

Hormone Receptors and Human Epidermal Growth Factor (HER2) Expression in Fine-Needle Aspirates from Metastatic Breast Carcinoma – Role in Patient Management

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Abstract

Introduction: Estrogen receptors (ER), progesterone receptors (PR), and epidermal growth factor (HER2) are prognostic and predictive factors for breast carcinoma. We determined them by immunohistochemistry (IHC) on cell blocks from fine-needle aspirates (FNA) of metastatic breast carcinoma to axillary lymphnodes and compared them with that reported in the primary breast carcinoma (PBC) to document any change in their expression for future management. **Materials and Methods:** ER, PR, and HER2 by IHC and HER2 oncogene by fluorescent in-situ hybridization (FISH) were studied on cell blocks of FNA of axillary lymphnodes in 53 of 94 PBC cases from 2012 to 2016. **Results:** In 25 of 38 (65.8%) ER, PR negative PBC the metastasis on FNA was ER, PR+, whereas the 15 (28.3%) ER, PRPBC remained negative. In 10 of 11 (91%) of HER2-IHC+, PBC the metastatic tumor was HER2-IHC+. 7 of 32 (21.9%) HER2-IHC negative PBC were HER2-IHC+ in metastatic tumor. HER2-FISH was performed in 37 cases on FNA. Six of 37 were HER2 amplified/positive, whereas 9 and 19 remained equivocal and negative for HER2 copy number, and 3 were not interpretable. All the 6 HER2-FISH+ cases were positive by IHC. In our study, 34.2% of ER, PR+ cases of PBC became ER, PR– in the metastatic tumor and 21.9% of HER2-IHC negative PBC became HER2-IHC+ in the metastatic aspirate. **Conclusion:** ER, PR, and HER2 by IHC in cell blocks of metastatic lymphnodes are reliable. Change in receptor (34.2%) and HER2 status (21.9%) was documented, which is of clinical significance as these patients warrant a change of management.

Keywords: Fine-needle aspirates, hormonal receptors, HER2, metastatic lymphnodes, primary breast carcinoma

INTRODUCTION

Breast carcinoma is a morphological and genetically heterogenous entity.^[1] Hormone therapy offers several advantages and determination of estrogen receptor (ER) and progesterone receptor (PR) form an important component of morphological evaluation of newly diagnosed and recurrent/metastatic carcinoma as it helps direct the appropriate use of endocrine therapies for these tumors.^[2,3] Hormone receptor – positive tumors benefit from the addition of postoperative endocrine treatments such as tamoxifen for premenopausal women, and in post menopausal women, the aromatase inhibitors such as anastrozole, letrozole, exemestane. Hormone receptor negative tumors do not benefit from hormonal therapy^[4] and have a relatively worse prognosis. The amplification of the HER2 receptor of the epidermal growth factor (EGFR) family or overexpression of

its protein product is seen in 18% to 20% of primary invasive breast carcinoma and acts as an independent prognostic and predictive marker.^[5,6] Women with HER2 positive breast carcinoma have a more aggressive course of disease with increased recurrence, distant metastasis, and shorter survival. HER2 status is also predictive of response to treatment with the humanized monoclonal antibody, which target the HER2 receptor (trastuzumab, pertuzumab, and lapatinib).^[7] HER2 overexpression in tumor tissue is associated with a poor prognosis. Hence, ER, PR, and HER2 are important predictive factors as they provide valuable information on response

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to a given therapeutic modality and are extensively used in management of breast carcinoma, both in the adjuvant treatment and metastatic setting.^[8,9] Neubauer *et al.*^[10] have shown that ER, PR, and HER2 expression can change in post chemotherapy surgical specimens and recommend that primary breast carcinoma (PBC) cases should be re-evaluated after chemotherapy. In the literature, there are conflicting reports about the conversion of these markers. Few studies have shown a 30% change in the metastatic site, whereas others have concluded that though there are some changes in the ER, PR, and HER2 status between the primary and metastatic disease still these changes were not statistically significant.^[5] The College of American Pathologists (CAP) recommend that these markers should be evaluated on every PBC, and retesting of biomarkers should be done in recurrent/metastatic tumor.^[11]

