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Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor 2 Expression in Breast Cancer FNA Cell Blocks and Paired Histologic Specimens: A Large Retrospective Study

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BACKGROUND: Molecular analysis represents an increasingly important component of the pathologic examination of tumor specimens. Notably, the characterization of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression in breast cancer specimens provides critical prognostic and predictive information. The objective of the current study was to compare the concordance of these markers as determined on fine-needle aspiration (FNA) cell blocks compared with tissue blocks prepared from surgical specimens. **METHODS:** A total of 134 cases of breast carcinoma were identified from 2002 through 2014 with both FNA cell blocks (fixed in 10% formalin) and corresponding available tissue blocks and ER, PR, and HER2 were characterized in both specimens. Negative and positive concordances were determined for ER and PR in cell blocks compared with tissue blocks, and for HER2 immunohistochemistry on cell blocks and tissue blocks versus the corresponding reference method, fluorescence in situ hybridization (FISH). **RESULTS:** Concordance for ER expression evaluated on a cell block compared with the corresponding tissue block was 96.2%. Concordance for PR expression was 77.5%. Overall agreement of HER2 FISH testing between cell blocks and tissue blocks was 96.7%. For both cell blocks and tissue blocks, HER2 expression by immunohistochemistry demonstrated $\geq$98% positive and negative concordance with the FISH reference method. **CONCLUSIONS:** ER, PR, and HER2 determination on FNA-acquired cell block (fixed exclusively in 10% formalin) showed excellent agreement for ER and HER2 and moderate agreement for PR with the corresponding tissue block. These findings support the equivalency of ER and HER2 evaluation performed on FNA cell blocks compared with surgical tissue blocks. ***Cancer Cytopathol* 2016;124:828-35**. © *2016 American Cancer Society*.

KEY WORDS: breast cancer; estrogen receptor (ER); fluorescence in situ hybridization (FISH); human epidermal growth factor receptor 2 (HER2); immunohistochemistry; progesterone receptor (PR).

INTRODUCTION

Driven by the evolution of cancer treatments from broadly cytotoxic to molecularly targeted therapies, the role of the modern pathologist has expanded to include molecular in addition to cytomorphologic and/or histologic characterization of tumor specimens. This transition is clearly in evidence in breast pathology, in which estrogen receptor (ER) and progesterone receptor (PR) expression and human epidermal growth factor receptor 2 (HER2) amplification status provide critical prognostic and predictive information that drive clinical management. ER expression (present in 75%-85% of breast carcinomas) identifies patients who may benefit from hormone

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therapy. In addition, PR expression identifies a small number of carcinomas (in most series <5%, although recent work with optimized monoclonal antibodies has suggested that carcinomas that are ER negative/PR positive by immunohistochemistry [IHC] primarily represent false-negative ER results, with the number of true-negative results near or equal to zero[1]) that are PR positive and ER negative but that may respond to hormonal therapy.[2,3] Gene amplification (along with other less common mechanisms) leads to overexpression of HER2 in 15% to 25% of breast cancers. Carcinomas overexpressing HER2 are susceptible to therapies targeting the HER2 receptor (trastuzumab, pertuzumab, and lapatinib), and derive increased benefit from anthracycline-based adjuvant therapy. In addition, HER2 is a significant prognostic factor, whereas both ER and PR have minor prognostic significance.

To determine the ER, PR, and HER2 status of a tumor, pathologists rely on a combination of semiquantitative IHC stains and fluorescence in situ hybridization (FISH). These techniques typically are performed on sections of formalin-fixed, paraffin-embedded tissue blocks obtained from surgical resection or large-bore core needle specimens. Unfortunately, the invasive and potentially morbid nature of such procedures can delay or, in the case of poorly accessible sites even prohibit, the acquisition of diagnostic tissue.

