

Comparison of fluorescent *in situ* hybridization *HER-2/neu* results on core needle biopsy and excisional biopsy in primary breast cancer

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***HER-2/neu* status is critical for the therapy for breast carcinoma. Fluorescent *in situ* hybridization for gene amplification and immunohistochemical stains for protein expression are widely used methods to detect *HER-2/neu* status. Multiple studies have shown fluorescent *in situ* hybridization and immunohistochemical stain results to have high concordance rates. To our knowledge, a comparison between fluorescent *in situ* hybridization results for core needle biopsy and the subsequent excisional biopsy specimens has not yet been studied. We retrospectively evaluated the fluorescence *in situ* hybridization and immunohistochemical results in both the breast core needle and the excisional biopsy of 125 patients with invasive breast carcinoma from 2002 to 2005. There was complete concordance with respect to both immunohistochemical and fluorescence *in situ* hybridization results for core needle biopsy and excisional biopsy specimens in 87% of the patients evaluated. Comparison of fluorescent *in situ* hybridization results of the 129 core needle biopsies to the 131 excisional biopsies of all 125 patients showed a concordance rate of 92%. The immunohistochemical stain results of the same core needle and excisional biopsies showed a concordance rate of 98%. Comparison of the immunohistochemical stain results with the fluorescent *in situ* hybridization results for all 260 cases examined showed 95% concordance. On the basis of our study, we observed that repeating *HER-2/neu* testing by immunohistochemical stain and/or fluorescent *in situ* hybridization methods on excisional biopsy is not unreasonable, in particular in cases of intratumoral heterogeneity, indeterminate/borderline *HER-2/neu* results and after neoadjuvant chemotherapy.**

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Determination of tumor *HER-2/neu* receptor overexpression/amplification status is one of the most important clinical assessments in the standard workup for breast carcinoma because its presence warrants the addition of trastuzumab (Herceptin) to the patient's medical oncology regimen.

HER-2/neu testing to evaluate for trastuzumab therapy is a prototypical example of targeted treatment. Currently, there are two widely used methods for determination of *HER-2/neu* status, fluorescent

in situ hybridization for the gene amplification and immunohistochemical studies for protein overexpression. Common practice is to perform immunohistochemical stains on all breast cancer specimens and subsequently perform fluorescent *in situ* hybridization only on specimens with an *HER-2/neu* immunohistochemical stain score of 2 (on a scale of 0–3). At institutions following this protocol, fluorescent *in situ* hybridization is not performed on specimens with immunohistochemical scores of 0 or 1, which are interpreted as no overexpression of the *HER-2/neu* receptor, or with an immunohistochemical score of 3, which is interpreted as overexpression of the *HER-2/neu* receptor. Also, *HER-2/neu* status is often performed on either the core needle or the excisional biopsy specimen and usually not on both. The core needle biopsy sample is often used to study breast biomarkers when neoadjuvant chemotherapy is

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planned. To the best of our knowledge, no study has compared immunohistochemical stains and fluorescent *in situ* hybridization of *HER-2/neu* results on the core needle biopsy and excisional biopsy from the same patient. We sought to determine the correlation between *HER-2/neu* fluorescent *in situ* hybridization and immunohistochemical stain results on core needle biopsy and the corresponding excisional biopsy on a selected patient population.

In 2007, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) revised the guidelines for the evaluation of *HER-2/neu* overexpression by immunohistochemical stain in an attempt to optimize the accuracy of *HER-2/neu* testing in breast cancer, based on the notion that approximately 20% of immunohistochemical stain test results from community-based laboratories may have been inaccurate when compared to high-volume reference laboratories.¹ In addition, the guidelines for the evaluation of *HER-2/neu* amplification by fluorescent *in situ* hybridization have been updated, incorporating a 'borderline' category (fluorescent *in situ* hybridization ratios ≥ 1.8 and ≤ 2.2) that is neither amplified nor not amplified.

After review of the correlation data, discrepant cases were identified from our study. Three pathologists blindly re-reviewed these cases to try to rectify these discrepancies. We re-reviewed the *HER-2/neu* immunohistochemical stain slides of the cases with immunohistochemical stain/fluorescent *in situ* hybridization discrepancies, core needle biopsy/excisional biopsy discrepancies, and/or borderline fluorescent *in situ* hybridization ratios using ASCO/CAP 2007 guidelines to see if the new guidelines may provide more consistent results.