Testing for ER, PR, and HER2 by immunohistochemistry (IHC) has been developed and optimized for use on formalin-fixed, paraffin-embedded tissue obtained by excisional biopsies.^[8] Studies have shown a discordance for IHC expression of ER, PR, and HER2 between tissue samples of PBC and metastatic disease.^[1,11] Overall, the rates of ER, PR, and HER2 conversion were 13%, 28%, and 3%–10%, respectively in tissues.^[12,13] In a recent review article, changes in ER (from 10% to 30%) and PR (from 20% to 50%) have been reported in locoregional and distant metastases.^[9] High HER2 concordance between PBC and axillary lymph node (ALN) or distant metastases has been shown.^[9] In the discordant cases, it is frequent to have HER2 positive metastases with negative primary tumors.^[9,14] This phenomenon could be attributed to an enhanced tumor aggressiveness or an underestimation of HER2 expression in PBC by the pathologist.^[9] Thus, switch in the receptor status or expression of HER2 in the recurrent/metastatic tumor is important to document as it heralds a poor outcome and possibly a change in the therapeutic regime.

Fine-needle aspiration (FNA) is a safe, well established, minimally invasive procedure currently not used widely for diagnosis of PBC in several institutions in the developed countries. However, it plays an important role in the diagnosis of inoperable/recurrent breast carcinomas and determine metastatic lesions in ALN in women undergoing core needle biopsy (CNB) for establishing PBC. The tissue collected is used for diagnostic purposes as well as for a multitude of ancillary tests including prognostic and predictive markers. Of late, there is increasing use of ancillary prognostic testing on material obtained by FNA because of the availability of molecular testing on aspirated samples.^[6,15-18] Studies are reported where ER, PR, and HER2 are documented on aspirates.^[5,18-24] Formalin-fixed and paraffin embedded cell block preparations from fresh FNA and serous fluid have been found to be reliable in the expression of ER, PR, and HER2 in PBC cases as it can provide important prognostic and predictive information.^[1,18,24] According to the guidelines published by American Society of Clinical Oncology/ The College of American Pathologists (ASCO/CAP)^[25] IHC has been a commonly used method to detect ER, PR, and HER2 in formalin fixed, paraffin embedded

tissue slides whereas fluorescent in-situ hybridization (FISH) is an alternative standard test for gene amplification of HER2 and should be performed on every new case of disease recurrence.^[26] Many of the recurrent or metastatic lesions are sampled by FNA alone. Hence, it is clinically important to determine ER, PR, and HER2 in the aspirated material for better management. The current ASCO/CAP guidelines accept cytology samples for testing.^[1] Very few large series of testing ER, PR, and HER2 on FNA are available.^[5] This prompted us to evaluate ER, PR, and HER2 by IHC on cell blocks prepared from FNA of metastatic breast carcinomas and compare them with the corresponding PBC.

MATERIALS AND METHODS

Sample collection

Ultrasound guided FNA of ALN done on 94 known cases of invasive ductal carcinoma proven by core needle biopsy (CNB), lumpectomy, or mastectomy in a Cancer Center for Specialized Surgery from June 2012 to June 2016 were studied. This retrospective study was conducted after approval of the Hospital Ethics Committee, which adheres to the declaration of Helsinki's Ethical Principles for Medical Research involving human subjects. The axillary lymph nodes were selected on the basis of ultrasound findings namely length of lymph node, cortical thickness, loss of fatty hilum, and length/width ratio. In 21 cases, the aspirate revealed reactive lymphoid tissue. In 20 of 73 cases, FNA failed to show metastatic tumor in the cell block section. These 20 cases were excluded.

