New guideline takes on tough HER2 cases

written by CAP TODAY
October 20, 2013

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October 2013—In HER2 testing for breast cancer, the term "equivocal" verges on being a four-letter word. If the patient has a clearly positive test result, therapies targeting HER2 become a treatment option, and a highly successful one at that. If the result is clearly negative, HER2-targeting drugs are off the table; the patient isn't expected to benefit from the drugs, which are expensive and can be cardiotoxic.

But if the result isn't clear? David Hicks, MD, can still remember the first time he reported a HER2 breast cancer result as "equivocal."

"The medical oncologist called me and said, 'I liked it much more when it was positive or negative,'" recalls Dr. Hicks, professor of pathology and laboratory medicine and director of surgical pathology, University of Rochester (NY) Medical Center.

Dr. Antonio Wolff says that over the years, many physicians have asked about the so-called best, most accurate test for HER2. In the new guideline, "We have taken the tack that it's not an issue of which test is right, among those linked with clinical outcome, but whether the test selected is done

right."

Dr. Hicks is also a practicing breast pathologist and thus no stranger to the difficult HER2 cases that bedevil his physician colleagues and patients. Equivocal results aren't the only challenges. What's the best way to handle tumor heterogeneity? What about *HER2* genotypic abnormalities, like aneusomy of chromosome 17; colocalization of *HER2* and CEP17 signals that affect *HER2*/CEP17 ratio in dual-signal in situ hybridization assays; and genomic heterogeneity?

Such "rogue" cases, as Dr. Hicks calls them, should become easier for both pathologists and medical oncologists to manage with the arrival of a newly updated HER2 testing guideline issued by the American Society of Clinical Oncology and the CAP. The guideline, published online Oct. 7 in *Archives of Pathology & Laboratory Medicine* (archivesofpathology.org) and *Journal of Clinical Oncology* (jco.ascopubs.org), updates the previous guideline, which appeared in 2007.

"We provide clear instructions and clear recommendations on how to handle difficult cases and how to reduce areas of uncertainty,'' says Antonio Wolff, MD, who was the principal oncologist author of both guidelines.

The guideline also simplifies fixation time for HER2 specimens, bringing requirements in line with those for ER/PgR assays. This is a change that Dr. Hicks—another of the guideline's authors—predicts will be "helpful and welcome news" for pathologists. And it defines bright-field ISH as a valid test platform for assessing *HER2* amplification.

All these highly specific recommendations bring a new level of harmoniousness to HER2 testing—one that took considerable effort to achieve. HER2 testing puts in sharp relief two of medicine's rival geometries. Medical oncologists, naturally, prefer to travel in a straight line, from test result to treatment. Pathologists, just as naturally, find it difficult to stick to that unswerving path. As Elizabeth Hammond, MD, the principal pathologist author of both the new guideline and the earlier one, says, "Unfortunately, cancer biology doesn't work in a straightforward manner." But with their latest guideline, the authors hope the odds that both parties will arrive together at agreeable answers have just gone up.

Above all, the guideline emphasizes the need for action. HER2 testing acts as a traffic light for HER2-targeted therapy, and idling at a yellow light indefinitely is not an option.

Yet pathologists are understandably leery of expressing a higher level of certainty than they feel, says Dr. Hammond, professor of pathology, University of Utah School of Medicine, and consultant pathologist, Intermountain Healthcare, Salt Lake City. For them, an "equivocal" result, for example, may well be not only an accurate answer but also the responsible one. But on its own, it doesn't help the oncologist much. "The result you give needs to be something that helps the clinician make treatment decisions for the patient."

In that sense, the guideline underscores the notion that test results can be seen as the start of a treatment, not the end of an assay, and that the role of the pathologist in patient care is an active one. Or, as Dr. Hicks puts it, "We're not just someone who generates a result and throws it over the fence."

[dropcap]A[/dropcap]s the guideline's authors began their discussions in early 2012, they had plenty to talk about.

There has been, for example, no shortage of concern or confusion about how in situ hybridization tests were being counted, says Dr. Hammond. As a result, she says, “We spent a lot of time as a panel dealing with this.”

Dr. Hicks

The guideline’s writers had a tough challenge. According to Dr. Hicks, their first thought was to set HER2 positive at 10 percent of cells with strong staining. But the definition for FISH was an average number over 40 or 60 nuclei. The two methods—cell-by-cell evaluation versus counting multiple nuclei—aren’t necessarily simpatico, however. Because of heterogeneity, a FISH result will depend on which nuclei are being counted. If 10 percent are amplified and 90 percent are not, but nuclei in the nonamplified area are counted, the result will be a 10 percent IHC positive with a HER2-negative FISH result.