Materials and methods

Sample Collection

A total of 125 patients with invasive carcinoma of the breast present in both the breast core needle biopsy and the subsequent excisional biopsy (lumpectomy or mastectomy) were identified from 2002 to 2005 at our institution, including 5 patients who received neoadjuvant chemotherapy. A standard breast biomarker panel was performed on each specimen, composed of immunohistochemical stains for estrogen receptor, progesterone receptor, Ki-67 proliferative index, and *HER-2/neu* receptor as well as fluorescent *in situ* hybridization for *HER-2/neu* amplification. This panel was ordered reflexively on all core needle biopsy and on the excisional biopsy block containing the largest amount of invasive carcinoma. The immunohistochemical stains were evaluated by the attending pathologists within 2–3 days of ordering the breast biomarkers panel. Fluorescent *in situ* hybridization analysis was ordered on all cases before evaluation of the immunohistochemical stain and performed

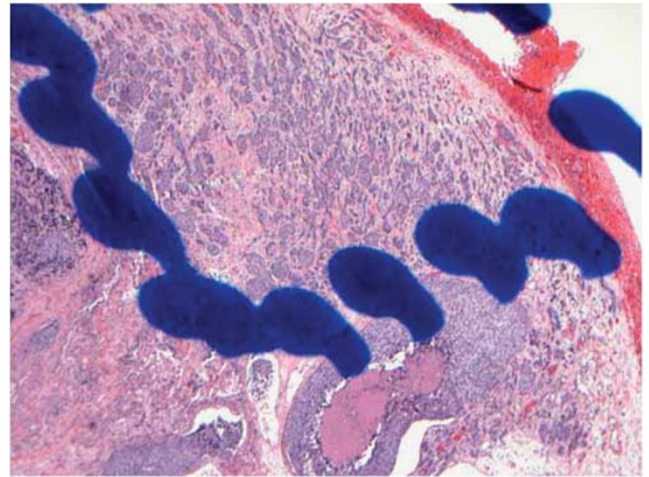


Figure 1 H&E slide with invasive carcinoma designated by the attending pathologist.

regardless of and independent of the corresponding immunohistochemical stain result. The fluorescent *in situ* hybridization analysis was signed out by the Cytogenetics laboratory at our institution approximately 2 weeks after the immunohistochemical stain scores were reported. At our institution, *HER-2/neu* tests are carried out using the FDA approved HercepTest kit for immunohistochemical stain and Vysis PathVysion for fluorescent *in situ* hybridization.

Immunohistochemical Stain for *HER-2/neu* Using HercepTest

Paraffin sections were cut at 4 μm thickness and baked for 1 h at 60°C. Immunohistochemistry was performed using the HercepTest kit (Dako Corp., Carpinteria, CA, USA) according to kit instructions with positive and negative control slides included in each run.

Areas of invasive carcinoma were assessed by the attending pathologists and scored on a scale of 0–3 according to the original FDA approved guidelines before ASCO/CAP 2007.

HER-2/neu Fluorescent *In Situ* Hybridization Using Abbott-Vysis PathVysion

An H&E slide and four unstained paraffin slides were cut from the core needle biopsy block and from the block of tumor representing the largest volume of invasive carcinoma from the excisional biopsy. Two of the unstained slides were cut at a 4 μm thickness. The invasive component of the carcinoma that did not include ductal carcinoma *in situ* (DCIS) was designated on the H&E slide by the attending pathologist (Figure 1). The corresponding area with invasive carcinoma was studied with the fluorescent *in situ* hybridization protocol. Dual-color fluores-

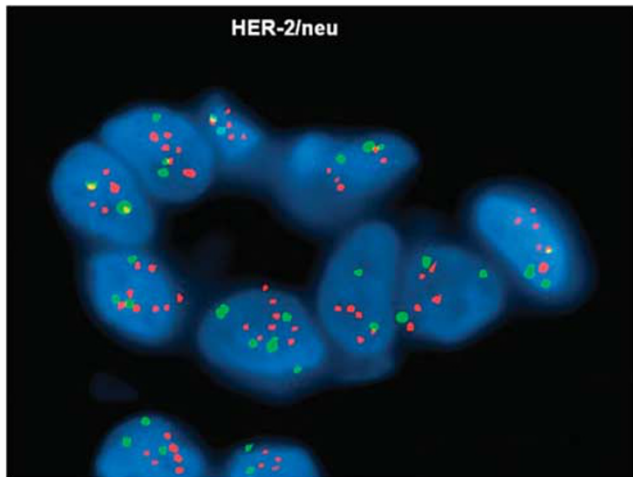


Figure 2 Dual-color FISH with HER-2 (red) and chromosome 17 centromere (green) probes with no amplification.