Thus, cell blocks from 53 cases of metastatic carcinoma were studied to demonstrate ER, PR, and HER2 by IHC and HER2 oncogene by FISH where necessary. Results of the FNA were correlated with the ER, PR, and HER2 status reported on the PBC.

Cell block preparation

Cell blocks were prepared from fresh aliquots of aspirated material from ALN obtained by FNA using the plasma thrombin procedure.

IHC FOR ER AND PR

Formalin-fixed (10% buffered formalin), paraffin embedded sections of cell blocks were stained for ER and PR using the primary monoclonal antibodies ER (Clone ID 5) DAKO, Glostrup, Denmark (Cat No. M 7047), PR (Clone PgR 636) DAKO, Carpinteria – CA (Cat No. M 3569) at a dilution of 1:50 according to the manufacturer's specification. The ASCO/CAP guideline recommendations^[26] were used for evaluation of the hormone receptors. Tumor cells were considered positive for ER or PR status, if $\geq 1\%$ of tumor cells demonstrated nuclear staining; $< 1\%$ is negative. Normal breast tissue was used as positive external control in all cell block preparations.

IHC FOR HER2

Formalin fixed, paraffin embedded sections of cell blocks were stained for HER2 using HER-2/neu (polyclonal), DAKO,

Glostrup, Denmark (Cat No. A 0485) at a dilution of 1:50 according to the manufacturer's specification. Stains were evaluated using the ASCO/CAP 2013 guidelines.^[25] HER2-IHC 3+ was considered when more than 10% of tumor cells show homogeneous dark circumferential (chicken wire) pattern. Incomplete and/or weak/moderate membrane staining and within >10% of tumor cells or complete membrane staining that is intense and within ≤10% of the tumor cells was interpreted as equivocal (HER2-IHC 2+). Incomplete membrane staining that was faint/barely perceptible and within >10% of the tumor cells (HER2-IHC 1+), and HER2-IHC -0 was defined by no staining observed, or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the tumor cells. HER2-IHC-1+ and HER2-IHC-0 were interpreted as HER 2 negative. The cell blocks were interpreted by one pathologist (KK), and whenever, a discrepancy was observed the cases were evaluated by both the pathologists (KK & IF) to reach a consensus.

Enumeration of HER2 DNA amplification by FISH

HER2-FISH was performed using PathVysion HER2 DNA Probe kit [Vysis LSI HER-2/neu Spectrum Orange/CEP17 Spectrum Green, Catalog No. 02J01-030] of Abbott Molecular Inc, IL, USA, following manufacturer's recommended protocol. The cell block sections were deparaffinized and fixed prior to assay with the Vysis Paraffin Pretreatment Reagent Kit (Catalog No. 02J02-032) of Abbott Molecular Inc, IL, USA.

HER2 FISH samples were analyzed using the fluorescent microscope (Zeiss Axio Imager MI). A total number of HER-2/neu and CEP 17 signals in 20–60 interphase tumor cells were counted. The HER-2/CEP (Chromosome Enumeration Probe) 17 ratio was calculated by dividing the total counts of the HER-2/neu signals by the total counts of CEP 17 signals. FISH positive cases were according to – Dual-probe HER2/CEP17 ratio ≥2.0 with an average HER2 copy number ≥4.0 signals/cell; Dual-probe HER2/CEP17 ratio ≥2.0 with an average HER2 copy number <4.0 signals/cell; and Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥6.0 signals/cell. FISH equivocal cases were according to- Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals/cell. FISH negative cases were according to- Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell. Polysomy 17

was defined as the occurrence of three or more copy numbers of centromeres for chromosome 17 per cell, according to Salido *et al.*^[27]

Number of cases selected for FISH: All negative and equivocal cases of HER2-IHC were selected for FISH. In addition, 10 cases positive for HER2 by IHC but negative for ER and PR were also included.

