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Original contribution

Breast cancer biomarkers before and after neoadjuvant chemotherapy: does repeat testing impact therapeutic management?☆,☆☆

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Summary In patients treated with neoadjuvant chemotherapy (NAC), there is no consensus on retesting biomarkers within the excision specimen. Our aim was to investigate the clinical relevance of biomarker changes post-NAC at a large tertiary medical center. A retrospective search was performed to identify cases from 2012 to 2015 with needle biopsy-confirmed invasive breast carcinoma treated with NAC and subsequent excision containing residual invasive tumor. Biomarkers (estrogen receptor [ER], progesterone receptor [PR], and HER2/neu [HER2]) were performed on all pre-NAC biopsies. One hundred fifty-four NAC-treated cases were identified in which 83 (54%) had repeat testing of at least 1 biomarker on the surgical specimen. Twenty-five (30%) of 83 repeated cases demonstrated changes in pre-NAC biopsy versus post-NAC resection biomarker status. There was no impact of age or grade on biomarker status changes. Tumors that were triple negative at biopsy were more likely to remain triple negative. Clinically relevant changes were identified including the following: (1) ER negative to ER positive, 2 (3%) of 75; (2) PR negative to PR positive with ER negative both pre- and post-NAC, 2 (3%) of 73; and (3) HER2 negative to positive, 1 (1%) of 77. Four of 5 of the changes led to modifications of the adjuvant treatment regimen, including the addition of adjuvant tamoxifen, anastrazole, or trastuzumab. In summary, post-NAC biomarker repeat testing in patients with breast cancer impacts therapeutic management in a small subset of patients and therefore, repeat testing may be considered for patients that are hormone receptor and/or HER2 negative before NAC.

1. Introduction

Neoadjuvant chemotherapy (NAC) is used to decrease tumor size and improve surgical conditions in the treatment of breast cancer. Biomarker (estrogen receptor [ER], progesterone receptor [PR], and HER2/neu [HER2]) status plays an important role in the choice of neoadjuvant regimen. Previous studies have determined that the biomarker status of the resection specimen post-NAC may differ from the results reported in the biopsy specimen [1-27]. A change in receptor status

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may dictate a change in adjuvant treatment. For example, if the reported hormone status was to switch from ER negative to positive, a patient could be a candidate for endocrine therapy, and if the reported biomarker status was to switch from HER2 negative to positive, a patient could be a candidate for trastuzumab. However, if biomarker status does not change or if the reported differences are not clinically relevant, then repeat testing is an additional unnecessary health care cost.

Currently, there are no national guidelines regarding whether the post-NAC residual tumor should be retested for ER, PR, or HER2. To establish national guidelines regarding repeat testing, it is necessary to demonstrate if there are differences in biomarker status pre-NAC and post-NAC, and whether the changes in biomarker status post-NAC have an impact on clinical management of patients. The aims of this study are to investigate the rate of reported biomarker differences post-NAC, determine if clinically actionable changes are observed, and establish the impact of the detected differences on the adjuvant regimen at our institution. The frequency of repeat biomarker testing, tumor characteristics that guide testing, and pathologist practice variability regarding repeat testing are for the first time analyzed.

2. Materials and methods

A retrospective cohort composed of women diagnosed via needle core biopsy with invasive breast carcinoma treated with NAC followed by subsequent surgical resection performed at The Ohio State University Wexner Medical Center from January 1, 2012 to May 6, 2015 was studied. Institutional review board approval was obtained and carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), with a waiver of informed consent. Pathology reports were analyzed to identify patients treated with NAC in which residual invasive carcinoma or lymph nodes metastasis was identified in the excision specimen. The pre-NAC core biopsy and post-NAC surgical resection ER, PR, and HER2 results, clinicopathological features, type of NAC received, and adjuvant therapeutic regimen were recorded. Biopsy and resection biomarker results were correlated for tumor location, focality, and histologic type. Comparison of repeat biomarkers was limited to cases that were considered the same primary tumor. Clinically relevant biomarker changes (as defined in the results section) were identified.