The current authors recognized that earlier guidance was not specific enough in cases where tumor heterogeneity was an issue. “The method that we were advocating before was not the right method for finding small populations of tumor cells that might be amplified,” Dr. Hammond says, given that those small populations might have important implications.

In the past, pathologists were mostly doing a random counting of the tumor population, she says. Now, per the new guideline, the pathologist needs to scan the entire ISH slide prior to counting at least 20 cells; areas of potential amplification must be included; and those areas of amplification must be separately counted and reported. “This gives a much better chance that a patient will have an opportunity to get a positive result if there are any amplified or heterogeneous elements in their tumor by this FISH or ISH test method,” Dr. Hammond says.

Dr. Hicks, who has been using the new approach, says it should eliminate discordant cases due to intratumoral heterogeneity. “I think we got that right. Time will tell.” Panel members wanted the guideline to be as evidence-based as possible. “But sometimes, even when there are limitations in our knowledge, guidance needs to be provided. You have to start someplace. As Liz finally said, ‘Well, I think this is the best we can do,’” until more data become available.

Tumor heterogeneity appears to occur more frequently than previously thought, and there’s been concern and plenty of questions about how physicians can be certain the tumor block selected for testing is representative of the patient’s tumor. Dr. Hammond says, “We can never know that. So you have to make a rational decision about how much of a tumor you need to examine to get the right answer.”

The new guideline also takes on the equivocal category. Most notably, it aligns the ASCO/CAP equivocal category with that recommended by the FDA.

Dr. Hammond

When the first guideline was written, says Dr. Hammond, “Our principal concern with HER2 testing was false-positives.” That concern led to one of the more vexing issues in HER2 testing. In the clinical trials for Herceptin, patients were considered positive for HER2 if their tumors contained more than 10 percent cells staining 3+ for HER2. The 2007 guideline writers chose a higher cutoff—more than 30 percent of cells because the panelists unanimously felt that almost all truly positive cases would have higher percentages of staining cells. Says Dr. Hammond: “We felt that tightening the threshold and being more specific about what constituted a positive would help limit the numbers of false-positives.”

Even as they did so, they knew the differing cutoffs might be confusing for practitioners, says Dr. Wolff, professor of oncology, Johns Hopkins University, Baltimore. But they had reasons for doing so.

Dr. Hammond calls setting the threshold at 30 percent “highly conservative. I still believe that’s the case.” In fact, she says, if proper specimen handling procedures are followed, the group of patients who fell into the previous equivocal category (that is, between 10 and 30 percent staining as 3+ positive) would be quite low. One retrospective paper (Perez EA, et al. *J Natl Cancer Inst*. 2012;104:159–162) considered patient eligibility for one of the trastuzumab adjuvant trials using both cutoffs and found that 3.7 percent of patients who met the FDA cutoff would have been declared ineligible by the ASCO/CAP criterion. “This high percentage would happen, though, only if recommendations from the 2007 guideline were ignored and reflex testing wasn’t done,” she says.

Therefore, Dr. Wolff says, physicians can set those concerns to rest. In a letter published in *JNCI* last year (Wolff AC, et al. 2012;104:957–958), he says, “We reanalyzed those data, and the actual number of patients affected would be about 0.2 percent of all patients newly diagnosed with breast cancer”—less, he adds, than the observed variability of various commercial assays in clinical use.

Dr. Hammond points out that the study population in the Perez paper consisted of patients who were put on clinical trials in the early 2000s before any specimen handling requirements were implemented. Wide acceptance of those requirements has lowered the false-positive rate since publication of the guideline in 2007. “We don’t believe this [the new guideline] is going to make any significant increase in the number of false-positives,” she says. It may even help curb them further, she adds.

Likewise, laboratories have shown a “meaningful increase” in participating in HER2 proficiency testing, Dr. Wolff says, adding that efforts to improve HER2 testing have gone beyond pathologists and oncologists, with health systems at large increasingly comprehending the need to provide the resources to implement accurate, standardized, and reproducible predictive biomarker testing, including proper handling of tissue specimens before they reach the pathology lab.

Might such improvements alone have led to a drop in equivocal cases, even without changing the threshold?

“That’s a great question, and I wish I knew the answer to it,” Dr. Hammond says. “Data from some large centers suggest so, and in my own laboratory, that is in fact the case.” Implementing careful specimen handling has had a dramatic effect on producing better pathology results at Intermountain, both by IHC and FISH, she says. In the past, perhaps up to 10 percent of cases would need a repeat FISH test. Her lab also has a “very, very small” number of patients who fell into the equivocal categories under the old guideline either by FISH or IHC, she says.

