Achieving 95% Cross-Methodological Concordance in HER2 Testing

Causes and Implications of Discordant Cases

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Abstract

We were interested in determining our concordance between fluorescence in situ hybridization (FISH) and a previously validated immunohistochemical HER2 assay to identify possible reasons for discordance and to determine if all reasons for discordance were addressed by the American Society of Clinical Oncology/College of American Pathologists guidelines. We reviewed 697 cases (2004-2007) in which HER2 immunohistochemical and FISH testing were concurrently done. Overall concordance between nonequivocal immunohistochemical and FISH results was 96%. Of the 19 discordant cases, 13 (68%) were interpreted as positive immunohistochemically but negative by FISH. The primary reason for this discordance was immunohistochemical interpretation. Weak stain intensity, granular staining, and interpretation in areas of crush artifact were identified as the most common issues. Of the 6 cases interpreted as immunohistochemically negative but FISH-positive, 2 were from patients known to be receiving trastuzumab at the time of biopsy, 1 was very close to the FISH equivocal category, and 4 cases had fewer than 1.5 CEP17 signals per cell (1 patient in this group was also receiving trastuzumab). Focusing on issues with HER2 immunohistochemical interpretation can improve concordance rates for immunohistochemically positive cases, but biologic reasons may explain some discordant immunohistochemically negative cases.

The human epidermal growth factor receptor 2 protein (ERBB2, CERB-B2, or HER2) is overexpressed in 10% to 25% of all breast carcinomas.1-4 The HER2 protein, a transmembrane tyrosine kinase receptor, is part of a complex pathway of signal transduction that affects cell proliferation, survival, motility, and adhesion.5 Breast carcinomas with HER2 overexpression have increased proliferation, altered motility and invasive potential, regional and distant metastases, accelerated angiogenesis, and reduced apoptosis.5 Amplification of the ERBB2 (HER2) gene is the primary mechanism of HER2 protein overexpression in most cases.6-8 Clinically, HER2 overexpression is associated with a more aggressive clinical course and increased resistance to nonanthracycline-, nontaxane-containing chemotherapy regimens.9 However, overall and recurrence-free survival improve with regimens that include anthracycline-based chemotherapy and HER2-targeted antibody therapies like trastuzumab and lapatinib. Correct assessment of HER2 status in breast carcinoma is therefore essential in guiding therapy-related decisions.

Three central methods to evaluate HER2 status are currently in clinical use: immunohistochemical analysis, fluorescence in situ hybridization (FISH), and chromogenic in situ hybridization (CISH). Immunohistochemical analysis detects the amount of HER2 protein at the cell membrane in a semiquantitative manner: incomplete or weak labeling of cell membranes typically equates with fewer than 185,000 receptors at the cell surface and no detectable amplification of the HER2 gene; strong, circumferential membrane staining correlates with more than 500,000 receptors at the cell surface, a level of expression almost always associated with...
HER2 amplification. FISH uses a fluorescent-labeled DNA probe to detect the relative copy numbers of the HER2 gene per cell. While less sensitive to technical factors that influence the reliability of immunohistochemical analysis (such as tissue fixation), FISH is more time- and labor-intensive and requires the use of a fluorescence microscope. CISH, like FISH, detects the HER2 gene copy number but uses a chromogenic detection method scored using standard bright-field microscopy. In current practice, immunohistochemical analysis is commonly used as the initial test for HER2 status, with FISH and CISH reserved for assessment of equivocal (2+) immunohistochemical results. However, studies indicate that as many as 20% of immunohistochemically positive (3+) breast carcinomas do not exhibit HER2 gene amplification in FISH assays performed at a centralized testing facility.11,12

In an attempt to further standardize HER2 testing and reduce discordance between laboratories and testing methods, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) published guideline recommendations for HER2 testing in 2007.9 These guidelines established standard testing and interpretive criteria for immunohistochemical analysis and FISH. Through these guidelines and enforcement of new inspection checklist items for laboratory practice, the CAP has effectively required all CAP-accredited laboratories performing HER2 testing to document 95% overall concordance with an external or internal validated method (the former typically the same basic testing platform, such as immunohistochemical analysis vs immunohistochemical analysis; the latter more often cross-platform, such as immunohistochemical analysis vs FISH) for non-equivocal results. So that we might gain a better understanding of how laboratories may improve concordance between in-house immunohistochemical and FISH methods for HER2 status, we investigated the causes of immunohistochemical-FISH discordance in our own practice.