At this center, breast biopsy and resection specimens are diagnosed by a subspecialized breast pathology service. Core biopsy was performed either at an outside institution with slide review at our facility, including review of all biomarker slides, or was performed at our institution. Biomarker studies for each needle core biopsy performed at our hospital included ER immunohistochemistry (IHC), PR IHC, and both HER2 IHC and *HER2* fluorescence in situ hybridization (FISH). After NAC, patients underwent surgical treatment (partial mastectomy or mastectomy). Post-NAC specimens (breast or lymph node) were retested at the attending pathologist's discretion. All pre- and post-NAC biomarker slides from cases with discrepant results between biopsy and resection were additionally reviewed for this study.

Hormone receptor (ER/PR) IHC was evaluated using clone 1D5 or SP1 for ER and PgR 636 for PR (Dako, Carpinteria, CA; Spring Bioscience, Pleasanton, CA). Percentage of positive nuclei was determined by the following microscopic estimation: less than 1% negative and at least 1% positive. HER2 IHC was evaluated using clone 4B5 (Ventana, Tucson, AZ). Membrane staining was evaluated by the following microscopic estimation and semiquantitatively scored per the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines: 0, 1+ negative; 2+ equivocal; and 3+ positive [28,29]. *HER2* FISH was evaluated using PathVysion HER2 DNA Probe Kit (Abbott Molecular, Abbott Park, IL) and duet scanning imaging workstation (BioView, Billerica, MA). Until November 2013, a positive result was *HER2*/chromosome 17 centromeric probe ratio greater than 2.2, negative less than 1.8, and equivocal 1.8 to 2.2 [28]. After November 2013, a positive result was ratio at least 2.0 and/or *HER2* copy number at least 6.0, negative ratio less than 2.0 and copy number less than 4.0, and equivocal ratio less than 2.0 and copy number at least 4.0 and less than 6.0 [29].

Statistical analyses were performed in Minitab Express Version 1.4.0 (Minitab, State College, PA) using a 95% confidence interval with a P value <.05 considered significant. Unequal variances 2-tailed 2-sample t test was performed to compare the mean age and grade in cases with and without repeated biomarkers. A χ^2 test was performed to compare numbers of cases without biomarkers repeated to cases with biomarkers repeated.

3. Results

Cohort characteristics are depicted in Table 1. One hundred fifty-four breast surgical resections with post-NAC residual invasive breast carcinoma in the surgical resection from 153 patients (1 patient had 2 breast resections from 2 separate breasts) were identified in which 54% (n = 83) had repeat testing of at least 1 biomarker. Of cases without repeat biomarkers, 37% (n = 26) were ER+/PR+/HER2−, 1% (n = 1) was ER+/PR−/HER2−, 11% (n = 8) were ER−/PR−/HER2+, 18% (n = 13) were ER+/HER2+, 31% (n = 22) were ER−/PR−/HER2−, and 1% (n = 1) was other at biopsy. In cases with repeated biomarkers, 27% (n = 22) were ER+/PR+/HER2−, 10% (n = 8) were ER+/PR−/HER2−, 5% (n = 4) were ER−/PR−/HER2+ or equivocal, 13% (n = 11) were ER+/HER2+, 42% (n = 35) were ER−/PR−/HER2−, and 4% (n = 3) were other at biopsy. Patients with biomarkers repeated received doxorubicin and cyclophosphamide with or without paclitaxel or docetaxel (52%, n = 43), trastuzumab in combination with other agents (20%, n = 17), carboplatin with or without doxorubicin,

cyclophosphamide, and/or paclitaxel (14%, n = 12), or other regimens (13%, n = 11). Almost all repeat testing was performed on breast tissue (90%, n = 75) with a minority on lymph node metastases.

There was no significant difference in patient age, mean grade of biopsy, or resection node status in cases with biomarkers repeated and those without. At resection, tumors with biomarkers repeated were more likely to be at least ypT1a than cases with no repeat (without repeat, 76% ≥ypT1a versus ER repeated ≥ypT1a, 92%, $P = .01$; PR repeated ≥ypT1a, 93%, $P < .01$; HER2 repeated ≥ypT1a, 94%, $P < .01$).