Fine-needle aspiration (FNA) is a minimally invasive procedure with little to no associated morbidity that is well established and validated[4–7] for the cytomorphologic characterization of breast lesions. The ability to perform FNA in the outpatient setting, the opportunity for immediate feedback to the clinician, and the diagnostic accuracy of the procedure have made it the first-line diagnostic intervention in many cases at the study institution. Although FNA is not widely used for the primary diagnosis of breast lesions, many institutions use FNA to diagnose metastatic lesions, which often are small and difficult to approach.

ER, PR, and HER2 testing performed on FNA-generated cell blocks is important for the care of patients with breast cancer because it provides important prognostic and predictive information. Early work addressing the accuracy of ER, PR, and HER2 IHC in FNA samples examined ethanol-fixed cell blocks[7–11] and demonstrated good correlation between tissue blocks and cell blocks for ER, but more equivocal results for PR and HER2. More recently, several small series using cell blocks fixed by various methods (ethanol and formalin fixation as well as others) suggested a strong correlation not only of ER but also of PR and HER2 IHC between FNA cell blocks and tissue blocks.[4,9,10] The current American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 guidelines recommend that biomarker assays should be performed on every new case of disease recurrence.[2] Many of these lesions are sampled by FNA only, and therefore the use of FNA samples for determining hormone receptor status by IHC is clinically important. The current guidelines accept cytology samples for testing but, to the best of our knowledge, there is an absence of large series comparing the ER, PR, and HER2 status of FNA cell blocks with corresponding histologic material.

The objective of the current study was to compare the concordance of these markers determined on FNA formalin-fixed cell blocks compared with tissue blocks prepared from surgical specimens retrospectively.

MATERIALS AND METHODS

Sample Acquisition

FNA was performed using a 23-gauge needle, and a portion (usually at least 1 dedicated sample/pass during the procedure) of the aspirated material was fixed immediately in 10% neutral-buffered formalin (the remainder of the specimen was used to prepare conventional cytology smears). Before the completion of the FNA procedure, formalin vials were checked for the presence of tissue fragments visible without magnification; repeat sampling was performed when possible in cases in which no tissue fragments were visible. Tissue acquired for cell block was allowed to be fixed in formalin for no more than 48 hours, and for no less than 6 hours in all samples collected since 2007 (as per the ASCO/CAP guidelines released in that year[12]).

Cell Block Preparation

After fixation, cell blocks were prepared using the collodion bag technique.[13] Briefly, collodion was poured into a 15-mL glass tube under a fume hood and allowed to set for 10 to 15 minutes. Tubes were rotated while pouring excess liquid collodion back into the reagent bottle to ensure an even coating of the inside of the tubes. Thereafter, the tubes were inverted and allowed to dry. Collodion-coated tubes were stored for up to 1 week, filled with distilled water, and capped to prevent fungal contamination. To prepare a cell block, the water was discarded and the 10%

neutral-buffered formalin containing the sample (acquired as described above) was poured into the tube and centrifuged at 2500 revolutions per minute (relative centrifugal force, 1125G) for 10 minutes. The supernatant fluid was subsequently discarded and the collodion bag containing the pelleted tissue removed by reaming the top of the tube with a scalpel. Finally, the bag was tied with a cotton string just above the pellet and processed and embedded in paraffin according to standard histologic techniques. Special attention was paid to the orientation of the collodion bag in the cell block, with the long axis of the bag oriented perpendicular to the plane of section (resulting in sections spanning both the pellet in the bottom of the bag and the knotted top of the bag).

Tissue Block Preparation

Tissue blocks from surgical specimens were fixed in 10% neutral-buffered formalin for 6 to 48 hours according to the ASCO/CAP guidelines beginning in 2007.[12] Tissue blocks were fixed, processed, and embedded in paraffin according to standard histologic techniques.

