RESEARCH ARTICLE

Alterations in Hormonal Receptor Expression and HER2 Status between Primary Breast Tumors and Paired Nodal Metastases: Discordance Rates and Prognosis

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Abstract

Background: We aimed to evaluate the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression discordance in matched pairs of primary breast cancer and lymph node metastasis specimens and determine the effect of discordance on prognosis. Materials and Methods: Among all patients diagnosed with lymph node metastases from 2004 to 2007, primary tumors and paired lymph node metastases were resected from 209 patients. The status of ER, PR, and HER2 expression was analyzed immunohistochemically in 200, 194, and 193 patients, respectively. Discordance was correlated with prognosis. Results: Biomarker discordance between primary tumors and paired lymph node metastases was 25.0% (50/200) for ER status, 28.9% (56/194) for PR status, and 14.0% (27/193) for HER2 status. ER positivity was a significant independent predictor of improved survival when analyzed in primary tumors and lymph node metastases. Patients with PR-positive primary tumors and paired lymph node metastases displayed significantly enhanced survival compared to patients with PR-positive primary tumors and PR-negative lymph node metastases. Patients with ER- and PR-positive primary tumors and paired lymph node metastases who received endocrine therapy after surgery displayed significantly better survival than those not receiving endocrine therapy. Similalry treated patients with PR-negative primary tumors and PR-positive paired lymph node metastases also displayed better survival than those not receiving endocrine therapy. Conclusions: Biomarker discordance was observed in matched pairs of primary tumors and lymph node metastases. Such cases displayed poor survival. Thus, it is important to reassess receptor biomarkers used for lymph node metastases.

Keywords: Breast cancer - biomarker - lymph node metastases - discordance - survival

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Introduction

Breast cancer is one of the most common causes of cancer-related deaths worldwide. The prediction of clinical outcome and the selection of patients for adjuvant therapy are currently based on prognostic factors [age, hormone receptor status, human epidermal growth factor receptor 2 (HER2) expression, tumor size, and lymph node involvement] according to international guidelines (Cinieri et al., 2007). Among these factors, the number of metastatic axillary lymph nodes is the most powerful prognostic factor (Nemoto et al., 1980). Axillary lymph node metastasis is affected by many factors, and the factors interact. The presence of axillary lymph node metastasis determines the prognosis of patients, affects the treatment options, well as the attitude of patients.The incidence of lymph node metastases is affected by chronic disease, scale of tumor, age, ER expression and pathologic diagnosis (Li et al., 2012).

Moreover, survival after relapse is reduced in node-positive patients compared to node-negative patients, indicating that nodal metastasis may also serve as a marker of aggressive phenotype and not simply a marker of disease recurrence (Jatoi et al., 1999).

Adjuvant therapy decisions are based on the molecular pathology of diagnostic core biopsy or resection specimens obtained from the primary tumor. For example, chemotherapy is commonly used in estrogen receptor (ER)- or progesterone receptor (PR)-negative or lymph node-positive patients. In contrast, endocrine therapy and anti-HER2 (trastuzumab) therapy have recently been recommended for ER-positive and HER2-positive patients (Goldhirsch et al., 2009). However, 60% of patients do not benefit from endocrine therapies, and only 30-40% of trastuzumab patients receive a clinical benefit (EBCTCG, 2005; Smith et al., 2007). One important cause of treatment failure may result from differing biomarker status between primary tumors and metastatic disease (e.g., ER-positive breast tumor *vs*. ER-negative node). Recently, an increased proportion of cases were shown to display

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disparate receptor status between primary and nodal disease compared to the proportions in previous studies (Masood et al., 2000; Simon et al., 2001; Tanner et al., 2001; Gancberg et al., 2002; Tsutsui et al., 2002; D'Andrea et al., 2007; Gomez-Fernandez et al., 2008). Furthermore, clinical outcome based on lymph node biomarker status has not yet been reported, and no information on adjuvant treatment has been provided (Cardoso et al., 2001; De la Haba-Rodriguguez et al., 2004; Dikicioglu et al., 2005; Ataseven et al., 2012; Raica et al., 2014).