Forty-nine percent of cases had ER repeated, 47% had PR repeated, and 50% had HER2 repeated (either IHC or FISH, or both). Nine (35%) of 26 ER+/PR+/HER2− cases, 2 (50%) of 4 ER−/PR−/HER2+, 4 (50%) of 8 ER+/PR−/HER2−, 5 (45%) of 11 ER+/HER2+, 3 (9%) of 35 ER−/PR−/HER2−, and 2 (67%) of 3 with other biomarker profiles demonstrated changes on excision repeat testing.

3.1. All biomarker changes

In tumors with repeated biomarkers, a subset (30%, 25/83) demonstrated changes pre-NAC versus post-NAC (Table 2, Fig. 1). Of these 25 cases, 16% (n = 4) had changes in the status of 2 biomarkers (2 cases changed ER and HER2 and 2 cases changed PR and HER2). There was no impact of age, grade, resection at least ypT1a, node status, tumor triple positivity, or location at which biopsy biomarkers were performed (outside or our institution) on biomarker stability. Tumors that did not demonstrate any changes in biomarker status were more likely to be pre-NAC triple negative (triple negative with no changes, 91% versus not triple negative, 58%, $P < .01$).

In tumors retested for ER, 6 (8%) of 75 demonstrated a change in pre-NAC versus post-NAC. Five percent (n = 4) were reported as positive at biopsy but negative at resection, whereas 3% (n = 2) changed from negative to positive. Of the tumors that switched from ER positive to negative, 3 were low positive (<10%), 3 were weak intensity, and 2 were grade 3 pre-NAC (Fig. 2A and B). In tumors that changed from ER negative to positive, both were grade 3 pre-NAC. One tumor was low positive, and both were weak-intensity post-NAC (Fig. 2C and D). No relationship between high grade at biopsy and switching from ER positive to negative or negative to positive was identified.

In tumors retested for PR, 13 (18%) of 73 demonstrated a change in pre-NAC versus post-NAC. Twelve percent (n = 9) were positive at biopsy but negative at resection, whereas 5% (n = 4) changed from negative to positive. Of the tumors that switched from PR positive to negative, 2 were low positive at biopsy, all had moderate (n = 6) or strong (n = 3) intensity, and 4 were grade 3 (Fig. 2E and F). For tumors that changed from negative to positive, 2 were grade 3 pre-NAC. Two tumors were low positive, 2 were strong, and 2 were weak-intensity post-NAC (Fig. 2G and H). One of the cases that were PR negative at biopsy but strongly positive at resection was found upon re-review to be an interpretation error in which the biopsy was actually partially strongly positive. No relationship between high grade at biopsy and switching from PR positive to negative or negative to positive was identified.

In tumors retested for HER2, 10 (13%) of 77 demonstrated a change in pre-NAC versus post-NAC. Three percent (n = 2) switched from positive to equivocal, 4% (n = 3) from positive to negative, 1% (n = 1) from equivocal to negative, 4% (n = 3) from negative to equivocal, and 1% (n = 1) from negative to positive (Fig. 2I-L). All patients reported to have changed from HER2 positive to equivocal or negative received neoadjuvant trastuzumab. The patient who switched from HER2 equivocal to negative did not receive neoadjuvant trastuzumab. There was no impact of high grade at biopsy and switching from HER2 positive to equivocal or negative, or negative to equivocal or positive. There were no changes in HER2 status due to biopsy interpretation by the 2007 ASCO/CAP guidelines and resection interpretation by the 2013 ASCO/CAP guidelines.