Case Selection

After approval of the Institutional Review Board, we searched the archives of the department of pathology of the University of California at San Francisco for cases of invasive breast cancer diagnosed from 2002 to 2014 and for which paired cell blocks prepared from FNA specimens (universally fixed in 10% formalin at the study institution) and surgical tissue blocks were available. We included in the current analysis only those cases in which IHC for ER, PR, and HER2 as well as FISH for HER2 had been performed on both cell block and tissue block material. Overall, 134 cases met the criteria for inclusion in the current study. Eleven cases in which the HER2 FISH result was interpreted as "borderline" were excluded from analysis of HER2 concordance. Overall, the demographic features of these cases were representative of the patient population of the University of California at San Francisco cytology clinic (data not shown). Of 134 cases, 116 were primary cancers and 18 were metastatic cancer cases. Of the histologic specimens from 116 primary cancers, 57 were core needle biopsy specimens, 40 were partial mastectomy (lumpectomy) specimens, and 19 were mastectomy specimens.

Immunohistochemistry

IHC tests for ER (SP1 rabbit monoclonal antibody at a dilution of 1:50; Lab Vision Corporation, Fremont, Calif), PR (PGR 636 at a dilution of 1:250; Dako North America Inc, Carpinteria, Calif), and HER2 (CB11 at a dilution of, 1:250; Novocastra, Newcastle-upon-Tyne, UK) were performed by manual morphometry and compared on paired cell blocks and tissue blocks from the same patient.

ER and PR status was interpreted as positive when $\geq$1% of invasive tumor cells demonstrated any nuclear staining (Figs. 1 and 2). When present, normal breast tissue was used as a positive internal control in tissue block specimens. External controls were used in the absence of normal breast tissue and in all cell block specimens.

HER2 scoring was performed according to the ASCO/CAP guidelines of 2007.[12] Intense, homogeneously dark membrane staining of >30% of invasive tumor cells was interpreted as positive (3+) (Fig. 3A). Complete membrane staining, either nonuniform or weak in intensity, with obvious circumferential distribution in $\geq$10% of invasive cells was interpreted as equivocal (2+) as was intense complete membrane staining in $\leq$30% of tumor cells (Fig. 3B). Weak incomplete membrane staining in any percentage of invasive tumor cells or weak complete membrane staining in <10% of tumor cells was interpreted as negative (1+), whereas no observable staining also was interpreted as negative (0+) (Fig. 3C).

Cases in which the results for ER expression (6 total) were discrepant between cell blocks and tissue blocks were reviewed by 2 pathologists (P.V. and B.M.L.) and reassigned as discrepant, nondiscrepant, or excluded from further analysis as indicated in the results section.

HER2 FISH

All cases selected for the current study were submitted for FISH analysis using the dual-probe assay (Vysis, Des Plaines, IL[12]). Interpretation of *HER2* gene amplification was based on the ratio of HER2 to centromere 17 copy number as defined in the 2007 ASCO/CAP guidelines.[12] HER2 FISH was reported as amplified (*HER2*/CEP17 ratio >2.2), equivocal (*HER2*/CEP17 ratio $\leq$2.2 but $\geq$1.8), or negative (*HER2*/CEP17 ratio <1.8). Cases with a "borderline" result on HER2 FISH (7 cell block and 8 tissue block cases) were excluded from the analysis of HER2 IHC versus HER2 FISH concordance.

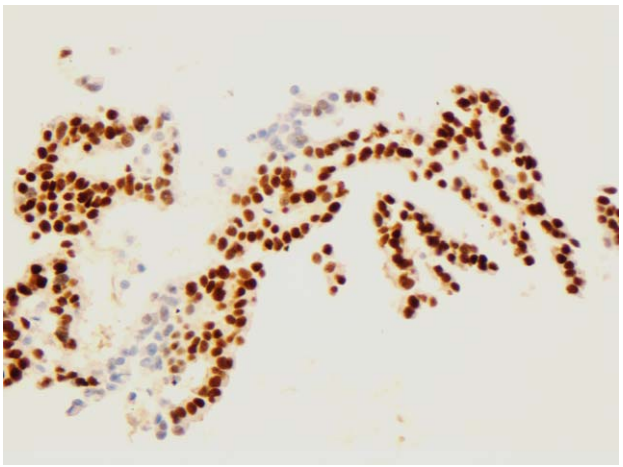

Figure 1. Strong nuclear estrogen receptor expression in tumor cells on cell block preparation.