cent *in situ* hybridization was performed with the *HER-2* probe labeled with spectrum red and chromosome 17 specific centromere (D17Z1) probe labeled with spectrum green on sections cut from the same block. Deparaffinization, *in situ* hybridization, and staining were performed using the PathVysion kit (Abbott-Vysis Lab, Abbott Park, IL, USA) as per the manufacturer's protocols. Fluorescent signals in at least 60 nonoverlapping interphase nuclei with intact morphology were scored using a Zeiss Axioplan 2 microscope with a $\times 100$ planar objective, using a triple band-pass filter that permits simultaneous blue, green, and red colors. Only tumor cells from the site designated on the H&E slide by the pathologist were scored for the number of red (*HER-2*) and green (chromosome 17) signals. As recommended by the FDA-approved PathVysion *HER-2* kit at the time of original interpretation, a case was scored as amplified if the ratio of the number of fluorescent signals of *HER-2* to chromosome 17 (R/G) was ≥ 2.0 (Figures 2 and 3).

***HER-2/neu* Immunohistochemical Stain Slide Re-Review**

After the results were tabulated, we selected 18 patients with 36 corresponding core needle biopsy or excisional biopsy cases with discrepant *HER-2/neu* results and/or a borderline fluorescent *in situ* hybridization ratio (defined as a fluorescent *in situ* hybridization ratio ≥ 1.8 and ≤ 2.2) for re-review. Six cases were unavailable because the slides were returned to the original institution; therefore, 30 cases were re-reviewed. Cases were determined to be discrepant if the fluorescent *in situ* hybridization results, immunohistochemical stain results, or both were inconsistent for the core needle biopsy or the excisional biopsy. Also, cases with immunohistochemical stain results that did not concur with the fluorescent *in situ* hybridization results were iden-

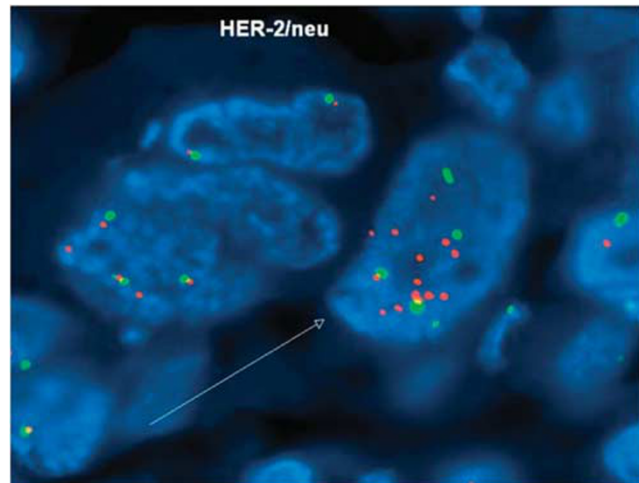


Figure 3 Dual-color FISH with HER-2 (red) and chromosome 17 centromere (green) probes with amplification.

tified as discrepant. Slides were retrieved from our storage area after obtaining institutional IRB approval. A total of three pathologists reviewed and scored all available slides independently using the ASCO/CAP 2007 guidelines. An average score was obtained from the three scores on each case.

The 'old' FDA immunohistochemical stain scoring system:

- 0 = no or incomplete membrane staining in $< 10\%$ of tumor: no overexpression
- 1 = weak, partial membrane staining: no overexpression
- 2 = weak to moderate, complete membrane staining in $> 10\%$ of tumor: indeterminate
- 3 = strong, complete membrane staining in $> 10\%$ of tumor: overexpression

The 'new' 2007 ASCO/CAP guideline scoring system:

- 0 = no staining is observed in invasive tumor cells: no overexpression
- 1 = weak, incomplete membrane staining in any proportion of invasive tumor cells, or weak, complete membrane staining in less than 10% of cells: no overexpression
- 2 = complete membrane staining that is nonuniform or weak but with obvious circumferential distribution in at least 10% of cells, or intense complete membrane staining in 30% or less of tumor cells: equivocal
- 3 = uniform intense membrane staining of more than 30% of invasive tumor cells: overexpression.

Table 1 Discordant cases between CNB and EXC

| Case no. | CNB IHC | CNB FISH | EXC IHC | EXC FISH |
|----------|--|------------------------------------|---------|----------|
| 75 | 1 | 1.3 | 3 | 11.8 |
| 108 | 0 | 1.6 | 3 | 3.7 |
| 9 | 1 | 2.4 | 1 | 0.9 |
| 16 | 1 | 2.4 | 0 | 1.4 |
| 47 | 3 | 2.1 | 2 | 1.6 |
| 52 | 1 | 3 | 1 | 1.0 |
| 68 | 3 | 2.3 | 2 | 1.0 |
| 46 | 3 | 1.4 | 3 | 2.7 |
| 54 | 1 | 1.4 | 1 | 2 |
| 43 | 2 | 4.2 | 2 | 0.9 |
| 66 | 1 | 1.3 | 3 | 1.5 |
| | FISH amplification IHC overexpression | No overexpression or amplification | | |

CNB, core needle biopsy; EXC, excisional biopsy; FISH, fluorescent *in situ* hybridization; IHC, immunohistochemical stain.