The aim of this study was therefore to assess biological markers in breast cancer axillary lymph node metastases and compare these data to the primary tumor status. Furthermore, the effect of biomarkers discordance on prognosis and therapy was also examined retrospectively.

Materials and Methods

Patients

The patients included in this study (median age=50 years, range=30-74 years) were diagnosed with stage II-III (pT1-3N1-3M0) breast carcinoma at the First Affiliated Hospital of China Medical University from 2004 to 2007, as previously described (Table 1). All 1, 156 surgical breast resections were matched to corresponding lymph nodes from axillary lymph node dissections, of which 517 contained metastases. The inclusion criteria for this study included the involvement of 10 or more regional lymph nodes, the absence of distant metastasis up to 6 months after diagnosis, and a minimum of 6 years of follow-up. A total of 209 formalin-fixed, paraffin-embedded primary tumor tissues and paired nodes metastases were available for this study. Post-surgical follow-up and examinations were performed every 3 months until 3 years post-surgery, every 6 months for 3-5 years post-surgery, and every 12 months thereafter.

Table 1. Baseline Demographic and Clinical Characteristics

Characteristic	n (%)
Median age (range) in yrs.	50 (30-74)
Median size (range) in cm	3.0(1.0-6.0)
No. of positive nodes at the primary tumor	
1-3	102 (49%)
4≤	207 (51%)
Stage	
2	100 (45%)
3	121 (55%)
ER status	
Positive	126 (60%)
Negative	74 (36%)
Unknown	9 (4%)
PR status	
Positive	112 (53%)
Negative	83 (40%)
Unknown	14 (7%)
HER 2 status	
Positive	30 (14%)
Negative	163 (78%)
Unknown	16 (8%)
Systemic treatment	
No systemic treatment	23 (11%)
Hormones	1
Chemotherapy	107 (51%)
Chemotherapy and hormones	78 (37%)

Table 2. BiomarkerDistribution in Primary Tumors and Paired Lymph Node Metastases

Variable Skewness	n	+/-	-/+	+/- or -/+	% Discordant	*P*
ER	200	31	19	50	25	0.119
PR	194	25	31	56	28.87	0.504
HER2	193	17	10	27	13.99	0.248

Immunohistochemical staining

Immunohistochemical staining was quantified on an interval scale and categorized according to the standardized cut-off levels for each marker.

Four-micrometer-thick dewaxed sections were incubated in 3% H_2O_2 diluted in wash buffer (Tris-buffered NaCl solution with Tween 20, pH 7.6) for 30 min. After washing, the sections were treated with serum-free Protein Block (DAKO Cytomation, Milan, Italy) for 30 min, followed by incubation with the corresponding antibodies overnight at 4°C. After three washes, the sections were treated with peroxidase-conjugated EnVision™ + dual link (DAKO Cytomation, Milan, Italy) for 30 min. The sections were then developed using the DAB substrate-chromogen system (DAKO Cytomation, Milan, Italy) and counterstained with hematoxylin.

The number of positive cells was counted in 10 random optic fields using a light microscope equipped with a 50× objective. Slides were reviewed by light microscopy, and the degree of positive tumor cell staining was represented as an approximate percentage of positive cells. The scoring was also classified as negative or positive according to the indicated cut-off for each marker. There is support for a cut-off value of 1% for endocrine treatment and thus the detection of any ER positive cell in the tumour will define it as an ER responsive tumour (Goldhirsch A et al., 2009). ASCO/PAP guidelines support the 1% cut-off (Hammond et al., 2010) but the guidelines are questioned in a recent study (Deyarmin et al., 2013). In our study, ER and PR status was considered positive if >1% of the cells were positively stained for the respective biomarker. HER2 status was considered positive for all 3+ tumors and negative for 0, 1+, and 2+ tumors.

Statistical methods

McNemar's test was used to evaluate whether the differences in dichotomized variables measured in the present study in both directions (+/- and -/+) were equally common when comparing primary tumors and lymph nodes. The Kaplan-Meier method was used to estimate survival, and the log-rank test was employed to evaluate null hypotheses of equal survival in two patient strata.