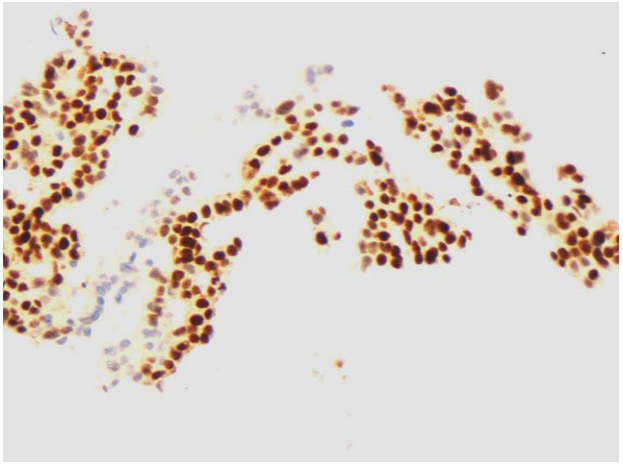

Figure 2. Strong nuclear progesterone receptor expression in tumor cells on cell block preparation.

Statistical Analyses

Raw agreement, concordance, and statistical significance (using the Fisher exact test) were calculated using Microsoft Excel (Microsoft Corporation, Redmond, Washington).

RESULTS

ER Expression Determined by IHC on Paired Cell Blocks and Tissue Blocks Is Highly Concordant

Among our 134 cases, 3 had ER results recorded as "borderline" in the database, and were excluded from the current analysis. Among the remaining cases, 97 of 102 cases that were positive for ER expression on tissue block (96.0% concordant) also were found to be positive on cell