Nevertheless, she agrees using consistent criteria will help create standardized behavior. And, she adds, “We don’t want to create confusion.”

Concerns were raised about the 2007 guideline’s equivocal category for FISH, Dr. Hammond says. In the FDA guideline and in the clinical trials, a threshold of 2.0 was set to qualify patients for treatment. In the ASCO/CAP guideline, however, the authors said that, based on package inserts provided by assay manufacturers, a *HER2*/CEP17 ratio of 1.8 to 2.2 would be better considered equivocal rather than positive or negative, since they were within two standard deviations of the standard error of that measurement, says Dr. Hammond.

Now, the result is considered equivocal when the dual-probe *HER2*/CEP17 ratio is < 2.0 with an average HER2 copy number ≥ 4.0 and < 6.0 signals/cell.

As an example, Dr. Wolff says confusion occurs in cases in which there is evidence of coamplification in the region recognized by the CEP17 probe. “If that happens,” he says, “you could end up in a situation where you have, say, an average HER2 signal copy number of six or seven, but also an increase of the average number of CEP17 copies detected to, say, four.” The result will be a ratio of less than two. Relying solely on that ratio, the pathologist runs the risk of calling the tumor nonamplified. “When in reality, we now recognized the occasional coamplification of the CEP17 region identified by the centromere probe. The new guideline mandates that such cases will have another HER2 test performed.

“The biggest message,” Dr. Wolff continues, “is that we ask physicians not only to look at the ratio alone, but actually to look at the individual average number of HER2 copies, which is provided in the numerator, because this can help with difficult cases.”

Again, the 2007 authors had their reasons for choosing the cutoffs they did. The equivocal category was created not to exclude patients from treatment, but to trigger additional testing. Unfortunately, it also led to confusion and controversy.

“Because it was within the error of the measurement,” says Dr. Hammond, “we felt it was useful for oncologists and pathologists to understand that the accuracy of a FISH estimate in that range might be affected by variability. But many of our colleagues later expressed concerns and preferred that we follow that same threshold provided in the clinical trial.”

Their desire is simple to understand, she says. “They want so much to find patients who are going to be HER2 positive.”

[dropcap]M[/dropcap]edical oncologists might have been happier if the authors had just bid adieu to the equivocal category with this latest guideline. “We actually tried to get rid of it,” Dr. Hammond says. “But we could not do so. There always is an equivocal category, but we think we narrowed it considerably.”

That might have a bright side. The problem might not be with an equivocal result per se, but with viewing it as a final answer or the end of the discussion. As the authors of the new guideline emphasize, some difficult cases (including those with an equivocal result) will continue to exist and should start conversations.

Indeed, that's what happened when they began to revise the guidelines and tried to answer the tough questions they face routinely in their own practices. "Most of us are practicing oncologists and practicing pathologists" as well as researchers, Dr. Wolff says.

Dr. Hammond calls the discussions "lively" but says they led to a strong document. It wasn't unusual, she says, for her and Dr. Wolff to disagree about a subject initially or to reflect the differing opinions between medical oncologists and pathologists. "But when we talked it through, we were very much aligned, because both of us just wanted an accurate test result for the patient." Yes, these conversations take time, and yes, they can be—temporarily—disconcerting. "But it's worthwhile," says Dr. Hammond. "It's better than talking to yourself."

Fortunately, not every case requires donnish oversight. "The cases we struggle with are very rare—one, two, three percent of the breast cancer population," says Dr. Hicks. But little wonder the discussions were animated—there are few data on how to help this small subset of patients with unusual tumors.

Dr. Hicks would like to see those discussions continue in clinical practice. "It would be wonderful if after the guidelines are out, at every tumor board across the country, a pathologist sat up there and presented the changes and led a discussion about how this is going to affect the management of breast cancer in that institution going forward."

As physicians familiarize themselves with the new guideline, they'll be able to ask better questions about what test results mean. Dr. Hicks draws attention to the language in the guideline that asks medical oncologists and pathologists to confer on difficult cases, including thinking about HER2 results in the context of the patient's histology. It's a new spin on personalized medicine, distilled to answering a basic question: *Does this make sense for this particular patient?*

That may sound like an obvious question to ask, but in practice it wasn't happening enough. "We describe the importance of ensuring that the assay results are concordant with the other histopathologic features," Dr. Wolff says. Take a tubular breast cancer that is low grade and ER/PgR positive, for example. If the HER2 result for the tumor is positive, "those data don't match in principle," he says. In situations with such apparent histopathologic discordance, "We ask the pathologist and the oncologist to look back and examine the case as a whole" and consider additional testing if appropriate.