Results

The total number of specimens evaluated from 125 patients was 260, composed of 129 core biopsies and 131 excisional biopsy specimens. Data analysis was carried out using the criteria before the ASCO/CAP 2007 guidelines for *HER-2/neu* testing.

Discordant Core Needle Biopsy/Excisional Biopsy Data for Fluorescent *In Situ* Hybridization and Immunohistochemical Stain

There was complete concordance with respect to both immunohistochemical stain and fluorescent *in situ* hybridization results for biopsy and excisional biopsy specimens in 87% of the patients evaluated (109 of 125) (Table 1). Of the remaining 13% of patients, 2% (2 of 125) showed discordance in core needle biopsy vs excisional biopsy results, with the immunohistochemical stain and fluorescent *in situ* hybridization results for the core needle biopsy concurring, yet opposing those of the excisional biopsy specimen; 6% (8 of 125) showed inconsistencies in only the fluorescent *in situ* hybridization results between the core needle biopsy and excisional biopsy; 1% (1 of 125) had only disparate immunohistochemical stain results between the core needle biopsy and excisional biopsy; and 4% (5 of 125) had concordant fluorescent *in situ* hybridization results and concordant immunohistochemical stain results, yet the fluorescent *in situ* hybridization and immunohistochemical stain results opposed each other. Of the 260 specimens evaluated, 5% (13 of 260) showed inconsistencies between the immunohistochemical stain and fluorescent *in situ* hybridization results, accounting for 9% of our patient population (11 of 125). Of these, 4% (5 of 125) showed neither immunohistochemical stain nor fluorescent *in situ* hybridization discrepancy, and 5% (6 of 125) had either immunohistochemical stain or fluorescent *in situ* hybridization results that did not concur.

Two patients (2% of 125) showed complete discordance between their core needle biopsy and

excisional biopsy results. For both patients (cases 75 and 108, Table 1), the biopsy immunohistochemical stain showed no overexpression of the *HER-2/neu* and the fluorescent *in situ* hybridization analysis similarly showed no amplification, whereas the examination of the excisional biopsy specimens of both cases showed both overexpression by immunohistochemical stain and gene amplification by fluorescent *in situ* hybridization. Polysomy of chromosome 17 was identified by fluorescent *in situ* hybridization on the biopsy specimen in one case (case 108). Slide re-review revealed that these two cases show differences in tumor nuclear grade between the core needle biopsy and excisional biopsy, with the excisional biopsy showing more poorly differentiated tumor cells. The inherent small nature of the core needle biopsy may not provide complete representation of the tumor, resulting in discordance due to sampling. Furthermore, the excisional biopsy may show more aggressive population of tumor with the higher nuclear grade, resulting in discordance due to qualitative morphological differences within the same tumor, as seen in cases 75 and 108. Heterogeneity of *HER-2/neu* status in a tumor sample may occur.

Comparing Fluorescent *In Situ* Hybridization Data Between Core Needle Biopsy and Excisional Biopsy

Fluorescent *in situ* hybridization testing showed 85% of all specimens to show no *HER-2/neu* amplification and 15% to show *HER-2/neu* gene amplification (see Tables 1 and 2). Of the 125 patients evaluated, 115 (92%) had fluorescent *in situ* hybridization core needle biopsy results that were concordant with the corresponding excisional biopsy results. Ten patients (8% of 125 patients) showed inconsistent fluorescent *in situ* hybridization results between their core needle biopsy and excisional biopsy specimens. Two of these cases (cases 75 and 108), referred to above, show complete discordance of *HER-2/neu*

Table 2 FISH concordant and discordant data between core needle and excisional biopsies

| | | Number of patients | | Patients (%) |
|------------|-----|-----------------------|-----|--------------|
| Concordant | 92% | Concordant negative | 102 | 82% |
| | | Concordant positive | 13 | 10% |
| Discordant | 8% | Discordant: CNB+/EXC- | 6 | 5% |
| | | Discordant CNB-/EXC+ | 4 | 3% |
| Total | | | 125 | 100% |

CNB, core needle biopsy; EXC, excisional biopsy; FISH, fluorescent *in situ* hybridization.