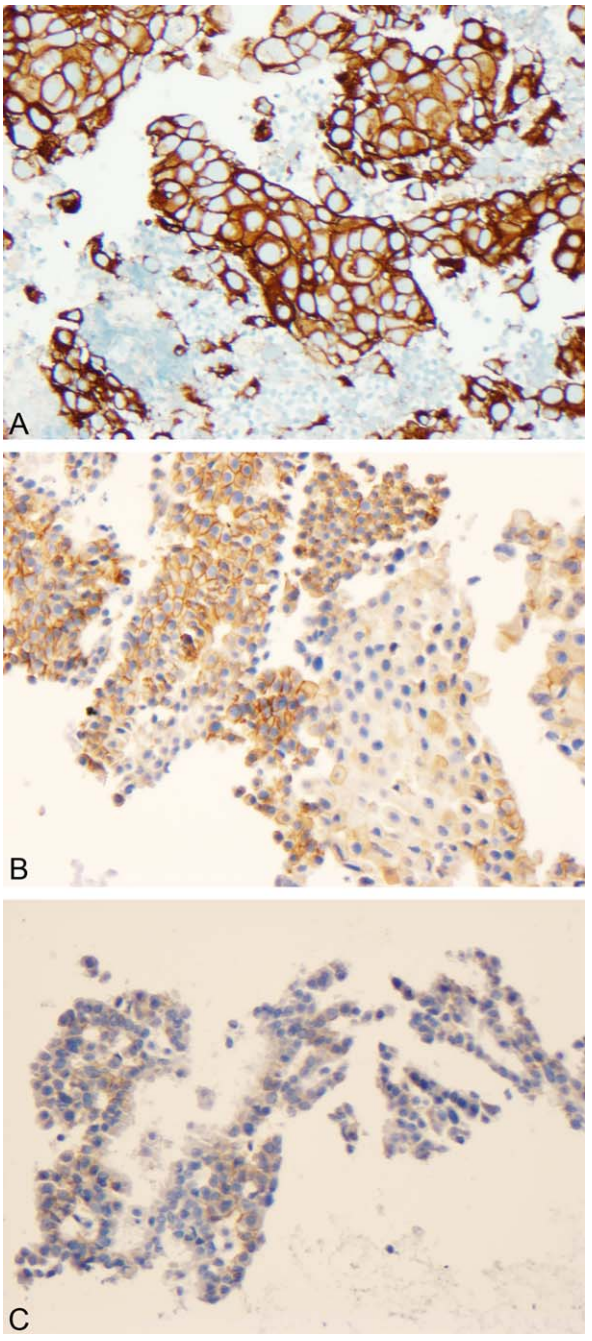

Figure 3. Human epidermal growth factor receptor 2 (HER2) immunostaining in a formalin-fixed breast carcinoma cell block section. (A) Strong, circumferential staining in >30% of tumor cells was scored as 3 (positive). (B) Moderate, circumferential, membranous staining in >10% of tumor cells or strong and circumferential staining in <30% of tumor cells was scored as 2 (equivocal). (C) Weak and incomplete membranous staining in tumor cells was scored as 1 (negative).

TABLE 1. Concordance of ER Expression by IHC on Paired CBs and TBs

		CB		
	ER	Positive	Negative	Concordance
TB	Positive	97	4	96.0%
	Positive (following review)	98	1	99.0%
	Negative	1	29	96.7%
	Negative (following review)	0	29	100.0%

Abbreviations: CB, cell block; ER, estrogen receptor; IHC, immunohistochemistry; TB, tissue block.
Overall concordance was 99.2%.

TABLE 2. Concordance of PR Expression by IHC on Paired CBs and TBs

		CB		
	PR	Positive	Negative	Concordance
TB	Positive	60	17	77.9%
	Negative	12	40	76.9%

Abbreviations: CB, cell block; IHC, immunohistochemistry; PR, progesterone receptor; TB, tissue block. Overall concordance was 77.5%.

block. Of 29 cases that were negative for ER expression on tissue block, 28 cases (96.7% concordant) also were found to be negative on cell block. The overall concordance was 96.2%. Discrepant cases were subject to expert review. Three cases (1 "false-positive" and 2 "false-negative" results on cell block) were subsequently excluded from further analysis based on a large separation of time and/or body site location for the acquisition of the surgical versus the cytology specimens. The 2 remaining discrepant cases were found to be negative on cell block and positive on tissue block; after review, 1 case was noted to have weakly positive cell block staining not previously identified and was reassigned as concordant, whereas the remaining case represented a true "false-negative" cell block result. After review, positive concordance rose to 99.0% and negative concordance to 100%, with an overall concordance of 99.2%. These results are summarized in Table 1.

PR Expression Determined by IHC on Paired Cell Blocks and Tissue Blocks Demonstrates Moderate Concordance

Among our 134 cases, 5 had PR results recorded as "borderline" in the database, and were excluded from analysis. Among the remaining cases (Table 2), PR was found to demonstrate 77.9% positive and 76.9% negative concordances (overall concordance of 77.5%). Given the limited prognostic and predictive contribution of PR in the clinical management of patients with breast cancer, expert review of discrepant cases was not performed. These results are summarized in Table 2.

HER2 Expression by IHC on Cell Blocks and Tissue Blocks Is Highly Concordant With HER2 *Gene Amplification by FISH*

To evaluate the accuracy of HER2 testing using cell block and tissue block IHC, we used amplification of HER2 as the standard of comparison.[14–16] Of the 134 cases initially identified, 123 were evaluated for HER2 IHC concordance between cell block or tissue block specimens and the FISH reference method. IHC performed on either cell blocks or tissue blocks demonstrated excellent negative concordance with FISH: 98% for cell block material (50 of 51 cases unamplified by FISH) and 100% for tissue blocks (57 of 57 cases unamplified by FISH). Positive concordance was 100% from cell block material (12 of 12 cases positive by FISH) and 91.7% from tissue block material (11 of 12 cases positive by FISH on tissue block) (Tables 3 and 4). Overall agreement between HER2 FISH testing on cell blocks versus tissue blocks was 96.7%; of the 18 cases with HER2 amplification confirmed by FISH, 14 were positive by FISH on both cell blocks and tissue blocks, 2 were positive on tissue block material only (false-negative result on cell block material), and 2 were positive on cell block material only (false-negative result on tissue block material). False-negative results were not found to be significantly associated with either block type by the Fisher exact test ($P = 1.0$) (Table 5). Among cases equivocal by IHC, both cell block and tissue block material yielded a similar number of amplified cases: 3 of 60 cases (5.0%) for cell block and 5 of 54 cases (9.3%) for tissue block.