Materials and Methods

After University of Washington Medical Center (UWMC; Seattle) Human Studies approval, we reviewed the HER2 status of 697 cases of breast carcinoma at UWMC on which immunohistochemical and FISH testing were performed between September 2004 and December 2007 (inclusive). Overall concordance rates between immunohistochemical and FISH results were determined, and all discordant cases were reviewed. Reasons for discordance were identified with an eye to improving performance of immunohistochemical and FISH methods and overall concordance between them.

The study population included cases referred directly for FISH testing (without prior HER2 testing by immunohistochemical analysis) as well as “in-house” cases at UWMC in which HER2 immunohistochemical analysis was performed and results were reported as equivocal. Our internal quality assurance protocol requires that all referral HER2 FISH cases also have immunohistochemical analysis for HER2 performed concurrently. The results of the latter immunohistochemical tests are recorded in our pathology database but are not reported. Cases without immunohistochemical and FISH results were excluded from further analysis.

Immunohistochemical analysis was performed using the avidin-biotin-peroxidase method (VectorStain Elite, Vector Labs, Burlingame, CA). Three 4-μm tissue sections cut from the formalin-fixed, paraffin-embedded tissue block were used. All slides were baked for 20 minutes at 60°C, dewaxed, and rehydrated through graded alcohols to xylene. The slides were rinsed and dehydrated through graded alcohols to buffer before immersion in a solution of 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase.

The first slide was run without heat-induced epitope retrieval (HIER). This “non-HIER” slide was blocked in normal goat serum (dilution 1:100; catalog No. S1000, Vector Labs) for 10 minutes and then incubated with the primary anti-HER2 antibody (polyclonal antibody, dilution 1:200; catalog No. A0485, DAKO, Carpinteria, CA) for 40 minutes at room temperature in a moist chamber. The second slide was run with HIER, 0.05 mol/L citrate buffer (pH 6.0), for 15 minutes at 98°C in a commercial microwave (model H2800, Energy Beam Sciences, East Granby, CT), cooled to room temperature (for 20 minutes), and then incubated with the primary antibody (diluted 1:400) in a manner otherwise identical to the non-HIER slide. The third slide, a methodological (negative) control, was handled the same as the HIER slide, except that the primary antibody was replaced by normal rabbit serum (dilution 1:8,000; MP BioMedicals, Solon, OH).

All slides were then subjected to sequential 30-minute immersion in biotinylated horse anti-rabbit antibody (1:500 dilution; Vector Labs) and avidin-biotin-peroxidase (1:100 dilution; catalog No. PK-6100, Vector Labs), followed by chromogen development, using 3,3′-diaminobenzidine tetrahydrochloride hydrate (catalog No. D5637-10G, Sigma, St Louis, MO) as the chromogen and hydrogen peroxide (3%; catalog No. 3306HP0316, MediChoice, Mechanicsville, VA) as the substrate. After hematoxylin counterstaining, slides were rinsed and dehydrated through graded alcohols to xylene and coverslipped using an automated coverslipper. Appropriate external positive control tests were performed concurrently with all HIER and non-HIER test slides.

Final HER2 immunohistochemical interpretation in cases requested for immunohistochemical testing was performed by members of a dedicated breast pathology service or by the immunohistochemical laboratory director. The HER2 immunohistochemical stains performed for quality assurance purposes in cases sent for FISH only were interpreted by the pathologist signing out the FISH results. HercepTest (DAKO)
criteria (10% of carcinoma cells with strong circumferential staining = 3+ positive) were used for HER2 stain interpretation before 2008. If nonneoplastic breast epithelium was present and was noted to have HER2 expression (assigned using the same criteria), the staining level of this internal control was “subtracted” from the level of staining in the carcinoma to avoid false-positive results.13

To control for overstaining during HIER (and as an additional quality assurance measure), our laboratory requires at least 1+ membrane-based staining for HER2 on the concurrently performed non-HIER slide if the same invasive carcinoma before a positive (3+) HER2 interpretation can be rendered on the HIER slide. Any HIER slide with apparent 3+ staining but without any membranous staining on the non-HIER slide is interpreted as equivocal and is referred for FISH testing.

