The Knowns and the Unknowns in HER2 Testing in Breast Cancer

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The 2007 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines for HER2 immunohistochemical and fluorescence in situ hybridization (FISH) testing in breast cancer address the potential impact of several “knowns” (eg, effect of fixative composition and fixation time).1,2 However, data continue to be generated that shed further light on the impact of “unknowns,” ie, selected preanalytic, analytic, and postanalytic (ie, interpretive) factors, on HER2 immunohistochemical testing.

The study by Manion and colleagues3 in the June 2011 issue of the Journal focuses on 1 analytic factor that was not addressed by the ASCO/CAP guidelines, namely, the impact of the choice of antibody clone used for HER2 immunohistochemical testing. Manion et al3 reported that by switching from the rabbit polyclonal antibody A0485 (the same antibody that is part of the HeceptTest kit, DAKO, Carpinteria, CA) to the rabbit monoclonal antibody SP3, they could essentially halve the number of 2+ cases, at the price of a probably statistically insignificant increase in the false-negative rate (ie, FISH-positive, immunohistochemically negative cases).

Can their results be extrapolated to other laboratories wanting to optimize HER2 immunohistochemical testing? As noted by the authors themselves, the apparent differences in performance of the A0485 rabbit polyclonal vs the SP3 rabbit monoclonal noted in their study have not necessarily been corroborated by other investigators.4-7 And in a study from our laboratory published in abstract form,8 involving a series of 416 breast cancer cases, we also found that the use of SP3 reduced the number of 2+ cases, albeit not by the magnitude reported by Manion et al. Because all of our cases had also been subjected to FISH analysis, we were able to determine that concordance rates between immunohistochemical and FISH testing were independent of the use of the SP3 or the A0485 antibody.

However, in subsequent but unpublished SP3 validation studies performed in our laboratory on 551 breast cancers, we found nearly identical proportions of cases showing 2+ immunostaining with the A0485 or the SP3 antibody. Among the cases showing amplification by FISH, there was a significant number of cases that had been classified as 2+ using A0485 that were scored as 3+ using SP3. But among the cases that were not amplified by FISH, there was a significant number of cases that had been classified as 0/1+ using A0485 but as 2+ using SP3. These 2 groups “balanced out,” and, hence, the number of 2+ cases was nearly identical in both antibody groups. What happened to our initial findings, which seemed to parallel those of Manion et al?

Could this be another example of the phenomenon of the “decline effect,” ie, the frequent failure of follow-up studies to corroborate the initially reported magnitude of scientific results (as exemplified by the “disappearing” benefits of second-generation antipsychotics)?9 Alternatively, with HER2 immunohistochemical testing there is the potential confounding factor of a “learning curve” of HER2 immunohistochemical interpretation, a phenomenon that seems to emerge from our published HER2 data. Contrary to the statement by Manion et al1 that our 2004 study of more than 2,900 carcinomas represents the largest published series of HER2 immunohistochemical-FISH concordance,10 in fact, our follow-up 2008 study included 6,604 cases, more than twice as many.11 While our methods were the same, we found a positive concordance rate (3+/amplified) of 92.8% in the 2004 study but a 94.7% concordance in the follow-up study in 2008. As our interpretation of the immunohistochemical test is continually informed by the daily performance of parallel FISH studies on the same cases, it is quite possible that this drift in positive concordance represents the result of a subtle learning curve. And
there could be subtle learning curve effects when laboratories switch from the A0485 to the SP3 clone.

Particularly in this era in which controlling medical costs is essential, a reduction of the number of cases scored as 2+ and, thus, the number of breast cancer cases submitted for reflexive FISH testing following immunohistochemical testing, is not an insignificant reason for preferring one clone over another. However, in the absence of compelling data that the use of SP3 rather than A0485 improves the immunohistochemical-FISH concordance rate, we suspect this may not of and by itself convince laboratories to switch antibodies.

Our laboratory, however, having transitioned from the A0485 to the SP3 antibody reagent more than 2 years ago following extensive technical and clinical validation studies, and having run nearly 9,000 cases in the interim, has noted additional benefits of the use of the SP3 rabbit monoclonal antibody, some of which were alluded to by Manion and colleagues. Clearly SP3 is a more robust reagent, and in the setting of a reference laboratory, in particular, it seems more forgiving of a wide range of preanalytic variables. (Indeed, the question of the ability of more robust reagents to ameliorate or even negate the impact of selected preanalytic factors is certainly worth much more study.)

Our laboratory uses 2 sets of positive control samples for HER2 testing: fixed, embedded cell pellets generated from cell lines with known HER2 expression levels and breast cancer tissues with known HER2 gene status with 1+ and 3+ immunostaining levels. This is admittedly a high performance bar for any antibody to attain, but our experience with the 2 antibodies has been that we have far fewer runs in which one or more of the controls have failed using the SP3 compared with the A0485 antibody.

But the use of the SP3 clone will not guarantee accurate and precise HER2 immunohistochemical testing. The choice of anti-HER2 antibody, eg, between A0485 and SP3, may be less important than other preanalytic factors, including “cold ischemia time,” ie, the time before the breast biopsy specimen is immersed into formalin, as well as the duration of formalin fixation. And these factors may well be less important, in turn, than details of the immunohistochemical procedure, including, eg, epitope retrieval method, antibody incubation time, and choice of polymer-based detection system. At the end of the day, however, the details of the immunohistochemical procedure may well prove less important than the ability of the pathologist to “bin” the tumors into the categories of 0/1+, 2+, and 3+, whether it is done by eye or with the assistance of an image analysis system.

The jury is still out on whether strict adherence to the ASCO/CAP guidelines will, of and by themselves, permit laboratories to attain 95% concordance between immunohistochemical studies and FISH for HER2 testing. But we suspect that at best, the ASCO/CAP guidelines will be necessary, but not sufficient, to reach this goal. This is because of other factors, some known and some unknown, that, like the choice of antibody clone, may have dramatic effects on HER2 immunohistochemical testing. Donald Rumsfeld, the former Secretary of Defense, perhaps best described the situation with HER2 testing, albeit in a totally different context:

“[T]here are known knowns; there are things we know we know.

“We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also unknown unknowns—the ones we don’t know we don’t know.”

From PhenoPath Laboratories, Seattle, WA.

References