Discrepant cases were reviewed. The single "false-negative" cell block result on IHC demonstrated insufficient tumor cells in the cell block. The single "false-positive" tissue block on IHC demonstrated a small number of tumor cells present within a background of numerous stromal cells, suggesting that, in this case, the HER2 ratio on FISH may have been falsely depressed by the presence of many nontumor nuclei.

We also reviewed 12 cases of primary breast cancers in the current study that were reported as having extensive ductal carcinoma in situ (DCIS) on histologic sections. In

TABLE 3. Concordance of HER2 Expression on CBs in Paired IHC and FISH Studies

HER2 in CB		IHC		
		Positive	Negative	Borderline
FISH	Positive	12	1	3
	Negative	0	50	57
	Concordance	100%	98%	

Abbreviations: CB, cell block; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

TABLE 4. Concordance of HER2 Expression on TBs in Paired IHC and FISH Studies

HER2 in TB		IHC		
		Positive	Negative	Borderline
FISH	Positive	11	0	5
	Negative	1	57	49
	Concordance	91.7%	100%	

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; TB, tissue block.

11 of the 12 cases, the HER2 findings were concordant between the cell block and the tissue block. In the 1 remaining case, the cell block was negative and the tissue block demonstrated focal positivity in 5% of tumor cells in only 1 of 2 blocks using FISH. Thus, in our limited number of breast cancer specimens with extensive DCIS, overall concordance remained >90%; there were no cases of false-positive HER2 on cell block preparations.

DISCUSSION

In the current study, the usefulness of cell blocks prepared from FNA material obtained from breast carcinomas as a substrate for the characterization of ER, PR, and HER2 expression by IHC was investigated. There was excellent concordance for ER and HER2 and moderate concordance for PR expression as determined by IHC on cell blocks compared with the same expression determined on tissue blocks. For HER2, in which the reference method was HER2 amplification by FISH, we found excellent positive and negative concordance (using both cell block and tissue block material) between IHC and FISH results. The false-negative rate for HER2 amplification did not differ significantly between cell block versus tissue block material. Overall, to the best of our knowledge, the current study is

TABLE 5. Concordance of HER2 Expression by FISH on Paired CBs and TBs

HER2 FISH		TB	
		Positive	Negative
Cell Block	Positive	14	2
	Negative	2	105
			$P = 1.0$
		Overall agreement	96.74796748

Abbreviations: CB, cell block; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; TB, tissue block.
Overall concordance was 96.7%. False-negative results were not found to be significantly associated with either CB or TB samples by the Fisher exact test ($P = 1.0$).

the first large series to evaluate the diagnostic accuracy of ER, PR, and HER2 IHC and FISH studies performed on FNA samples collected during the course of clinical care and fixed exclusively in 10% formalin.

Perhaps most significantly, the current study data indicate excellent concordance of HER2 IHC performed on cell block compared with the FISH reference method, as well as excellent concordance between HER2 FISH performed on cell block compared with tissue block. These results support the functional equivalence of cell block and tissue block material for the evaluation of HER2 in breast lesions. We recognize that the current analysis was slightly limited by the use of the 2007 ASCO/CAP guidelines (which were used for consistency in our database, which spans cases from 2002 to 2014).[12] However, recent data have demonstrated only very infrequent changes to HER2 classification when using the 2007[12] versus 2013 guidelines, and therefore we would not expect statistically significant changes to our results if cases were reclassified using 2013 criteria.[17,18]

The results of the current study also provide data regarding the hypothetical risk of false-positive HER2 results from FNA samples due to sampling of HER2-positive DCIS within the background of a HER2-negative invasive carcinoma. This possibility is of concern because to the best of our knowledge tumor cells from DCIS cannot be distinguished from the invasive component in FNA specimens, and because treatment with anti-HER2 antibodies should be restricted to patients with HER2 amplification within invasive carcinoma; HER2 status within DCIS is not considered clinically relevant for drug treatment. However, such a "false-positive" HER2 result on cell block was not observed among our 134 cases, including 12 cases of primary breast cancer with extensive DCIS.