3.2. Clinically actionable biomarker changes

Five (6%) of 83 patients with repeat testing had clinically actionable changes identified (Table 3, Fig. 1). The following changes were identified as clinically relevant in tumors with repeated biomarkers: (1) ER negative to ER positive, 2 (3%) of 75 (Fig. 2B), (2) PR negative to PR positive with ER negative both pre- and post-NAC, 2 (3%) of 73 (Fig. 2D), and (3) HER2 negative to HER2 positive (via FISH testing), 1 (1%) of 77 (Fig. 2F). There were no patients who were HER2 equivocal at biopsy and HER2 positive at resection. Four of 5 changes led to modifications of the adjuvant treatment regimen. One ER and 1 PR change resulted in the addition of

Table 1 Comparison of tumor characteristics in cases without repeat biomarker testing versus with repeat

	Age (y)	Biopsy				Resection	
		Median grade	ER negative	PR negative	HER2 negative	≥ypT1a	≥ypN1mi
No repeated biomarkers (n = 71)	51.4	3	44%	52%	68%	76%	51%
Biomarkers repeated							
ER (n = 75)	51.8	3	47%	60%	77%	92%	55%
PR (n = 73)	52.0	3	48%	60%	79%	93%	53%
HER2 (n = 77)	52.2	3	51%	60%	84%	94%	56%

Table 2 Changes observed in ER, PR, and HER2 status from pre-NAC biopsy to post-NAC resection

	Pre-NAC biopsy	Post-NAC resection	% (n)	Biopsy median tumor grade
ER	+	+	48 (36)	3
	+	−	5 (4)	2.5
	−	+	3 (2)	3
	−	−	44 (33)	3
PR	+	+	26 (19)	2
	+	−	12 (9)	2
	−	+	5 (4)	2.5
	−	−	55 (40)	3
	Not tested	−	1 (1)	2
HER2	+	+	8 (6)	3
	+	Equivocal	3 (2)	3
	+	−	4 (3)	2
	Equivocal	−	1 (1)	3
	−	Equivocal	4 (3)	3
	−	+	1 (1)	3
	−	−	79 (61)	3

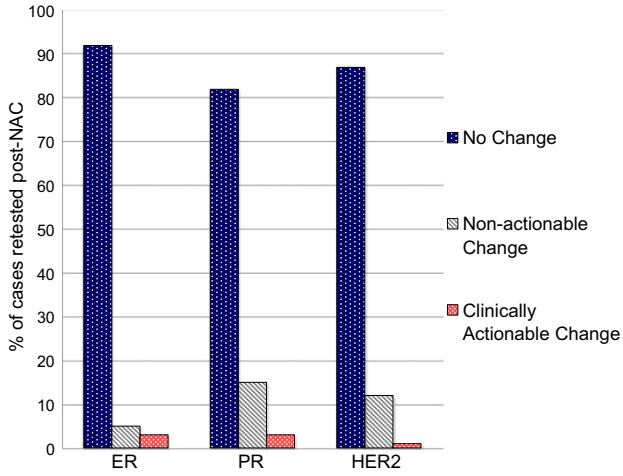

Fig. 1 ER, PR, and HER2 changes observed in cases with repeat biomarker testing post-NAC.

tamoxifen to the adjuvant regimen, although in these cases the post-NAC result was only 1% to 2% tumor reactivity. The second ER change resulted in the addition of anastrazole. The second PR change did not result in any addition due to pregnancy, with endocrine therapy being considered for after the postpartum period. For all 4 of the ER and PR reported differences, the post-NAC biomarker staining intensity was weak. The HER2 change resulted in the addition of trastuzumab. Of note, the HER2 ratio pre-NAC (1.98) was very close to the current threshold for HER2 positive, yet *HER2* copy number was less than 4 both pre- and post-NAC.

3.3. Practice patterns

The 154 resections were diagnosed by 7 pathologists, designated pathologists 1 to 7. Pathologist 7 only diagnosed 2 cases and was excluded from practice pattern analyses. There was an observed difference in practice patterns regarding repeat testing with the following 3 patterns identified: frequent testing, infrequent testing, and testing in half of the cases ($P < .01$) (Table 4).