How have discordant results been handled up to now? "It really varied," Dr. Wolff says. More experienced practitioners would catch the discrepancy, he says, and realize "something wasn't quite right." By formalizing this as a recommendation, even those who don't specialize in breast pathology or breast oncology will know what to do.

Conversation is critical, says Dr. Wolff, but it "doesn't happen as easily as we would like it to happen. By putting it in the document, we want to make that the norm."

Similarly, the guideline gives specific recommendations listing the individual steps that pathologists and oncologists need to take as HER2 testing unfolds. "You can't duck responsibility," says Dr. Hammond.

It also provides specific guidance for patient and clinician conversations about HER2 testing. “Words matter,” says Dr. Wolff. “We spent a lot of time going over the meaning of everything we wrote.” Nothing, it seems, was left to chance.

That being said, tough cases, like the equivocal category, aren’t disappearing anytime soon. And just as this guideline reflects better knowledge and growing experience, so, too, will future changes create more challenges.

“We tried to do our best, but we’re very honest in saying we may not have covered everything,” Dr. Wolff says, “and we may not have gotten everything right. We did our best with the information available.” It’s inevitable that new classes of HER2 test results not covered in the guideline will become apparent once more data are available. Hence the need to keep the conversation going.

[dropcap]S[/dropcap]ome of those conversations will be sparked by new technologies, another topic that’s taken up in the new guideline.

While there’s plenty of interest in HER2 serum testing and RT-PCR, the only new platform endorsed in the guideline is FDA-approved bright-field in situ hybridization. The evidence for other methods was insufficient to warrant a recommendation right now. Conversely, the guideline notes, bright-field ISH measures gene amplification, which has clear clinical utility—it was the biological criterion used in prospective clinical trials. It shows high concordance levels with other ISH methods using FISH, and it appears to be reproducible across laboratories.

Bright-field offers another option for labs that currently offer only IHC testing. “That might be a much more interesting way for pathologists to operate,” Dr. Hammond says. While it brings challenges, it will also allow such labs to thoroughly examine the tumor in a way they likely didn’t before, she says.

Labs will also have more flexibility in specimen handling, thanks to other changes in the guideline.

Recognizing the need to further standardize preanalytical variables, the authors have extended the fixation time for HER2 specimens. Now, the fixation time is six to 72 hours, bringing it in line with the times used for ER/PgR testing. In the 2007 guideline, the time was six to 48 hours.

Another change addresses sample procurement. While the 2007 guideline implied that a tumor excisional sample was preferable because it provided more cells for study, says Dr. Hicks, HER2 testing on needle core biopsies has increased in recent years. The new guideline says the needle core biopsies are acceptable, though with clearly defined caveats.

“I think what’s been happening is people are doing the HER2 on the needles—if they’re negative, they’re calling them HER2 negative, and they’re not repeating the test,” says Dr. Hicks. “That may be fine 90, 95 percent of the time. But for that small number of patients who really are HER2 positive, and the needle didn’t get to that part of the tumor, they’re missing an opportunity to have a potentially life-prolonging or life-saving treatment.”

As with other difficult cases, the authors spent plenty of time grappling with this issue. Ultimately, they called for repeat testing in cases where the tumor is high grade; if there’s a limited amount of tissue on the needle core biopsy; if the needle core result falls into the equivocal range; or if the excisional sample contains a different component from that found on the core. The guideline also specifies the type of case when repeat testing is not warranted: if the test is negative and the tumor is grade one and

strongly ER/PgR positive.

[dropcap]E[/dropcap]ven as the new guideline aims for clarity, no one expects it to be the final word on HER2 testing.

If anything, pressure to improve testing will only grow down the road. New studies are exploring the possibility of treating patients with HER2-targeted antibodies without chemotherapy, Dr. Wolff says. “In that situation we have to be incredibly clear and certain that the tumor is HER2 positive or not. Because we could be in a situation where a patient might only receive HER2-targeted treatments in the future.”

When it comes time for another update, the authors will have a good model to follow, says Dr. Hicks: this most recent panel. “More than anything, we are grateful for all the hard work by the guideline panel members and staff from CAP and ASCO.”

No one expects guidelines to be chiseled in stone like commandments. Even if nothing changed— and when was the last time that happened in medicine?—“At some point people start saying, ‘This is an old guideline. It can’t be valid anymore,’” says Dr. Wolff. With the 2007 guideline, “We knew all along that at some point we would need to update the document. And we’ve been more or less waiting for that moment. Now was the time.”

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