Statistical methods

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) Version 24.0 (IBM Corp., Chicago, Illinois, USA). Descriptive statistics have been presented as number and percentage and mean for age. Sensitivity, specificity, and positive and negative predictive values were computed for hormone receptors and HER2 against PBC and FNA from metastatic lymph nodes. The probability “p” < 0.05 was considered as statistically significant.

RESULTS

The median age of 53 PBC was 50 years (range 30 to 80 years). The diagnosis of invasive ductal carcinoma was made on CNB in 41 (77.3%) cases, mastectomy 10 (18.9%) cases, and excision biopsy with axillary clearance in 2 (3.8%) cases.

The size of the tumor in 2, 8, and 2 cases was less than 2 cm, 2–4 cm, and more than 4 cm, respectively. Majority 34 (64.1%) PBC cases were grade 3, whereas 18 (34%) and 1 (1.9%) were grade 2 and grade 1, respectively. In 21 (39.6%) cases, ALN were aspirated at the time of the CNB, whereas in 27 (50.9%) cases ALN were aspirated within 3 months of detection of PBC by CNB. In 7 of these 27 cases, mastectomy was done. Two cases each were detected in less than 1 year and less than 5 years, respectively. In these cases, mastectomy was performed in 2, and excision biopsy with axillary clearance in 2. One case who had a mastectomy performed was detected with ALN after an interval of more than 5 years. In all these five cases, chemotherapy was administered.

Table 1 correlates the expression of ER, PR in FNA of metastatic lymphnodes with PBC. ER and PR were positive in 38 (71.7%) PBC cases. All ER and PR negative cases in PBC remained negative in the FNA. However, 13 of 38 (34.2%) cases positive for ER and PR in PBC were found to be negative in the FNA of metastatic lymphnodes.

Table 1: Correlation of ER and PR expression by immunohistochemistry (IHC) in primary breast carcinoma with their expression in aspirates from metastatic axillary lymph nodes (FNA-LNM) [n=53]

ER and PR - IHC Primary breast carcinoma	Number of cases	ER and PR - IHC (FNA - LNM)			
		ER - Positive	ER - Negative	PR - Positive	PR - Negative
ER - Positive	38 (71.7)	25 (65.8)	13 (34.2)		
ER - Negative	15 (28.3)	0	15 (100)		
PR - Positive	38 (71.7)			25 (65.8)	13 (34.2)
PR - Negative	15 (28.3)			0	15 (100)
Total	53	25 (47.2)	28 (52.8)	25 (47.2)	28 (52.8)

FNA=Fine-needle aspiration, LNM=Lymph node metastases, ER=Estrogen receptor, PR=Progesterone receptor, () Figure in parenthesis indicates percentage

In the PBC, HER2-IHC was positive in 11 (20.8%) and negative in 32 (60.4%) cases. There were 10 (18.8%) equivocal cases where HER2-FISH was done. HER2 was amplified in 1 case, equivocal in 2 cases, and not amplified in 7 cases. HER2 by IHC was positive (3+) in 26 (49.1%) cases, equivocal (2+) in 14 (26.4%) cases, and negative (1+/0) in 13 (24.5%) cases of metastatic lymphnodes [Table 2]. In the 14 equivocal and 13 negative cases of HER2 – IHC, HER2 was also determined by FISH. Ten of 26 cases, positive for HER2-IHC were also evaluated by FISH [Table 3]. Two each of 10 HER2 -IHC positive cases were negative and equivocal by FISH. All the negative cases remained negative. The 14 HER2-IHC equivocal cases were equivocal and negative by FISH in 7 and 5 cases, respectively, whereas two cases were not interpretable. When the 5 cases (HER2-FISH negative and HER2-IHC equivocal) in the metastatic tumor were correlated with the PBC HER2-IHC was found to be negative in four and positive in one case. These 5 cases were not studied by FISH in the PBC as HER2-FISH on tissue in PBC is routinely limited to equivocal cases only. The 7 cases equivocal by HER2-IHC and HER2-FISH in the metastatic tumor were negative for HER2 in the PBC in one case where FISH was done. There was no significant change in the HER2 status in the positive and equivocal cases. However, 7 (21.9%) and 12 (37.5%) of the HER2-IHC negative PBC cases were positive and equivocal, respectively in the FNA of metastatic lymphnodes.