Pathologists 1 and 2 repeated testing in most of their specimens (84% repeated, 38/45), and half of the cases not repeated had triple positive biomarkers before NAC (without repeat, triple positive, 57% versus repeated, triple positive, 13%, $P < .01$). Five percent of the cases in this subgroup had clinically actionable changes, which may more accurately reflect the true percentage of identifiable clinically actionable changes if robust repeat testing is performed. Pathologists 3 and 4 did not repeat biomarker testing in most of their specimens (28% repeated, 9/32). They repeated biomarker testing on tumors with a higher grade, although this difference was not statistically significant (without repeat, mean grade 2.3 versus repeated, mean grade 2.6, $P = .14$). Unlike the entire cohort, they did not retest more commonly in tumors that were at least ypT1a. Pathologists 5 and 6 repeated testing in approximately half of their cases (48% repeated, 36/75). They had less repeat biomarker testing in cases that were triple positive biomarkers before NAC, but this difference was not significant (without repeat, triple positive, 15% versus repeated, triple positive, 5%, $P = .17$).

4. Discussion

There is controversy regarding the frequency of biomarker conversion in breast cancer pre- and post-NAC [1-9,30-32]. Furthermore, only a small number of studies have assessed patient outcomes after NAC in tumors with biomarker status changes [7,10-13] and none have evaluated changes in management due to gain of hormone receptor or HER2 biomarker status or analyzed clinical practice patterns of repeat testing between pathologists.

We identified differences in ER, PR, and HER2 reported status pre- and post-NAC in 8%, 18%, and 13% of cases. Previous studies comparing biomarker status in tumors before and after NAC have reported a wide range of differences, with calculated median frequencies of change for the published literature of 13% for ER, 21% for PR, and 12% for HER2, comparable to our findings [1-3,8-27,30,31]. We found the most frequent change in PR, as described by others [1-3,11,14-17]. There were much higher rates of changes from positive to negative in ER, PR, and HER2 than negative to positive. We further observed that tumors without any biomarker changes post-NAC were more likely to be triple negative at biopsy, consistent with a prior report [13].

In our study, changes in biomarker status from negative to positive were observed in 3% of tumors retested for ER, 5% for PR, and 1% for HER2. Previous studies have documented