P-values less than 0.05 derived from two-sided tests were considered significant. The statistical software package Stata 19.0 (StataCorp., IBM, SPSS, USA) was used for statistical calculations.

Results

ER, PR, and HER2 expression discordance was estimated in 200, 194, and 193 matched pairs of primary

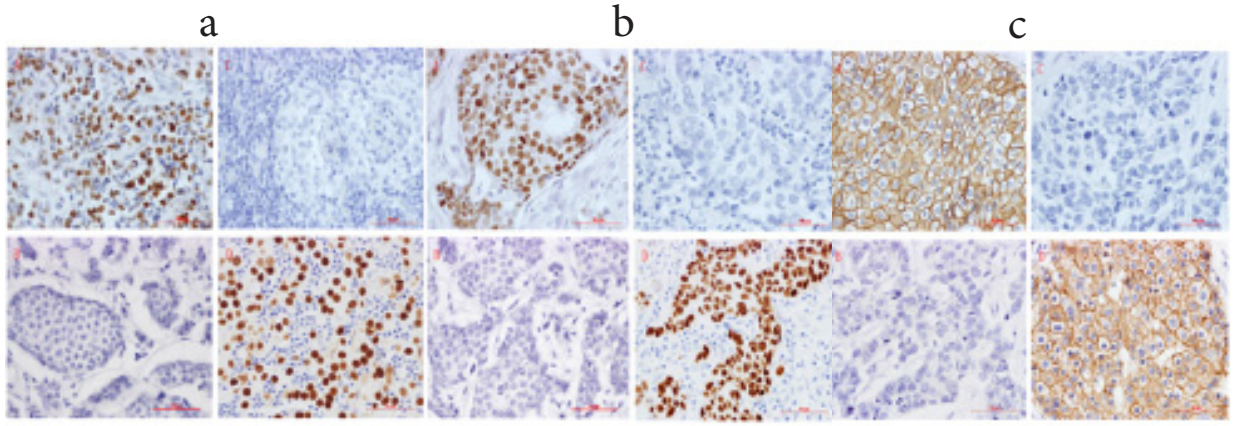

Figure 1. Immunohistochemical Findings a. A: ER express positive in the primary tumor; B: ER express negative in the paired lymph node metastases. C: ER express negative in the primary tumor; D: ER express positive in the paired lymph node metastases; b. A: PR express positive in the primary tumor; B: PR express negative in the paired lymph node metastases. C: PR express negative in the primary tumor; D: PR express positive in the paired lymph node metastases; c. A: HER2 express positive in the primary tumor; B: HER2 express negative in the paired lymph node metastases. C: HER2 express negative in the primary tumor; D: HER2 express positive in the paired lymph node metastases

