidelines, other than our present study, that have documented 95% concordance between HER2 testing by IHC and FISH. The single study cited in the guidelines[2] was misrepresented as documenting a 4% discordance rate between IHC and FISH, when in fact the paper documents a 14% discordance rate within the 'central laboratory,' ie, cases that were IHC 3+ but FISH negative (Table 3 of reference Reddy *et al*[2]).

(3) Hanna and co-workers state that 'strict adherence to scoring criteria and calibrations of the test, plus use of internal controls and participation in external QA programs can result in excellent IHC/FISH concordance results of >90%; many labs have achieved that level according to published QA programs.' Two publications are cited to justify this comment;