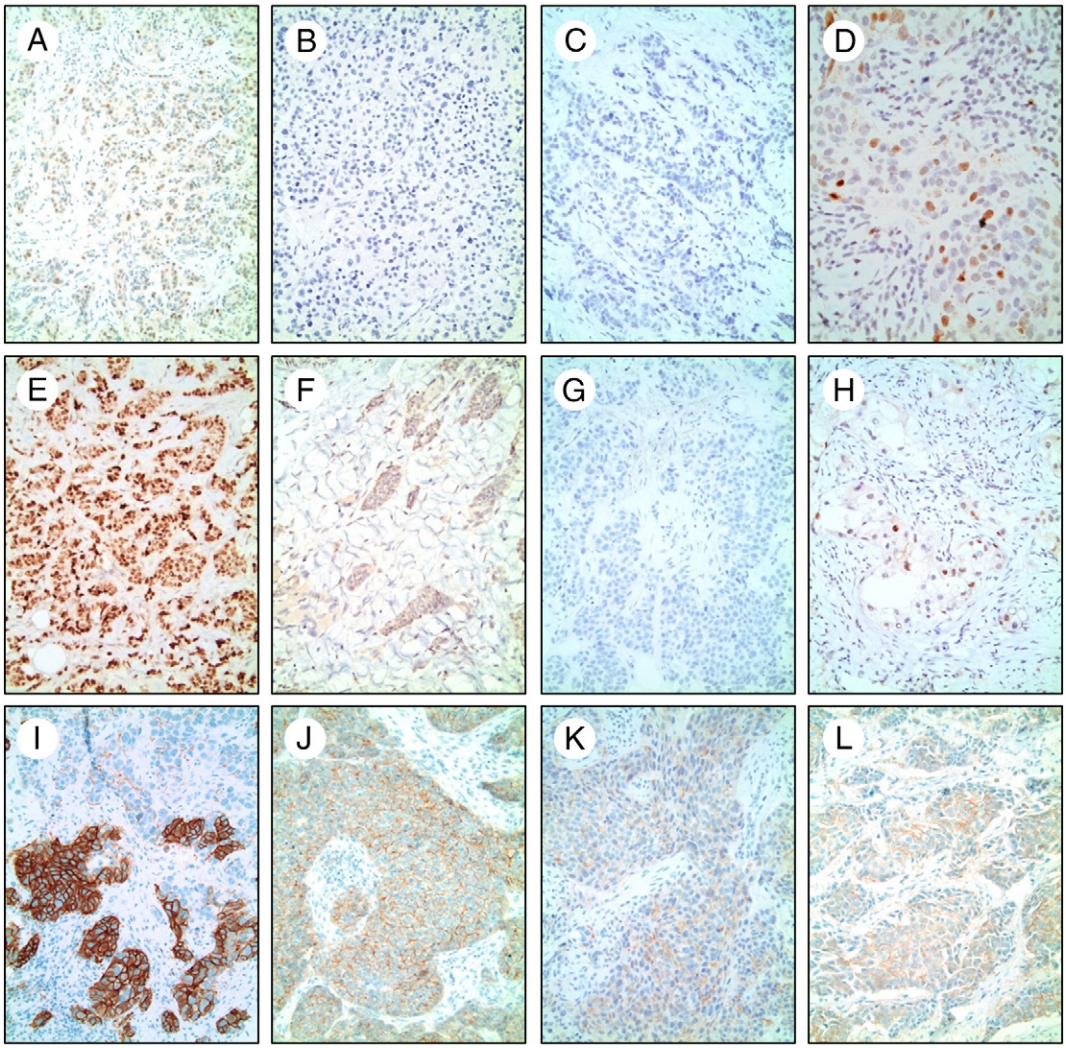

Fig. 2 Photomicrographs of biomarkers pre- and post-NAC. A and B, ER low positive with weak intensity pre-NAC and ER negative post-NAC (A and B, original magnification, ×20). C and D, ER negative pre-NAC (C, original magnification, ×20) and ER positive with weak intensity post-NAC (D, original magnification, ×40) (patient 1 in Table 3). E and F, PR positive with strong intensity pre-NAC and PR negative ~~with moderate intensity~~ post-NAC (E and F, original magnification, ×20). G and H, PR negative pre-NAC and PR low positive with weak intensity post-NAC with ER negative both pre- and post-NAC (G and H, original magnification, ×20) (patient 3 in Table 3). I and J, HER2 positive (IHC 3+; FISH ratio 3.9, copy 12.4) with heterogeneity (weak staining on top, strong staining on bottom) pre-NAC and HER2 equivocal (IHC 2+; FISH ratio 1.43, copy 4.6) post-NAC (I and J, original magnification, ×20). K and L, HER2 negative (IHC 1+; FISH ratio 1.98, copy 3.2) pre-NAC and HER2 equivocal by IHC but positive by FISH (IHC 2+; FISH ratio 2.4, copy 3.6) post-NAC (K and L, original magnification, ×20) (patient 5 in Table 3).

Table 3 Clinically actionable changes in tumor biomarker status with alterations in therapeutic management

Patient	Change	Pre-NAC biopsy	Post-NAC resection	Therapy change	Adjuvant addition
1	ER− to ER+	ER− (0%)	ER+ (15%, weak)	Y	Anastrazole
2	ER− to ER+	ER− (0%)	ER+ (2%, weak)	Y	Tamoxifen
3	PR− to PR+ (with ER− in Bx)	PR− (0%)	PR+ (1%, weak)	Y	Tamoxifen
4	PR− to PR+ (with ER− in Bx)	PR− (0%)	PR+ (30%, weak)	D	Endocrine therapy delayed
5	HER2− to HER2+	HER2− (IHC 1+; FISH ratio 1.98, copy 3.2)	HER2+ (IHC 2+; FISH ratio 2.4, copy 3.6)	Y	Trastuzumab

Abbreviations: Bx, biopsy; D, delayed; Y, yes.