immunohistochemical stain and fluorescent *in situ* hybridization results between the core needle biopsy and excisional biopsy. If these 2 patients are excluded, 8 of the remaining 123 patients, representing 7% of our patient pool, had discordant fluorescent *in situ* hybridization results. Six of these cases (5% of the 123 patients; cases 9, 16, 43, 47, 52, and 68; Table 1) showed fluorescent *in situ* hybridization amplification on the core needle biopsy specimen with no *HER-2/neu* amplification on the excisional specimen. Two cases (2% of 123 patients, cases 46 and 54; Table 1) showed no gene amplification on core needle biopsy with amplification at the time of tumor excision. These patients fell within the borderline fluorescent *in situ* hybridization category, one with a core needle biopsy fluorescent *in situ* hybridization ratio of 2.07 and an excisional biopsy ratio of 1.6 and the other had a core needle biopsy fluorescent *in situ* hybridization ratio of 1.4 and an excisional biopsy ratio of 2.0. One possible source of discordance could be 'borderline' cases. Before ASCO/CAP 2007 guidelines, a fluorescent *in situ* hybridization ratio ≥ 2.0 was scored as amplified according to FDA guidelines. The ASCO/CAP 2007 guidelines for *HER-2/neu* proposed the category of 'equivocal' for tumors with fluorescent *in situ* hybridization ratio of 1.8 and 2.2. On the basis of the ASCO/CAP guidelines, we found that our two patients will fall into this 'equivocal' category and may have not received trastuzumab therapy. Interestingly, the corresponding immunohistochemical stain score of one of these equivocal fluorescent *in situ* hybridization cases was distinctly positive (case 47 with an immunohistochemical stain score of 3) whereas the other was negative (case 54 with an immunohistochemical stain score of 1).

Comparing the Immunohistochemical Stain Data Between Core Biopsy and Excisional Biopsy Specimens

Immunohistochemical stains performed on the entire set of specimens showed 77% to be negative for overexpression ($n = 199$; 20% of all specimens scored as 0 and 57% scored as 1), 15% to be indeterminate for overexpression ($n = 40$; immuno-

Table 3 Immunohistochemical stain concordant/discordant data between core needle and excisional biopsies

| | No. of patients | Patients (%) |
|----------------------|-----------------|--------------|
| Concordant negative | 83 | 66% |
| Concordant positive | 6 | 5.0% |
| Indeterminate | 33 | 26% |
| Discordant CNB+/EXC- | 0 | 0% |
| Discordant CNB-/EXC+ | 3 | 2% |
| Total | 125 | 100% |

CNB, core needle biopsy; EXC, excisional biopsy.

histochemical stain score as 2), and 8% to show overexpression ($n = 21$; immunohistochemical stain score 3) (Tables 1 and 3).

Of 125 patients, 3 (2%, cases 66, 75, and 108) showed discordant immunohistochemical stain results between their core needle biopsy and excisional biopsy specimens. Two of these three cases, described above (cases 75 and 108), show complete discordance with respect to their core needle biopsy and excisional biopsy immunohistochemical stain and fluorescent *in situ* hybridization data. Excluding these two cases, our data show only 1% discordant core needle biopsy and excisional biopsy data by immunohistochemical stain. Our data show immunohistochemical stain studies to be more consistent than fluorescent *in situ* hybridization studies with 99.0% (122 of 123 patients) and 94.0% (115 of 123 patients) concordance, respectively.

Comparing the Immunohistochemical Stain and Fluorescent *In Situ* Hybridization Data

Of the 260 specimens, the concordance rate of fluorescent *in situ* hybridization and immunohistochemical stain was 95% (Tables 4 and 5). Thirteen specimens (5% discordant) representing 11 patients (9% of 125 patients) are subdivided as follows: Three of these cases represented overexpression of the *HER-2/neu* by immunohistochemical stain with no fluorescent *in situ* hybridization amplification. Ten cases showed no overexpression by immunohistochemical stain with amplification by fluorescent *in situ* hybridization. Of the three cases that showed immunohistochemical stain overexpression

without fluorescent *in situ* hybridization amplification (cases 33, 46, and 66), one showed polysomy of chromosome 17 that was determined at the time of fluorescent *in situ* hybridization analysis (case 66). No evidence of polysomy was identified in the other two cases. The two other cases showed no identifiable reason for discrepancy.