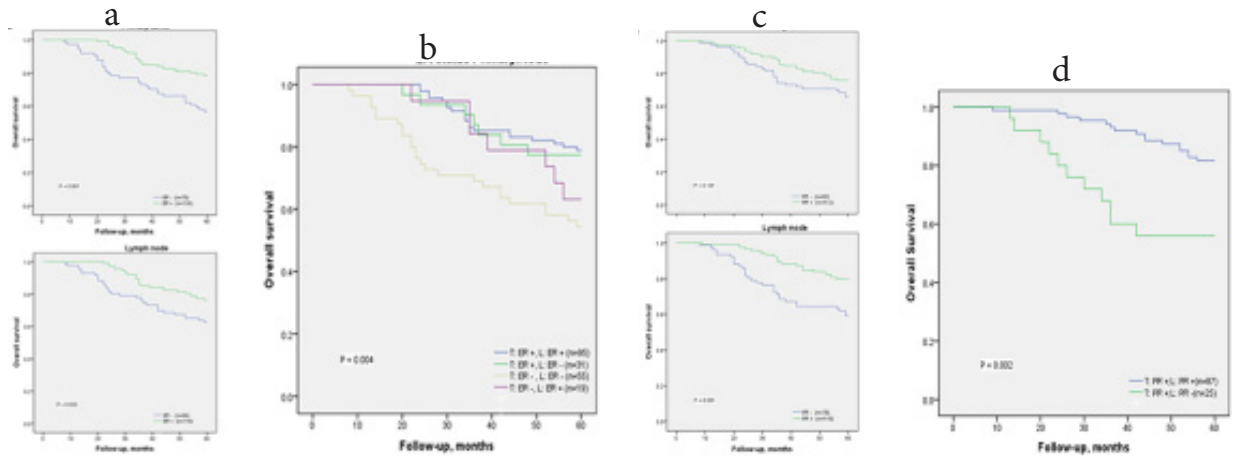

Figure 2. Kaplan-Meier estimates of OS based on ER status in the primary tumor and in lymph node metastases. a. Patients with ER-positive tumors were compared to patients with ER-negative tumors, and the P-value was calculated using a two-sided log-rank test; b. Kaplan-Meier estimates of overall survival (OS) for ER status as a combined variable of the ER statue in primary tumor and corresponding lymph node metastases. Patient with ER-positive primary tumor and/or lymph node metastases were compared with patients with ER-negative primary tumors and lymph node metastases. The P value was calculated using two-sided log-rank tests; c. Kaplan-Meier estimates of OS based on PR status in the primary tumor and in lymph node metastases. Patients with PR-positive tumors were compared to patients with PR-negative tumors, and the P-value was calculated using a two-sided log-rank test; d. Kaplan-Meier estimates of OS based on PR status as a combined variable in primary tumors and corresponding lymph node metastases. Patients with PR-positive primary tumors and lymph node metastases were compared to patients with PR-positive primary tumors and ER-negative lymph node metastases. The P-value was calculated using a two-sided log-rank test

tumors and lymph node metastases, respectively. The percentage of discordant pairs was 25.00% for ER status, 28.87% for PR status, and 13.99% for HER2 status. Statistically significant skewness was not observed for any of the biomarkers (Table 2). Immunohistochemistry images of ER, PR, HER2 express in primary tumor and paired lymph node metastases are provided inFigure1 a, b, c).

Association between biomarker status in primary tumors and lymph node metastases and overall survival (OS), as assessed by univariate analyses.

ER status

ER positivity was a significant independent predictor of enhanced survival in both primary tumors (c^2=12.039, *P*=0.001) and lymph node metastases (c^2=5.377, *P*=0.020) (Figure 2a). Significant OS predictions were also noted (c^2=13.583, *P*=0.004) when ER-positive cases were compared to ER-negative cases at both locations (+/+, +/-, and -/+ *vs*. -/-) (Figure 2b).

PR status

PR positivity in the primary tumor did not correlate with 5-year OS (c^2=5.377, P=0.101); however, different results were obtained for PR positivity in the lymph node (c^2=11.253, *P*=0.001) (Figure 2c). Moreover, patients with PR-positive primary tumors and paired lymph node metastases demonstrated significantly better survival than patients with PR-positive primary tumors and PR-negative lymph node metastases (c^2=9.803, *P*=0.002) (Figure 2d). These data suggest that PR at lymph node were significant factor for prognosis while PR becoming negative was a poor prognostic.