FISH was performed manually according to the vendor’s protocol using the US Food and Drug Administration–approved Vysis PathVysion (Abbott-Vysis, Des Plaines, IL). Interpretation of FISH cases as positive before 2007 required an HER2/CEP17 ratio of at least 2.0 (<2.0, no amplification; ≥2.0, amplification); however, all FISH cases were reclassified according to the ASCO/CAP guidelines for the purposes of this study. In the latter, HER2/CEP17 ratios less than 1.8 or fewer than 4 HER2 signals per nucleus were considered HER2 nonamplified, ratios of 1.8 to 2.2 or carcinomas with 4 to 6 HER2 signals per nucleus were defined as equivocal, and ratios of more than 2.2 or more than 6 signals per nucleus were considered amplified. By using a fluorescence microscope with an appropriate filter set, tumor cells were counted until there was at least a 99% confidence interval that the measured mean ratio differed from 2.0 (Student t test) or until 60 cells were counted in representative fields previously chosen by a pathologist.

Concordance studies excluded cases in which the immunohistochemical or FISH results were equivocal. Discordance was defined as cases in which a positive or negative immunohistochemical result was accompanied by the opposite FISH result. Immunohistochemical cases with equivocal FISH results were excluded from the following calculations.

Overall concordance was calculated as follows:

\[
\frac{(\text{Immunohistochemically Positive + FISH-Positive Cases}) + (\text{Immunohistochemically Negative + FISH-Negative Cases})}{\text{Total Immunohistochemically Nonequivocal Cases}} = \text{Concordance}
\]

Positive assay concordance (concordance for immunohistochemically positive cases) was calculated as follows:

\[
\frac{\text{Immunohistochemically Positive + FISH-Positive Cases}}{\text{Total Immunohistochemically Positive Cases}} = \text{Positive Assay Concordance}
\]

Negative assay concordance (concordance for immunohistochemically negative and FISH-negative cases) was calculated as follows:

\[
\frac{\text{Immunohistochemically Negative + FISH-Negative Cases}}{\text{Total Immunohistochemically Negative Cases}} = \text{Negative Assay Concordance}
\]

Data from discordant results were reviewed, and patient histories were obtained when possible. Any cases identified as discordant that had been diagnosed before publication of the ASCO/CAP guidelines were reviewed and scored according to the 2007 ASCO/CAP guidelines by a panel of 3 pathologists who routinely interpret HER2 immunohistochemical stains (P.E.S., S.M.D., and K.H.A.) who were blinded to the FISH results. After rescoring, cases were reviewed again to identify and categorize possible reasons for discordance.

Results

The database search resulted in 700 cases in which FISH and immunohistochemical analysis had been performed between September 2004 and December 2007. Three cases had morphologically heterogeneous populations of invasive carcinoma that were considered carcinomas with potentially different biologic potentials, and thereby, the morphologically distinct populations were individually analyzed by immunohistochemical analysis and FISH and not counted as discordant cases. After the exclusions, 697 cases were analyzed. Table II summarizes the data generated by our database search.

Overall assay concordance was 96% (see concordance equation in “Materials and Methods”); 19 of 458 nonequivocal cases had discordant results. The original immunohistochemical stains from all 19 discordant cases were reviewed by 4 of us (K.H.A., P.E.S., S.M.D., and E.E.G.) who were initially blinded to the FISH results. All cases initially called immunohistochemically negative were also called immunohistochemically negative by this panel. However, 12 of the 13 cases initially recorded as immunohistochemically positive (3+) in our database were called equivocal by the panel. The single immunohistochemically positive discordant case in which the panel agreed with the 3+ interpretation was an alcohol-fixed (not formalin-fixed) cytology specimen. After initial

### Table II

<table>
<thead>
<tr>
<th>HER2 Immunohistochemical and FISH Results in 697 Cases*</th>
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<tbody>
<tr>
<td><strong>Immunohistochemical Results</strong></td>
</tr>
<tr>
<td>FISH</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Equivocal</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

* Data are given as number (percentage).
rescoring, immunohistochemical cases were rereviewed to identify the possible reasons for discordant cases.

