EDITORIAL

The Quality of Her-2/*Neu* Predictive Immunohistochemistry: Something FISHy?

The American Cancer Society estimates that approximately 175,000 women will be identified in 1999 with new cases of breast cancer and approximately 43,000 will die of the disease (1). An increasing number of these cases are diagnosed at an early stage when there is no evidence of lymph node metastases. Tumor biology differs substantially among cases in such low-stage tumors, and it has become increasingly important to identify ancillary tools which allow the pathologist to reliably identify (1) Harbingers of aggressive biologic potential (early recurrence, metastases, death) and/or (2) genotypic or phenotypic features that can be linked to specific therapy based on blocking receptors or specific agents targeted to kill tumor cells.

The exact role for ancillary studies in the diagnosis and management of breast carcinoma remains controversial, even though many of these tools have been available and in use for over a decade. A national consensus does not exist as to which, if any, ancillary studies should be measured in newly diagnosed patients with breast carcinoma. There are certainly many reasons for uncertainty and lack of consensus. Chief among them are the plethora of univariate and poorly controlled literature reports of the correlation of ancillary studies with recurrence and other hallmarks of biologic behavior. A careful review of this literature is confusing at a minimum and generally depressing. For example, "breast cancer" is used not infrequently as an admission criterion to the study, with no exclusion of *in situ* (as opposed to invasive) ductal carcinoma, exclusion of lymphoma, sarcoma, or specific morphologic variants of invasive ductal breast carcinoma. Variation in methods (sampling, fixation, cell conditioning, instrumentation, reagent sources and stability, interpretation, and others) may also contribute to the confusion and lack of consensus.

Her-2/*neu* (c-*erb*-B2) is an oncogene related to the epidermal growth factor receptor family. It is overexpressed on a subset of *in situ* and invasive carcinomas of the breast. Genomic amplification of the Her-2/*neu* gene in patients with invasive breast carcinoma has been associated with a significant reduction in metastasis-free survival; similarly staged patients without Her-2/*neu* genomic amplification have a very high likelihood of remaining cancer free years after removal of the tumor (2–10). With the advent of a humanized monoclonal antibody to the encoded protein on the surface of mammary carcinoma tumor cells, the laboratory assessment of Her-2/*neu* status has assumed even more importance (11–14). As a predictive factor, Her-2 amplification/overexpression has been found to be related to benefit from adjuvant doxorubicin (15, 16) and lack of benefit from adjuvant tamoxifen (17) and CMF (18).

In the current issue of *Modern Pathology*, the controversial relationship between results derived from fluorescence *in situ* hybridization (FISH) and immunohistochemical detection of encoded protein is addressed (19). The authors utilized whole cell preparations for FISH rather than paraffin sections, the more standard approach for FISH Her-2/*neu* detection. The inability to distinguish between cells derived from *in situ* rather than invasive carcinoma could limit the practical application of such an approach. High levels of gene amplification were associated with strong membrane immunostaining. Gene copy greater than four and less than 10 was usually associated with chromosome 17 polysomy. The authors used two-color FISH to simultaneously assess Her-2/*neu* and chromosome 17 ploidy; given the low frequency of intermediate level Her-2/*neu* gene amplification, sequential single-color FISH for such cases may be more cost effective. *In situ* studies evaluating mRNA expression will be needed to properly understand those cases which show moderate immunostaining ("2+") without genomic amplification. Reportedly, genomic amplification is usually associated with increased expression of messenger RNA and the encoded oncoprotein; discordance between expression and genomic amplification occurs in about 3 to 15% of cases. The clinical significance of lower levels of staining ("2+") and such discordance between genomic amplification and oncoprotein expression, including response to serotherapy, is uncertain.

Overall, the authors of the current study found very good concordance between genomic amplification and encoded protein detection by immunohistochemistry (19). Conclusions from other studies have not been as sanguine (20, 21). A particular problem has been an unexpectedly large number of positive results with immunohistochemistry using a polyclonal antibody approved by the FDA for determining Her-2/*neu* status. Surprisingly high percentages of Her-2/*neu* of positive cases were identified within a large series of breast carcinoma. When the same cases have been evaluated with a monoclonal antibody to the Her-2/*neu* encoded protein or with fluorescence *in situ* hybridization,

the immunohistochemical results with the polyclonal antibody appear to be inappropriately high (21–23). Denying the potential clinical benefits of therapy must be carefully balanced against the significant cardiotoxicity associated with Herceptin administration. In particular, the prudent therapeutic choice for cases with "2+" staining is uncertain.

The intense interest and controversy surrounding the laboratory detection of Her-2/*neu* amplification is manifest in many ways. One need only look at the recently published abstracts from the annual United States and Canadian Academy of Pathology meeting to recognize the intense interest focused on this issue. FISH is inherently quantitative; results are expressed as average number of gene copies over multiple sampled fields. Yet FISH protocols are as variable as immunohistochemical methods, and special equipment is necessary. Because pretesting for technical and interpretative competence is required for both FISH FDA-approved systems before reagents can be purchased for clinical use, FISH variability is probably minimized as long as laboratories adhere to approve protocols. To our knowledge, such competence pretesting is not required for immunohistochemistry.

For immunohistochemistry, issues that have largely been ignored for some time are now forcibly brought to the fore for consideration. Should immunohistochemistry ever be considered a quantitative assay? Or are there at least limitations to be placed on quantitation of cytoplasmic membrane antigens (as opposed to nuclear determinants)? But even for relatively straightforward quantitation such as MIB/or Ki-67-based proliferation assays, numerous problems still remain if one considers intensity of the reaction as part of the quantitation. One could argue that immunohistochemistry has been developed as a *qualitative* adjunctive tool supplementing morphologic interpretation and was never really intended to be a quantitative technique. Certainly, given the enormous variability in procedural and interpretive protocols, intensity may simply be a variable that is unable to be clinically evaluated. In run cell-line quantitation control standards may improve reproducibility and reliability. Adherence to FDA-approved protocols for FDA-approved tests is an issue—deviation from such approved protocols, whether for "epitope retrieval," staining, or interpretation invalidates the FDA-approved status of the testing. The test becomes an in-house Analyte Specific Reagent (ASR)-based test. Quality assurance programs administered by our profession's self-governing bodies still do not actively grade immunohistochemistry interlaboratory comparisons. One would think that in the third decade of use of diagnostic immunohistochemistry, a laboratory performing clinical assays should at least be able to consistently and reproducibly detect a core group of antigens such as cytokeratins, S100 protein, and basic lymphoid differentiation antigens. Yet there remains no graded rigorous quality assurance standard against which laboratories' results can be measured. The same can be said for interphase fluorescent and chromogenic *in situ* hybridization. More rigorous quality assurance programs for both immunohistochemistry and molecular morphology (FISH and chromogenic *in situ* hybridization) are absolutely essential.

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