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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,