Of 94 immunohistochemically positive cases with non-equivocal FISH results, 81 (86%) were concordant. Initially, 13 cases (1.9% of 697) were recorded as immunohistochemically positive and FISH-negative in our database. Of these discordant cases, 12 were received for FISH testing only and the immunohistochemical interpretations were recorded in our database for quality assurance purposes but were excluded from the final pathology report. The last of these was reported as 3+ positive immunohistochemically but with a comment that FISH would be ordered because the case was a low-grade invasive cancer that was not expected to be HER2+. This case was later called equivocal after review.

Reasons identified for immunohistochemically positive, FISH-negative discordance are listed in Table 2. The most common of these (occurring in 11 of 13 discordant cases) involved interpretation of the immunohistochemical staining characteristics. The most common issues with interpretation were interpreting weak to moderate circumferential staining as intense staining (7/11 [64%]), interpretation in areas of crush (3/11 [27%]), and interpreting granular staining as uniform membranous staining (2/11 [18%]). The ASCO/CAP guidelines, which were published during the end of the data collection period, define 3+ staining as “uniform, intense membrane staining in >30% of invasive tumor cells.” However, the new 30% threshold for 3+ staining did not change categorization of any of the cases in the discordant group because the character (intensity, granularity) rather than the quantity of the cells stained was the reason for misinterpretation. Examples of these misinterpretation errors are shown in Image II.

Another issue with the immunohistochemically positive, FISH-negative cases was interpretation of the non-HIER HER2 control. In 4 cases, the non-HIER stain lacked at least 1+ staining, which, according to our laboratory protocol, should have resulted in an interpretation no greater than a 2+ on the HIER slide (see the “Materials and Methods” section). However, in 3 of the 4 cases, the staining characteristics of the diagnostic HIER slide also had interpretation issues identified.

Of 364 immunohistochemically negative cases with non-equivocal FISH results, 358 (98.4%) were concordant. All 6 discordant cases were originally referred to UWMC for FISH analysis only; immunohistochemical interpretations were recorded in our database for quality assurance purposes only (as noted earlier) but were not included in the final report. It is interesting that 2 nonmethodology-based reasons for discordance were identified in this group of immunohistochemically negative, FISH-positive cases: (1) cases in which the patients were known to have received HER2-targeted antibody therapy, and (2) cases in which the patients had apparent chromosome 17 haploid genotypes. Clinical records were available for only 2 of the 6 cases, and both patients were receiving trastuzumab at the time of the biopsy. Patient histories were not available in the remaining 4 discordant cases. It is interesting that in 4 of the 6 immunohistochemically negative, FISH-positive cases, patients also had carcinomas with fewer than 1.5 CEP17 signals per cell, suggesting an apparent chromosome 17 haploid genotype. Of these 4 cases, 2 also had fewer than 4 HER2 signals per cell (possible “false-positive” FISH results by ratio alone). One case (case 16, Table 3) was near the equivocal range of 1.8 to 2.2 with a ratio of 2.36 and was counted twice by 2 different technologists.

### Table 2

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Inappropriate Interpretation of Immunohistochemical Staining Characteristics</th>
<th>Nonformalin Fixation</th>
<th>Quality Assurance Control Not Interpreted Correctly*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High background cytoplasmic staining; crush artifact</td>
<td>Alcohol-fixed cytology fluid</td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>2</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>3</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>4</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>5</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>6</td>
<td>Stain intensity not strong; granular staining</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>7</td>
<td>Crush artifact</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>8</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>9</td>
<td>Crush artifact</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>10</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>11</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>12</td>
<td>Stain intensity not strong</td>
<td></td>
<td>Non-HIER slide negative</td>
</tr>
<tr>
<td>13</td>
<td>Granular staining</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

* Non-HIER, non-heat-induced epitope retrieval (microwaved) slide.