Table 2: Correlation of HER2 expression by immunohistochemistry (IHC) in primary breast carcinoma with their expression in aspirates from metastatic axillary lymph nodes (FNA-LNM) [n=53]

HER2 - IHC Primary breast carcinoma	Number of cases	HER2 - IHC (FNA - LNM)		
		Positive 3+	Equivocal 2+	Negative 1+/0
Positive 3+	11 (20.8)	10 (38.5) [90.9]	1 (7.1) [11.1]	0
Equivocal 2+	10 (18.8)	9 (34.6) [90]	1 (11.1) [10]	0
Negative 1+/0	32 (60.4)	7 (26.9) [21.9]	12 (85.7) [37.5]	13 (100) [40.6]
Total	53	26 [49.1]	14 [26.4]	13 [24.5]

FNA=Fine-needle aspiration; LNM=Lymph node metastases; () Figure in parenthesis indicates percentage in y-axis, []Figure in parenthesis indicates percentage in x-axis

The evaluation of HER2 by IHC between PBC and FNA of metastatic lymphnodes when the equivocal cases are combined with the negative cases [Table 4] show that 16 (38.1%) of the negative cases became positive for HER2 in the FNA of the metastatic lymph node, whereas 1 (9.1%) case positive for HER2 became negative for HER2 in the metastatic lymphnode. The sensitivity, specificity, positive predictive value, negative predictive value were 38.5%, 96.3%, 90.9%, and 61.9%, respectively. However, evaluation of HER 2 by IHC between PBC and FNA of metastatic lymphnodes when the equivocal cases are combined with the positive cases [Table 5] show that 19 (59.4%) of the HER2 negative case in the PBC became positive in the FNA of the metastatic lymphnodes. The sensitivity, specificity, positive predictive value, negative predictive value were 52.5%, 100%, 100%, and 40.6%, respectively.

The time interval, hormonal status, and HER2 expression of the five cases with recurrence is shown in Table 6. The 3 cases ER, PR positive in PBC were negative and positive in 2 and 1 case, respectively in the ALN aspirates, whereas the 2 negative cases remained negative in the Lymph node metastases (LNM). In PBC, 4 cases were HER2 negative, and 2 of these 4 cases were positive for HER2 in the metastatic lymph node. The case of PBC equivocal for HER2 was positive for HER2 in LNM.

DISCUSSION

In our study, we also found a high concordance for ER and PR expression as observed by Shabaik *et al.*^[6] In 65.8% ER/PR positive, PBC cases were also positive for ER/PR in the metastatic aspirates, whereas 34.2% became ER/PR negative in the metastases [Table 1]. This is important as it signifies that these patients are less likely to respond to hormonal therapy. Rossi *et al.*^[9] found changes in ER and particularly in PR in locoregional and in distant metastasis reaching a rate of 10% to 30% for ER and 20% to 50% for PR. They found that a loss of PR was more frequent than a loss of ER. In our study, ER and PR positivity showed concordance, and we had no ER negative, PR positive case. All the hormonal negative PBC remained negative in the metastatic aspirate. This is consistent with previous studies.^[1,11]

High HER2 concordance between primary tumors and ALN or distant metastasis has been shown in several studies.^[9] Most of these studies are on tissue sections, and in the discordant HER2 cases, it is more frequent to have a

Table 3: Correlation of expression of HER 2 by immunohistochemistry (IHC) and Fluorescent in-situ hybridization (FISH) in aspirates from metastatic axillary lymph nodes [n=37]