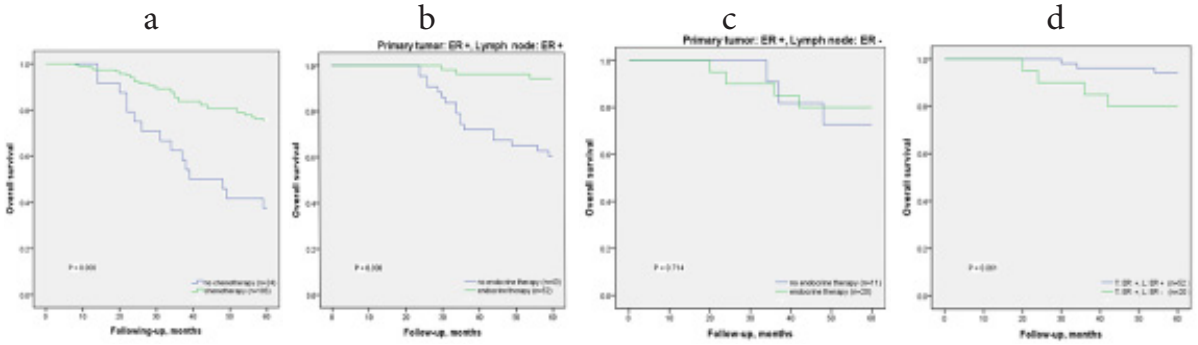

Figure 3. Kaplan-Meier estimates of OS. a. Patients who did not receive chemotherapy compared to patients who received chemotherapy. The P-value was calculated using a two-sided log-rank test; b. Kaplan-Meier estimates of OS for patients with ER-positive primary tumors and corresponding lymph node metastases. Patients who received endocrine therapy were compared to patients who did not receive endocrine therapy. The P-value was calculated using a two-sided log-rank test; c. Kaplan-Meier estimates of OS for patients with ER-positive primary tumors and ER-negative lymph node metastases. Patients who received endocrine therapy were compared to patients who did not receive endocrine therapy. The P-value was calculated using a two-sided log-rank test; d. Kaplan-Meier estimates of OS for patients with ER-positive primary tumors who were administered endocrine therapy. Patients with ER-positive lymph node metastases were compared to patients with ER-negative lymph node metastases. The P-value was calculated using a two-sided log-rank test

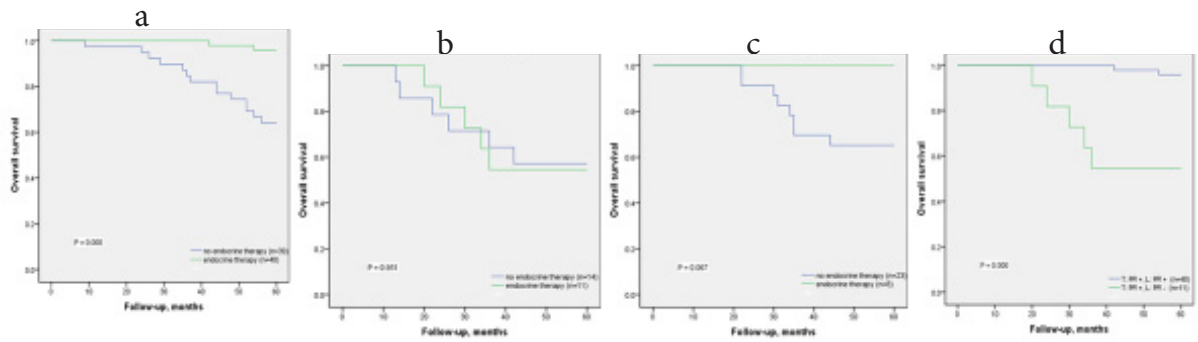

Figure 4. Kaplan-Meier estimates of OS. a. Patients with PR-positive primary tumors and corresponding lymph node metastases. Patients who received endocrine therapy were compared to patients who did not receive endocrine therapy. The P-value was calculated using a two-sided log-rank test; b. Kaplan-Meier estimates of OS for patients with PR-positive primary tumors and PR-negative corresponding lymph node metastases. Patients who received endocrine therapy were compared to patients who did not receive endocrine therapy. The P-value was calculated using a two-sided log-rank test; c. Kaplan-Meier estimates of OS for patients with PR-negative primary tumors and PR-positive corresponding lymph node metastases. Patients who received endocrine therapy were compared to patients who did not receive endocrine therapy. The P-value was calculated using a two-sided log-rank test; d. Kaplan-Meier estimates of OS for patients with PR-positive primary tumors who received endocrine therapy. Patients with PR-positive lymph node metastases were compared to patients with ER-negative lymph node metastases. The P-value was calculated using a two-sided log-rank test

HER2 status

The presence of HER2-positive primary tumors did not correlate with 5-year OS (P=0.180), and similar results were obtained for HER2-positive lymph nodes (P=0.070).