'Borderline' Cases by Fluorescent *In Situ* Hybridization

Five patients showed 'borderline' *HER-2/neu* amplification on fluorescent *in situ* hybridization (data shown in Table 6). At the time of fluorescent *in situ* hybridization interpretation, ratios greater than 2.0 were designated as amplified. Yet, their fluorescent *in situ* hybridization scores fall within the interval designated as borderline in the 2007 ASCO/CAP guidelines, with a ratio ≥ 1.8 and ≤ 2.2 . Two of the patients (cases 11 and 78) showed persistent borderline amplification on the core needle biopsy and excisional biopsy specimens with core needle biopsy/excisional biopsy fluorescent *in situ* hybridization scores of 2.28/2.0 and 2.16/2.04, respectively. Case 70 showed borderline amplification on the core needle biopsy specimen (ratio of 2.2) and definitive amplification on the excisional biopsy specimen (ratio of 2.79). Case 47 showed borderline

fluorescent *in situ* hybridization amplification on the core needle biopsy specimen (ratio of 2.07) and no amplification on the excisional biopsy specimen (ratio of 1.6). Case 54 showed no fluorescent *in situ* hybridization amplification on the core needle biopsy specimen (ratio of 1.4) with borderline amplification on the subsequent excisional biopsy specimen (ratio of 2.0).

Effect of Neoadjuvant Chemotherapy

Five patients received neoadjuvant chemotherapy; all showed no differences in *HER-2/neu* expression when evaluated by immunohistochemical stain or fluorescent *in situ* hybridization (data not shown). The one case (case 80) with *HER-2/neu* amplification by fluorescent *in situ* hybridization at the time of core needle biopsy showed persistent amplification after receiving chemotherapy at the time of tumor excisional biopsy. The four cases (cases 113, 116, 122, and 124) without *HER-2/neu* amplification on core needle biopsy continued to not have amplification on excision by fluorescent *in situ* hybridization.

Immunohistochemical Stain Re-Review of Discrepant Cases

From 18 patients, 36 were found to have discrepant *HER-2/neu* results by immunohistochemical stain,

Table 4 FISH data on the total number of patients

| | FISH+ | FISH- | Total | Total (%) | |
|-----------|-------|-------|-------|-----------|----------------|
| 0 | 0 | 52 | 52 | 20.0 | 0 or +1 77% |
| 1+ | 10 | 137 | 147 | 57 | |
| 2+ | 12 | 28 | 40 | 15 | 5 borderline |
| 3+ | 18 | 3 | 21 | 8 | 2 borderline |
| Total | 40 | 220 | 260 | | |
| Total (%) | 15.0 | 85 | | | Discordant |

FISH, fluorescent *in situ* hybridization.

Table 6 Borderline FISH results on core needle biopsy and excision

| Case no. | CNB FISH ratio | EXC FISH ratio |
|----------|----------------|----------------|
| 11 | 2.28 | 2.0 |
| 47 | 2.07 | 1.6 |
| 54 | 1.4 | 2.0 |
| 70 | 2.2 | 2.79 |
| 78 | 2.16 | 2.04 |

CNB, core needle biopsy; EXC, excisional biopsy; FISH, fluorescent *in situ* hybridization.

Table 5 Discordance between IHC and FISH

| Case no. | CNB IHC | CNB FISH | EXC IHC | EXC FISH |
|----------|--|--|---------|----------|
| 9 | 1 | 2.3 | 1 | 0.98 |
| 13 | 1 | 2.6 | 1 | 2.67 |
| 16 | 1 | 2.41 | 0 | 1.43 |
| 33 | 1 | 0.91 | 2 | 0.94 |
| 40 | 1 | 2.7 | 2 | 2.6 |
| 46 | 3 | 1.36 | 3 | 2.69 |
| 52 | 1 | 3 | 1 | 1.04 |
| 63 | 1 | 2.5 | 1 | 2.53 |
| 66 | 1 | 1.3 | 3 | 1.52 |
| 78 | 2 | 2.16 | 1 | 2.04 |
| 54 | 1 | 1.4 | 1 | 2 |
| | IHC overexpress, FISH no amplification | IHC no overexpress, FISH amplification | | |

CNB, core needle biopsy; EXC, excisional biopsy; FISH, fluorescent *in situ* hybridization; IHC, immunohistochemical stain.