* Per quality assurance policy, to control for possible overstaining due to antigen-retrieval methods, for an HER2 immunohistochemical result to be interpreted as 3+ positive on the HIER slide, the non-HIER slide must have at least 1+ staining.
**Image 1 A.** An HER2+ case by immunohistochemical analysis that was confirmed to be amplified by fluorescence in situ hybridization (FISH) (not a discordant case). **B-F.** Cases with problems in HER2 interpretation. Cases were initially interpreted as high overexpression by immunohistochemical studies but were not amplified by FISH. The review panel called all 5 cases equivocal (2+) by immunohistochemical studies. **B and C.** Cases with circumferential membranous staining in areas, but the review panel thought the staining intensity was not strong enough to qualify as 3+ (called 2+, or equivocal, by review panel). Of note, the carcinoma in **D** was well-differentiated, which is very uncommonly HER2+. **E.** Example of chatter artifact. **F.** Example of nonuniform “granular” membranous staining (**A-F.** x400).
Discussion

Accurate assessment of HER2 status is integral to the care of patients with invasive breast carcinoma. Several studies indicate that interlaboratory concordance rates for this growth factor receptor are less than 80%, raising considerable concern over the accuracy and precision of current HER2 testing. The ASCO/CAP guidelines now recommend that laboratories performing HER2 immunohistochemical studies show 95% concordance with another validated test for positive and negative assay values (an external comparison with the common method or an internal comparison with an alternative method) and provide standards for test performance and interpretation. In our practice, internal validation of FISH is based on concordance with a validated immunohistochemical assay. In our review of 697 cases in which immunohistochemical and FISH studies were performed at UWMC before these guidelines took effect (2004-2007), we achieved an overall positive and negative test concordance of 96%. We were interested in identifying causes for any discordant cases and examining whether the 2007 ASCO/CAP HER2 testing guidelines would have addressed all of these causes.

Among the 4% of cases that were discordant, immunohistochemically positive, FISH-negative cases accounted for the majority of discordant cases (13/19 [68%]). This pattern occurred in 13 (14%) of 94 immunohistochemically positive, nonequivocal FISH cases. Others have reported that false-positive immunohistochemical results are the most common problem with HER2 testing, but there is little detail in these studies regarding specific causes for the discordant result. The 2007 ASCO/CAP HER2 testing guidelines address a number of possible causes of immunohistochemical and FISH testing discordance, including time to fixation, method of tissue processing, total time in fixative, and type of fixation used. However, most of these factors are believed to contribute more reproducibly to a false-negative HER2 immunohistochemical result, an outcome that was much less common in our single-institution study.

In our analysis, HER2 immunohistochemical-FISH result discordance was considerably more likely to be an interpretive error than a technical error. The ASCO/CAP guidelines list the following interpretive criteria for a positive immunohistochemical result: (1) More than 30% of tumor must show circumferential membrane staining. (2) Membrane staining must be intense and uniform. (3) Membrane staining must be homogeneous (chicken wire). (4) Incomplete or pale membrane staining should be ignored.

The most common issue in immunohistochemical interpretation in our evaluation of discordant cases was the interpretation of weak to intermediate circumferential staining as intense staining (54% of immunohistochemically positive, FISH-negative discordant cases). To maximize positive immunohistochemical-FISH result concordance, we recommend strict adherence to the requirement for thick, homogeneous, dark circumferential membrane-based staining. Additional interpretive issues in our study included interpreting granular staining as uniform, and interpreting partial membrane staining of cells as complete circumferential staining. Interpretation of areas with significant crush artifact was also common. The current ASCO/CAP guidelines recommend that areas of crush artifact in HER2 immunohistochemical stains not be interpreted. Image 1 contains examples of these staining pitfalls.