Association between changes in biomarker status and type of adjuvant systemic therapy and OS. Chemotherapy was a significant independent predictor of improved survival regardless of ER, PR, and HER2 expression in the primary tumor and lymph node metastases (c^2=16.858, P=0.000) (Figure 3a).

Among patients with ER-positive primary tumors and paired lymph node metastases, those who received endocrine therapy following their initial diagnosis displayed significantly enhanced survival compared to patients who were not administered endocrine therapy (c^2=16.510, P=0.000) (Figure 3b). However, no significant difference in survival was observed between patients with ER-positive primary tumors and ER-negative paired lymph node metastases who received endocrine therapy compared to patients who did not receive endocrine therapy (c^2=0.134, P=0.714) (Figure 3c). Among ER+ patients receiving hormonal therapy, enhanced survival was observed in the ER concordant (ER+→ER+) group compared to the receptor discordant group (ER+→ER-) (c^2=3.512, P=0.061) (Figure 3d).

Similar results were observed for PR status (c^2=14.917, P=0.000; c^2=0.004, P=0.951) (Figure 4a, b). Interestingly, patients with PR-negative primary tumors but PR-positive lymph nodes who were administered endocrine therapy display a better prognosis than patients who did not receive endocrine therapy (c^2=3.343, P=0.06) (Figure 4c). Given that the data set for this category only included eight patients, no definitive conclusions can be drawn due to the lack of statistical power. Among PR+ patients who received hormonal therapy, significantly enhanced survival was observed in the PR concordant (PR+→PR+) group compared to the receptor discordant group (PR+→PR-) (c^2=18.500, P=0.000) (Figure 4d).

Discussion

The heterogeneous expression of molecular markers between breast cancer patients is well established. Although cancer therapies target metastases, hormone receptor and HER2 status are typically evaluated only for the primary tumor. In fact, this characterization may be inappropriate given that secondary disease acquires new biological characteristics to gain access to blood vessels/lymphatics and colonize remote sites (Chanbers AF et al., 2000). Some of these molecular alterations may be associated with changes in receptor status, given that endocrine and growth signaling pathways are involved in invasion and metastasis (Maynadier M et al., 2008; Huang TH et al., 2009). We therefore sought to investigate differences in receptor expression levels between primary tumors and paired lymph nodal metastases, which may serve as an alternative explanation for resistance to targeted therapy in breast cancer.

This study represents one of the largest series published to date demonstrating molecular phenotype discordance between primary tumors and paired lymph node metastases. The strength of this study lies in the acquisition of tissue from both primary tumors and paired lymph node metastases. In addition, survival analysis was performed in relation to biomarker status at both locations, and the primary tumor and lymph node samples were subjected to identical antibody staining conditions and scoring criteria. Additional strengths include pathologist blinding and the assessment of ER, PR, and HER2 status. In line with previous publications, few discordant cases for all analyzed markers were observed, with no significant distribution skewness. We observed biomarker discordance between the primary tumor and paired lymph node metastases in 25.00% (50/200), 28.87% (56/194), and 13.99% (27/193) of patients for ER, PR and HER2 status, respectively.

Extensive reports from 1984 compared ER, PR, and HER2 expression between lymph node metastases and primary tumors. However, data from high-quality prospective studies are limited. Furthermore, it remains unclear whether ER, PR, and HER2 assays using lymph node metastasis samples can accurately evaluate the status of the primary tumor.

We also analyzed the association between biomarker status in primary tumors and lymph node metastases and OS using Kaplan-Meier curves. Our results indicated that ER-positive tumors at any location displayed a better prognosis than tumors that were ER-negative at both locations (P=0.004). PR-positive primary tumors did not correlate with 5-year OS (P=0.101), although different results were observed for PR-positive lymph nodes (P=0.001). Interestingly, patients with PR-positive primary tumors and paired lymph node metastases demonstrated significantly better survival compared to patients with PR-positive primary tumors and PR-negative lymph node metastases (P=0.002). These data suggest that PR-positive lymph node status is a significant marker of good prognosis, whereas PR-negative status is associated with poor prognosis. Moreover, HER2 positivity in the primary tumor did not correlate with 5-year OS (P=0.180), and similar results were obtained for HER2 positivity in the lymph node (P=0.070).