Table 7 Comparison of *Her-2/neu* results between pre- and post-ASCO/CAP guidelines

| Case no. | Average 'new' IHC score | Original FISH | Original IHC |
|----------|-------------------------|---------------|--------------|
| 1 | 1 | 1.56 | 0 |
| 2 | 2 | 2.79 | 2 |
| 3 | 1 | 1.3 | 1 |
| 4 | 1 | 3 | 1 |
| 5 | 2 | 1.04 | 1 |
| 6 | 2 | 1.3 | 1 |
| 7 | 2 | 1.4 | 1 |
| 8 | 1 | 2 | 1 |
| 9 | 2 | 3.74 | 3 |
| 10 | 1 | 2.16 | 2 |
| 11 | 1 | 1.03 | 2 |
| 12 | 2 | 2.31 | 3 |
| 13 | 2 | 1.52 | 3 |
| 14 | 2 | 2.07 | 3 |
| 15 | 1 | 2.7 | 1 |
| 16 | 1 | 1.6 | 2 |
| 17 | 2 | 1.36 | 3 |
| 18 | 1 | 2.35 | 1 |
| 19 | 1 | 0.98 | 1 |
| 20 | 1 | 2.28 | 2 |
| 21 | 2 | 2 | 2 |
| 22 | 1 | 2.6 | 1 |
| 23 | 1 | 2.67 | 1 |
| 24 | 2 | 2.04 | 1 |
| 25 | 0 | 1.43 | 0 |
| 26 | 1 | 2.6 | 2 |
| 27 | 3 | 11.84 | 3 |
| 28 | 3 | 2.53 | 1 |
| 29 | 3 | 4.23 | 2 |
| 30 | 2 | 0.99 | 2 |

FISH, fluorescent *in situ* hybridization; IHC, immunohistochemical stain.

fluorescent *in situ* hybridization, or both between the core needle biopsy and excisional biopsy specimens and/or to have borderline fluorescent *in situ* hybridization ratios. Of these cases, 30 of the original immunohistochemical stain slides for *HER-2/neu* were available for re-review. The 2007 ASCO/CAP criteria for immunohistochemical stain evaluation of *HER-2/neu* were applied by three separate pathologists. The averages of these scores were taken and compared to the original immunohistochemical stain score (Table 7). The number of discordant immunohistochemical stain/fluorescent *in situ* hybridization cases is 7 vs 8 when you compare the 2007 ASCO/CAP criteria and the original immunohistochemical stain scores, respectively, which is not statistically different. With 2007 ASCO/CAP criteria score evaluation of *HER-2/neu*, all seven discrepant cases were undercalled by immunohistochemical stain and showed amplification by fluorescent *in situ* hybridization. Comparing the original immunohistochemical stain score with the fluorescent *in situ* hybridization ratios, two of the discrepant cases were overcalled as three on immunohistochemical stain, yet showed no amplification by fluorescent *in situ* hybridization and six cases were undercalled by immunohistochemical stain. Using the 2007 ASCO/CAP criteria, 12 of the

30 re-reviewed cases would result in reflex fluorescent *in situ* hybridization testing, whereas using the original FDA-approved immunohistochemical stain score resulted in 9 cases needing reflex fluorescent *in situ* hybridization.

Discussion

Determination of *HER-2/neu* status in breast cancer is important. It is critically important that *HER-2/neu* studies only be performed on the invasive component of the carcinoma and not on DCIS. DCIS is known to be more positive for *HER-2/neu* amplification/overexpression than its invasive counterpart in both immunohistochemical stain and fluorescent *in situ* hybridization tests. Further literature indicates that fluorescent *in situ* hybridization for *HER-2/neu* amplification can emerge in atypical ductal hyperplasia and most often appears first in these lesions. These emerging data suggest that amplification of *HER-2/neu* appears to be mainly involved in initiation of breast oncogenesis.^{2,3}

Extensive research has been performed to determine the accuracy of the various testing methods. Of the various methods used to determine *HER-2/neu* amplification status, immunohistochemical staining and fluorescent *in situ* hybridization are the two most widely accepted practices. Many pathologists perform the immunohistochemical stain for *HER-2/neu* receptor expression. Reflexive fluorescent *in situ* hybridization testing is carried out for a subset of cases with an immunohistochemical stain score of 2 and not for cases with immunohistochemical stain scores of 0, 1, or 3 in most laboratories.

The literature contains many reports on the accuracy of *HER-2/neu* testing by many different methodologies and new technologies are numerous;⁴⁻⁹ however, there is no published study evaluating the concordance of core needle biopsy and excisional biopsy specimen *HER-2/neu* results from the same patient. Our findings are novel and unique in that similar studies have not been previously published.

Overall, our data support good correlation (87%) between the immunohistochemical stain and fluorescent *in situ* hybridization *HER-2/neu* results of patients' core needle biopsies and excisional specimens. Yet within our population of 125 patients, we also did not obtain perfect concordance between their immunohistochemical stain and fluorescent *in situ* hybridization results for both core needle biopsy and excisional biopsy.