It is interesting that the increase in the threshold for a positive immunohistochemical result to 30% of cells (ASCO/CAP) from 10% (HercepTest) was not a reason for discordance in our study. The principal (but as yet unvalidated) argument for the 30% cutoff is that the requirement for a higher percentage of HER2+ cells decreases interobserver variability in the interpretation of immunohistochemical stains and improves immunohistochemical-FISH concordance. Two recent studies by Hameed et al and seem to support this claim but were limited by reporting immunohistochemically positive rates higher than expected and immunohistochemical-FISH result concordance well below optimum. However, the reported decrease in interobserver variability was relatively small (2% difference), and the increased concordance with FISH was not statistically significant. The fact that use of the 30% threshold did not result in recategorization of any discordant cases in our study may be related to study design because we did not systematically evaluate how this threshold would affect interpretation and concordance. Nevertheless, we believe that using a strict
percentage positive threshold may misleadingly exclude cases with heterogeneous populations of HER2 staining. More important, manipulations of threshold do not directly address problems with the semiquantitative interpretation of overall staining intensity and character, precisely the issues this study identified as most common (and potentially correctable).16

Another reason identified for immunohistochemically positive, FISH-negative discordance in our study was interpretations that did not follow our internal quality assurance guidelines with special attention to appropriate interpretations that did not follow our internal quality assurance-based interpretive criteria using non-HIER controls. Specifically, our institution controls for possible HIER-induced overstaining for HER2 by preparing a non-HIER slide in parallel for each patient. For externally reported results, we require at least 1+ staining on the non-HIER slide before its paired HIER slide can be interpreted as positive, even if it independently meets criteria for a 3+ stain. A 3+ HIER stain paired with a nonreactive non-HIER slide may represent a false-positive result due to epitope retrieval or some other condition of tissue processing or slide preparation that is exploited by HIER (neoantigens and conditional antigens). Institutions not comparing HIER and non-HIER slides might instead use a consensus HER2 immunohistochemical equivocal (2+) case as part of the external positive control (an element that is lacking in some commercial positive controls) to assess for technical irregularities that could lead to erroneous results. The production of a visibly positive or negative stain in the “equivocal” control sample might reasonably alert a pathologist to a possible technical irregularity that requires further investigation.

One immunohistochemically positive, FISH-negative case in our series was initially interpreted as HER2+ on the basis of the immunohistochemical stain but was referred for FISH testing because the invasive carcinoma was very well-differentiated (Nottingham grade 1 of 3) and strongly positive for estrogen and progesterone receptor proteins by immunohistochemical analysis. Although subsequent blinded rereview of this immunohistochemical result for the purposes of this study reclassified the immunohistochemical stain as equivocal, the case offers an important reminder that any testing paradigm that bases reflexive FISH testing on immunohistochemical results should remain flexible enough (or be used by pathologists sufficiently mindful of clinical, histologic, and complementary immunohistochemical findings) to allow decisions “on the fly” to test unexpected HER2 immunohistochemical results by FISH as well.

Based on our analysis of immunohistochemically positive, FISH-negative discordant cases, issues with immunohistochemical interpretation were identified as the primary reason for this discordance. We believe these problems can be avoided by strict adherence to the current ASCO/CAP guidelines with special attention to appropriate interpretations of staining intensity and use of quality assurance controls. Preanalytic variables such as fixation time were not directly addressed by this study (the majority of cases in this study were tested before adoption of the 2007 ASCO/CAP guidelines), but only 1 of the immunohistochemically positive, FISH-negative discordant cases that was an alcohol-fixed cytology specimen was thought to be a true false-positive immunohistochemical result on rereview.17 Ensuring that pathologists who interpret HER2 immunohistochemical stains see a high enough volume of these cases, are well-trained in interpretation, and are aware of diagnostic pitfalls will likely decrease problems with HER2 immunohistochemical interpretation. At UWMC during the period of this study (which achieved a 96% overall concordance rate), a limited group of pathologists (each with substantial experience with this technique) routinely interpreted HER2 stains, including members of a dedicated breast pathology service, the immunohistochemical laboratory director, and pathologists who also interpreted HER2 FISH tests.