Similar results have been obtained by other investigators. Latta et al found concordance of HER2 expression between in situ and invasive components in 90.4% of cases by IHC (CB11 antibody).[19] In discordant cases, the in situ component demonstrated overexpression in the majority of cases; however, in all but 1 case, the score was near the breakpoint of positive versus negative (equivalent to 2+ in the current study's scoring system). Overall, the findings of the current study suggest that an admixed in situ component discordantly positive for HER2 at most rarely could provide sufficient HER2 staining (by IHC or FISH) to trigger a false-positive HER2 result for the associated invasive carcinoma. Because the in situ component is almost never negative when the invasive component is positive, the risk of a false-negative result due to an admixture of an in situ component does not appear significant.

The results of the current study also demonstrate excellent concordance of ER IHC performed on tissue block and cell block material. After review, only a single discrepant case remained ("false-negative" cell block); correlation with the clinical findings in this case demonstrated that the patient had received chemotherapy in the interim between acquisition of the tissue block and cell block specimens, suggesting that in this case, the loss of ER expression could have represented a true alteration in the molecular characteristics of the tumor rather than an erroneous result on FNA.

Although the results of the current study demonstrated excellent concordance for ER and HER2, overall concordance for PR was only 77.5%. However, this concordance is similar to that observed by other investigators examining PR in multiple specimens (FNA and/or surgical) from the same tumor, including a recent comparison of core needle biopsy and excision specimens.[20–23] Overall, these previous studies support the intratumoral heterogeneity of PR expression as a major contributor to the moderate concordance of PR between multiple samples from the same tumor, and therefore we attribute the moderate PR concordance observed in the current study to the effects of intratumoral heterogeneity. In any case, because ER status is the primary determinant of hormonal treatment, the clinical significance of the more moderate concordance of PR between cell block and tissue block specimens is unclear. Nevertheless, it is possible that a small number of ER-negative, PR-positive cases will be missed by IHC on cell block, and therefore further research is needed to evaluate the significance of this possibility.

We did note the relatively low frequency of ER-positive cases in the current study. This can be explained by an overrepresentation of triple-negative cases in the current study cohort. Herein, 18 ER-negative cases were triple negative, most likely due to the selective referral of patients with clinically more aggressive breast cancer to the breast clinic at our tertiary cancer center. Nevertheless, sufficient numbers of all configurations of receptor positivity are represented among the current study cases for statistical analysis.

The results of the current study present a series of 134 cases correlating ER, PR, and HER2 determination on FNA-acquired cell block (fixed exclusively in 10% formalin) and tissue block samples. The results demonstrate excellent agreement for ER and HER2 and moderate agreement for PR IHC performed on cell block compared with tissue block material. All HER2-amplified cases (as determined by FISH) were detected as either equivocal or positive HER2 IHC staining in the same specimen on both cell and tissue blocks. These results support the diagnostic accuracy of ER, PR, and HER2 IHC studies performed on FNA samples.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Poonam Vohra: Conceptualization, methodology, formal analysis, investigation, data curation, resources, writing–original draft, writing–review and editing, and visualization. **Benjamin Buelow**: Conceptualization, methodology, formal analysis, investigation, data curation, writing–original draft, writing–review and editing, and visualization. **Yunn-Yi Chen**: Conceptualization, methodology, formal analysis, and writing–review and editing. **Maria Serrano**: Conceptualization, methodology, investigation, resources, and writing–review and editing. **Manjiv Singh Vohra**: Methodology, formal analysis, data curation, writing–review and editing, and visualization. **Anna Berry**: Methodology, validation, investigation, resources, and writing–review and editing. **Britt-Marie Ljung**: Conceptualization, methodology, investigation, resources, writing–review and editing, supervision, and project administration.

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