Adjuvant systemic therapy can significantly decrease breast cancer recurrence and mortality rates (EBCTCG, 2011; EBCTCG, 2012). In particular, endocrine therapy is recommended for most ER/PR-positive patients because of its efficacy and favorable safety (EBCTCG, 2011). Qi-Dong Ge et al. found that endocrine therapy could improve the survival time of ER/PR-positive patients regardless of endocrine therapy to give patients at initial diagnosis of breast cancer whether or not (Qi-Dong Ge et al., 2012). For HER2-positive disease, trastuzumab is considered the standard treatment (Slamon D et al., 2011). We analyzed the association between changes in biomarker status and type of adjuvant systemic therapy and OS using Kaplan-Meier curves, and we found that chemotherapy was a significant independent predictor of improved survival regardless of ER, PR, and HER2 expression in the primary tumor and lymph node metastases (P=0.000). Among patients with ER/PR-positive primary tumors and paired lymph node metastases, significantly enhanced survival was observed in patients who received endocrine therapy following their initial diagnosis compared to patients who did not receive endocrine therapy (P=0.000/ P=0.000). However, no significant survival difference was observed between patients with ER/PR-positive primary tumors and ER/PR-negative paired lymph node metastases who were administered endocrine therapy compared to those who did not receive endocrine therapy (P=0.714/P=0.951). Among ER+/PR+ patients receiving hormonal therapy, enhanced survival was observed in the ER/PR concordant group (ER+→ER+, PR+→PR+) compared to the receptor discordant group (ER+→ER-, PR+→PR-) (P=0.061, P=0.000). Given that a proportion of ER/PR-positive patients do not respond to endocrine therapy and demonstrate a poor outcome, it is possible that endocrine therapy is not appropriate for patients with ER/PR-positive primary tumors and ER/PR-negative nodes; in particular, this observation may prevent the use of unnecessary therapies and unpleasant side-effects. Interestingly, our data indicate that patients with PR-negative primary tumors but PR-positive lymph nodes who receive endocrine therapy may still experience a better prognosis than patients who do not receive endocrine therapy (P=0.06). However, because the data set for this category included only eight patients, no definitive conclusions could be drawn due to the lack of statistical power. Substantial *in vitro* and *in vivo* evidence suggests that PR expression may serve as a marker of endocrine dependence, thereby indicating a functional role for the PR. The response to anti-estrogen therapy also correlates with PR expression, and preclinical data indicate that the inhibition of PR function may inhibit proliferation and induce apoptosis (Jonat W et al., 2002). However, our study lacks a sufficient number of cases to compare patients with ER-negative primary tumors but ER-positive lymph nodes who received endocrine therapy to those who did not receive endocrine therapy, and our study also included a paucity of Her2-positive patients who received trastuzumab therapy.

Despite the strengths of our study, various limitations

of the study warrant discussion. Despite the relatively large sample size, the number of patients with discordant cases for all analyzed markers was limited; therefore, the conclusions may not generalizable to all patients presenting with lymph node metastases. Although every attempt was made to increase the sample size, the Kaplan-Meier OS results lack sufficient stringency for samples older than 6 years. In the future, the accumulation of greater numbers of samples should make these results more generalizable.

Another potential limitation of the current study was the possibility of antigen loss over time in formalin-fixed, paraffin-embedded tissue blocks. The tissue samples used to create both the primary tumor and paired lymph node metastasis samples were >6 years old in the majority of cases, and this could explain how ER-, PR-, and HER2-positive tumors became negative; however, the age of the samples does not explain the reverse observation. Of the 200 cases displaying molecular differences between the primary tumor and lymph node metastases, 60 (30%) exhibited a gain in receptor status.

In conclusion, receptor expression discordance between primary tumors and paired lymph node metastases occurred in 13.99-28.87% of our cases. These findings have significant implications for the selection of breast cancer treatment options and the evaluation of subsequent responses to therapy. However, future studies need to address the biology of this discordance and assess the clinical significance of biological marker alterations.

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