In our experience, negative immunohistochemical assay concordance is more consistently achieved than positive assay concordance. Our negative immunohistochemical assay concordance was 98.4% (6 of 364 cases were discordant). This smaller group of discordant immunohistochemically negative, FISH-amplified cases in our study did not seem to have issues with immunohistochemical interpretation. Instead, potential biologic reasons were identified that could explain this discordance in 4 of the 6 cases, including patients receiving HER2-targeted therapy before HER2 testing and cancers with fewer than 1.5 CEP17 signals per cell.

The effects of HER2-targeted treatments on HER2 testing are not well described in the literature, but it stands to reason that antibodies targeting the HER2 cell surface receptor may result in down-regulation of this receptor without affecting the gene amplification status. Because the lack of immunohistochemically detectable receptors may reflect the true biologic phenotype of these treated cancer cells, a negative immunohistochemical result in this setting should not be considered a clinically discordant result. While the benefit of additional HER2-targeted therapy in a previously treated and now immunohistochemically negative, FISH-positive cancer remains unclear, knowing the immunohistochemical and FISH results in such a case may have clinical usefulness. Therefore, we recommend that tissue sent for HER2 testing in patients who have already received HER2-targeted therapy should undergo immunohistochemical and FISH testing until the clinical significance of these results is known. Until then, efforts should be made to exclude such cases from concordance studies.

It is interesting that the immunohistochemically negative, FISH-positive group in our study was enriched for cases with fewer than 1.5 CEP17 signals per cell by FISH analysis, which occurred in 4 of the 6 cases. Monosomy seems to be
a rare event, occurring in 2% of cases in some series.18-20 Similar to polysomy, CEP17 monosomy often seems to reflect loss of the CEP17 locus rather than the entire chromosome.19,20 However, there are scarce data on response to HER2-targeted therapy in these cases. In fact, the discordant immunohistochemically negative, FISH-positive cases with apparent chromosome 17 monosomy could represent false-positive FISH results when positivity is defined by ratio alone. Further studies evaluating the clinical response to HER2-targeted therapy in this setting are required to determine the significance of these results.

One case from our immunohistochemically negative, FISH-positive group was borderline equivocal by FISH and had to be counted twice before it was interpreted as weakly amplified (case 16, Table 3). The equivocal or borderline FISH category was created by the 2007 ASCO/CAP guidelines; however, the likelihood of patient benefit from HER2-targeted therapy within this group remains unstudied. The ASCO/CAP guidelines recommend additional immunohistochemical testing in FISH-equivocal cases to determine if either assay can give a nonequivocal result from which therapy decisions can be made. More data on equivocal FISH cases are required to determine if the immunohistochemical results in these cases can predict which patients will reproducibly respond to HER2 therapy. FISH-equivocal cases should be excluded from concordance studies until the biologic significance of the immunohistochemical results in this setting is understood.

Because the majority of cases in this study were tested before requirements for formalin fixation time were mandated, we could not control for formalin fixation time as a factor. We cannot confirm or exclude that underfixation or overfixation was an additional factor in the discordant immunohistochemically negative, FISH-positive cases. Fixation issues should be minimized in the post-ASCO/CAP guidelines era, and we agree with the recommendation that fixation of all tissues (core biopsy specimens included) for a minimum of 6 to 8 hours will ensure more consistent testing results.

Our data suggest that an overall concordance rate of more than 95% for HER2 cross-methodological testing is achievable in a high-volume laboratory. We found that the most common type of discordance was immunohistochemically positive, FISH-negative cases and that issues with interpretation of immunohistochemical staining characteristics were the primary reasons for this discordance. Strict adherence to the ASCO/CAP guidelines during immunohistochemical interpretation, including knowledge of interpretation pitfalls, together with the added interpretive stringency provided by using controls that assess subtle issues in stain intensity variation, can significantly minimize cases of immunohistochemically positive, FISH-negative discordance. Immunohistochemically negative, FISH-positive discordance was significantly less common and is more often related to biologic phenomena, including concurrent trastuzumab therapy, fewer than 1.5 CEP17 signals per cell, and borderline equivocal FISH results. Cases with a biologic explanation for immunohistochemical and FISH discordance should be excluded from concordance studies.

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References