HER2 -IHC	Total number of cases	Number of cases with FISH	HER 2 -FISH			
			Amplified	Equivocal	Not amplified	Not interpretable
Positive 3+	26	10	6	2	2	-
Equivocal 2+	14	14	-	7	5	2
Negative 1+/0	13	13	-	-	12	1
Total	53	37	6	9	19	3

HER2 positive metastases with negative primary tumors.^[9] It must be remembered that reproducibility of HER2 staining in different laboratories is approximately 85%. In addition, concordance was higher for HER2-FISH testing than the HER2-IHC (88.1% and 81.6%), respectively.^[28] In our study, 91% of HER2-IHC positive cases in PBC were positive in metastatic ALN, 9 of 10 equivocal cases became positive in metastatic ALN, and 21.9% [Table 2] of the HER2 negative

cases in PBC were positive in the metastatic tumor. This is significant as these 21.9% of cases will benefit from targeted therapy and will have a worse prognosis.

In a meta-analysis including 39 studies assessing receptor conversion from primary breast tumors to paired distant breast carcinoma metastases, the incidence of receptor conversion varied largely between studies.^[29] They found that for ER α , PR, and HER2, the random effects pooled positive to negative conversion percentages of 22.5% (95% confidence interval [CI] = 16.4% to 30.0%), 49.4% (95% CI = 40.5% to 58.2%), and 21.3% (95% CI = 14.3% to 30.5%), respectively. Negative to positive conversion percentages were 21.5% (95% CI = 18.1% to 25.5%), 15.9% (95% CI = 11.3% to 22.0%), and 9.5% (95% CI = 7.4% to 12.1%). Receptor conversion for ER α , PR, and HER2 occurs frequently in the course of disease progression in breast cancer. Large prospective studies assessing the impact of receptor conversion on treatment efficacy and survival are needed.^[29]

We performed HER2-FISH in 37 cases on aspirated material from metastatic ALN and found a good correlation with HER2-IHC [Table 3]. All the FISH positive cases were positive by IHC, and the 9 equivocal cases were either equivocal (7 cases) or positive (2 cases). Two of 19 FISH negative cases were positive for HER2 on IHC. These results support the diagnostic accuracy of HER2-IHC. This finding is of importance for HER2-IHC can be determined in laboratories where facilities are not available to do FISH. When we compared the HER2-IHC of the metastatic tumors with the PBC taking the equivocal in both sites as negative, there were 38.1% cases, which had become positive for HER2 in the metastatic tumor [Table 4]. However, when the equivocal HER2-IHC cases were included with the positive cases then 19 (59.4%) cases became positive for HER2 in the metastatic tumor [Table 5]. Moreover, when we compared HER2-IHC with HER2-FISH [Table 3], we found that none of the equivocal HER2-IHC was positive by the FISH technique, which is considered as a gold standard. We thus felt that the equivocal cases should be further tested by FISH. This is of great significance as it shows that in nearly 30% of the tumors,

Table 4: Correlation of HER2 expression by immunohistochemistry (IHC) in primary breast carcinoma with HER2 expression by IHC in aspirates of metastatic axillary lymph nodes when equivocal cases are combined with negative [n=53]

HER 2 - IHC (Primary breast carcinoma)	Number of cases	HER 2 - IHC (FNA-LNM)	
		Positive	Negative and Equival
Positive	11	10 (90.9)	1 (9.1)
Negative and Equivocal	42	16 (38.1)	26 (61.9)
Total	53	26 (49.1)	27 (50.9)

FNA=Fine-needle aspiration; LNM=Lymph node metastases; () Figure in parenthesis indicates percentage, Sensitivity=38.5%; Specificity=96.3%; $P<0.002$, Positive predictive value (PPV) = 90.9%; Negative predictive value (NPV) = 61.9%

Table 5: Correlation of HER 2 expression by immunohistochemistry (IHC) in primary breast carcinoma with HER2 expression by IHC in aspirates of metastatic axillary lymph nodes when equivocal cases are combined with positive [n=53]