Amplification of *HER-2/neu* is reported 10–20% in literature and our study showed overall 15% amplification. The discordant rate up to 20% of *HER-2/neu* protein expression by immunohistochemical stain and gene amplification by fluorescent *in situ* hybridization has been documented in several studies. In our study population, there was

95% concordance rate between immunohistochemical stain and fluorescent *in situ* hybridization test.

The possible reasons for inconsistent immunohistochemical stain results are multifactorial: lack of reproducibility of immunohistochemical stain to evaluate *HER-2/neu* receptor expression with intraobserver and interobserver variability and differences in pre-analytic factors such as fixation time and laboratory-processing variables may have functions in inconsistent results.

Another factor that leads to different *HER-2/neu* status in both immunohistochemical stain and fluorescent *in situ* hybridization is the intratumoral heterogeneity due the sampling issue with the core needle biopsy. Two of our cases (cases 75 and 108) showed heterogeneity in tumor nuclear grade; in both, the excisional biopsy shows more poorly differentiated areas than sampled in the core needle biopsy.

Occasional breast cancers with *HER-2/neu* immunohistochemical stain scores of 3 may be negative for gene amplification by fluorescent *in situ* hybridization. Polysomy of chromosome 17 has been found as one of the reason for the strong immunohistochemical stain score of 3 and negative amplification by fluorescent *in situ* hybridization.^{10–12} Downs-Kelly *et al*¹³ suggested that polysomy 17 in the absence of *HER-2/neu* amplification does not have biological influence on *HER-2/neu* gene expression in breast carcinoma.

We observed no change in *HER-2/neu* receptor amplification status in the five patients who received neoadjuvant chemotherapy before their excision.

Although, this patient population is quite small, we note that these findings echo the results found in other studies of patients treated with chemotherapy.¹⁴ However, previous study performed by our group showed changes in the breast biomarkers including *HER-2/neu* status after neoadjuvant treatment.¹⁵

In an attempt to resolve some of the discrepancies between the *HER-2/neu* immunohistochemical stain results of core needle biopsy and excisional biopsy, we re-reviewed 30 discrepant and/or borderline fluorescent *in situ* hybridization cases applying the new ASCO/CAP 2007 guidelines. Using the 2007 ASCO/CAP criteria, immunohistochemical stain scores of 3 were seen in 17% of the re-reviewed cases, 2 in 53%, and 0 or 1 in 30% of cases, whereas re-review utilizing the guidelines before ASCO/CAP showed scores of 3 in 10%, 2 in 40%, and 0 or 1 in 50%. Using 2007 ASCO/CAP guidelines for fluorescent *in situ* hybridization, there are five cases of a borderline amplification for *HER-2/neu*.

Upon re-review of the available *HER-2/neu* slides using the 2007 ASCO/CAP guidelines, there are seven cases with immunohistochemical stain results that were discordant with the fluorescent *in situ* hybridization results. This is not statistically different from the eight cases with original pre-ASCO/CAP immunohistochemical stain scores that did not concur with the respective fluorescent *in situ*

hybridization results. We did not find significant changes in terms of resolving the discordant rate of *HER-2/neu* immunohistochemical stain results with fluorescent *in situ* hybridization results. Our findings are similar to those of Gilmore *et al*¹⁶ in their abstract presentation in 2008. In addition, our findings are similar to the Brunelli *et al*¹⁷ study that showed the ASCO/CAP scheme has a great concordance coefficient between strong immunohistochemical positivity (scores of 3) and *HER-2/neu* fluorescent *in situ* hybridization amplification.

In summary, the most cost-efficient and medically effective way to study breast biomarkers is to do them on the excisional specimen, because the excisional biopsy contains a larger tumor volume to avoid the problem of intratumoral heterogeneity. However, breast biomarkers should be performed on the core needle biopsy of patients who are to undergo neoadjuvant chemotherapy. Although we did not see changes in *HER-2/neu* status after neoadjuvant treatment in our limited number of cases, other studies have seen such changes; therefore, repeating *HER-2/neu* studies after neoadjuvant chemotherapy is reasonable. On the basis of our study, we observed that repeating *HER-2/neu* testing by immunohistochemical stain and/or fluorescent *in situ* hybridization methods on excisional biopsy is not unreasonable on an individual case, in particular if the excisional biopsy specimen shows different tumor characteristics or different morphology than the core needle biopsy. The *HER-2/neu* results for both fluorescent *in situ* hybridization and immunohistochemical stain reflected the tumor grade. If breast biomarkers have been performed on core needle biopsy and show indeterminate/borderline *HER-2/neu* results by immunohistochemical stain/fluorescent *in situ* hybridization, we recommend repeating the studies on the excisional biopsy.

Conflict of interest

The authors declare no conflict of interest.

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