Table 4 Comparison of pathologist practice patterns regarding tumor characteristics in cases without repeat biomarker testing versus with repeat

Pathologist	Age (y)	Biopsy		Resection	
		Tumor size	Triple positive	≥ypT1a	≥ypN1mi
1 and 2					
Biomarkers not repeated (n = 7)	50.0	1.0	57% *	57% *	43%
Biomarkers repeated (n = 38)	55.1	1.1	13% *	92% *	61%
3 and 4					
Biomarkers not repeated (n = 23)	52.1	1.1	9%	83%	70%
Biomarkers repeated (n = 9)	49.4	1.0	0%	78%	44%
5 and 6					
Biomarkers not repeated (n = 39)	51.3	1.0	15%	74% *	41%
Biomarkers repeated (n = 36)	50.2	1.1	6%	97% *	50%

* Statistically significant difference.

the percent of cases that switch from negative to positive, with median values for the literature that we have calculated as 7% for ER, 7% for PR, and 3% for HER2, similar to our results [2,3,8-18,21-24,26,27,30,31]. In our cohort of cases with biomarkers retested, 6% demonstrated a clinically actionable change of ER, PR, or HER2 post-NAC with 4 of 5 of these cases resulting in an addition to the adjuvant regimen based upon retesting. The patient who did not have a modification in adjuvant regimen is under consideration for future endocrine therapy. Although these changes in biomarker status impacted clinical management, the observed differences were small. The 2 ER and 2 PR changes switched from negative to positive but had weak reactivity and low percentage of nuclear positivity. In the HER2 change from negative to positive, the pre-NAC biopsy result was very close to the threshold for positive. In addition, although some of the changes in biomarker status were likely due to the receipt of NAC (eg, loss of HER2 after trastuzumab), others may be caused by intratumoral heterogeneity, differences in cold ischemic time, fixation time, or antibody clones, variability in pathologist's interpretation, or errors in processing/reporting. Our results may also be biased as only some of these cases with residual tumor were retested, and therefore, the percent of changes in biomarker status or modifications to the adjuvant regimen may be different when all cases are considered together. It is uncertain whether the small subset with actionable changes is sufficient to justify the health care costs of additional testing, as the clinical benefit of additions to adjuvant therapy in patients with focal/weak hormone receptor expression is unknown. Certainly, we recommend retesting HER2 if the pre-NAC biopsy result is near the threshold for positive.

Although a number of studies that analyzed the relationship between changes in biomarker status after NAC and patient outcomes have suggested that repeat testing may yield prognostic information, there is disagreement regarding the direction of change and its impact on overall or disease-free survival. One report correlated any change in hormone receptor status with improved overall survival, as well as gain in hormone receptor status with better disease-free survival [7], whereas 2 other articles observed that a loss of hormone receptor positivity led to worse overall and disease-free survival [10,12]. These contrast with 2 publications that observed no difference in overall or disease-free survival for patients with tumors that demonstrated changes in hormone receptor [11,13] or HER2 [13] status versus those with stable biomarkers. Importantly, there was no demonstrated survival benefit of continued endocrine therapy in patients who switched from hormone receptor positive to negative [10]. Therefore, loss of hormone receptor expression after NAC may represent another difference that has therapeutic relevance.

We identified different practice patterns at our institution between pathologists, including pathologists who repeated testing in most cases and others with infrequent repeat testing. Although retesting is performed at the discretion of the pathologist, oncologist, or surgeon, our study found that certain tumor characteristics appeared to guide pathologist practice patterns. For the cohort, repeat testing was more likely in larger tumors (≥ypT1a). However, there was no impact of biopsy triple negative or triple positive biomarkers, patient age, biopsy tumor grade, or node status. Limiting analysis to the subgroup with frequent repeat testing yielded 5% of cases with clinically actionable changes, which may more accurately reflect the true percentage of identifiable clinically actionable changes if robust repeat testing is performed. Further study of the factors that predict tumor biomarker stability post-NAC is needed to create guidelines for repeat testing after NAC, thus allowing for more uniform testing practices among pathologists.

In conclusion, our study showed that 6% of patients have clinically actionable reported differences in ER, PR, or HER2 biomarker status after NAC that resulted in additions to the adjuvant therapy regimen. Detection of these changes is contingent upon whether repeat testing is performed, which occurs at the physician's discretion as there are no guidelines regarding repeat testing, and we observed significant variability in the frequency of repeat testing between pathologists. Alterations in biomarker status post-NAC can lead to adjustments in the adjuvant regimen, but further investigation is warranted to assess the clinical benefit of these changes.

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