HER 2 - IHC (Primary breast carcinoma)	Number of cases	HER 2 - IHC (FNA-LNM)	
		Positive and Equival	Negative
Positive and Equivocal	21	21 (100)	0
Negative	32	19 (59.4)	13 (40.6)
Total	53	40 (75.5)	13 (24.5)

FNA=Fine-needle aspiration; LNM=Lymph node metastases; () Figure in parenthesis indicates percentage, Sensitivity=52.5%; Specificity=100%; $P<0.001$, Positive predictive value (PPV) = 100%; Negative predictive value (NPV) = 40.6%

Table 6: Hormonal status and HER2 expression by immunohistochemistry (IHC) of five cases with recurrence

Case number	Age (years)	Surgical procedure	Time interval	ER/PR/HER-2 - IHC (Primary breast carcinoma)	ER/PR/HER 2 - IHC (FNA-LNM)
1	41	Mastectomy	6 years	ER/PR Positive HER2 Negative	ER/PR Positive HER2 Positive
2	50	Mastectomy	2 years	ER/PR Negative HER2 Negative	ER/PR Negative HER2 Positive
3	39	Excision biopsy with axillary clearance	2 years	ER/PR Negative HER2 Negative	ER/PR Negative HER2 Negative
4	63	Excision biopsy with axillary clearance	4 months	ER/PR Positive HER2 Negative	ER/PR Negative HER2 Negative
5	40	Mastectomy	4 months	ER/PR Positive HER2 Equivocal	ER/PR Negative HER2 Positive

FNA=Fine-needle aspiration, LNM=Lymph node metastases, ER=Estrogen receptor, PR=Progesterone receptor

a change in the HER2 status may be observed in the metastatic tumor there by warranting an aggressive therapeutical approach with targeted monoclonal therapy. A high HER2 concordance between primary tumors and ALN or distant metastases has been shown.^[9,14] Previous studies suggest discordance rates of 0%–34% for HER2 between primary breast cancer and its paired metastatic tumor.^[1,9] A meta-analysis of 48 articles indicated that the prevalence of negative conversion outnumbered that of positive conversion (13 vs. 5%).^[30] Hou *et al.*^[11] reported a discordance rate of 3%, and all these cases switched from a positive HER2 status to negative in the metastatic tumor. Rossi *et al.*^[9] in their review of discordant cases report that it is frequent to have HER2 positive metastases with negative primary tumors. The discrepant results may be procedural or interpretational. Hanley *et al.*^[21] and Williams *et al.*^[23] did not show a good concordance between HER2-IHC and ER and PR in cell blocks of aspirates and tissue sections. However, their cell blocks were fixed in 50% ethanol. It is recommended that the cell blocks from the needle aspirates are made using the plasma thrombin clot procedure prior to fixing in formalin and embedding in paraffin. Cell blocks prepared similarly by Vohra *et al.*^[18] indicated that IHC for HER2, ER, and PR was reliable in predicting the expression of these markers when correlated with IHC and/or FISH performed on the corresponding tumor tissue.

CONCLUSION

ER, PR, and HER can be evaluated by IHC in cell blocks of FNA material from ALN with fair reliability. It is important to document them in recurrent or metastatic tumor as a change in their status may occur, which could affect the management of the patient. They also act as prognostic indicators. In our limited study, we found that HER2-FISH contributed in a limited manner as the equivocal cases remained equivocal or negative. HER2-FISH in the PBC is only done on cases equivocal by IHC. In the aspirates of metastatic ALN, HER2-IHC was effective in determining the change in status of HER2. However, our sample size is limited, and this observation needs to be substantiated by larger studies. Our observations suggest that the laboratories where sophisticated equipment for FISH is not available, can still document the change in the marker status by IHC, which has a significant bearing on the management of the breast carcinoma patients.

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Conflicts of interest

There are no conflicts of interest.

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