

Identifying breast cancer patients who require a double-check of preoperative core needle biopsy and postoperative surgical specimens to determine the molecular subtype of their tumor

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Purpose: Core needle biopsy (CNB) is a widely used procedure for breast cancer diagnosis and analyzing results of immunohistochemistry (IHC). Several studies have shown concordance or discordance in IHC results between CNB and surgical specimens (SS). A double-check (CNB and SS) is inefficient and costly to perform a double-check on all patients. Therefore, it is important to determine which patients would benefit from a double-check.

Methods: We collected the medical records of patients who underwent breast cancer surgery at Pusan National University Yangsan Hospital between April 2009 and June 2018 (n = 620). Molecular subtypes were classified as follows by hormone receptors (HR) and human epidermal growth factor receptor-2 (HER2): HR+/HER2+, HR+/HER2-, HR-/HER2+, HR-/HER2-. Clinicopathological factors including age, obesity, histological grade, preoperative CEA, CA15-3, T stage, N stage, and menopausal status were assessed to determine whether they were associated with subtype change.

Results: Increasing histological grade (P < 0.001; odds ratio [OR], 3.693; 95% confidence interval [CI], 1.941–7.025), preoperative CEA ≥ 5 ng/mL (P = 0.042; OR, 2.399; 95% CI, 1.009–5.707) and higher T stage (P = 0.015; OR, 2.241; 95% CI, 1.152–4.357) were significantly associated with subtype change. On multivariable analyses, subtype changes were more common in high-grade breast cancer (P < 0.001; OR, 1.077; 95% CI, 1.031–1.113) and CEA ≥ 5 (P = 0.032; OR, 2.658; 95% CI, 1.088–6.490).

Conclusion: Patients with moderate- to high-grade tumors or CEA ≥ 5 ng/mL are required a double-check to determine the molecular subtype of breast cancer.

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Key Words: Adjuvant, Biopsy, Breast neoplasms, Chemotherapy, Immunohistochemistry, Large-core needle

INTRODUCTION

Because breast cancer is the most frequently diagnosed cancer and the second most frequent cause of cancer-related death in women, preoperative assessments of diagnostic and prognostic factors are becoming more important in

management. Core needle biopsy (CNB) is routinely used for preoperative assessment of patients with breast cancer [1-4].

Breast cancers are heterogeneous tumors that arise from epithelial cells around the mammary ducts. The heterogeneity of mammary tumors has been well documented based on histopathology and clinical outcomes, and these differences

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have become the basis of disease classification. Today, this pathology-based classification has been refined and replaced with molecular classifications that have the potential to combine disease classification and clinical outcome measures [5]. Breast cancer treatment is varied depending on the molecular classification of the tumor [6].

Adjuvant systemic therapy can significantly reduce the rates of breast cancer recurrence and mortality [7,8]. Endocrine therapy is recommended for most patients with hormone receptors (HR) positive tumors because of its efficacy and safety [8]. For human epidermal growth factor receptor-2 (HER2) positive disease, trastuzumab is considered the standard treatment [9]. In addition, chemotherapy is considered an effective option for HR negative tumors or HER2 positive disease [7].

In general, patients with breast cancer undergo a double-check of their histopathologic results by comparison of a preoperative CNB and a postoperative surgical specimen (SS). CNB is a procedure widely used for diagnosis of breast cancer and to provide tissue for immunohistochemistry (IHC) [10-12]. The CNB test has the advantage of providing definitive results for hormone receptor and HER2 expression to inform treatment with neoadjuvant drugs before surgery. CNB also provides the only suitable material for preoperative molecular testing [1]. Another advantage of CNB is that the tissues obtained give better fixation results compared with SS. Fixation protocols are also more standardized for CNB, while many different protocols exist for the fixation of SS [10]. The 2015 European Society of Medical Oncology Breast Cancer Clinical Practice Guideline recommends a preoperative pathological examination of CNB that includes determination of the status of the estrogen receptor (ER), progesterone receptor (PR), and HER2 by IHC or fluorescence *in situ* hybridization [10,13].

Previous studies have highlighted controversies about concordance or discordance between the results for preoperative CNB and postoperative SS [1-3,10,14-17], and about whether a double-check is necessary when the results of both tests are concordant. Such a double-check is inefficient, and because a large number of women are diagnosed with breast cancer every year, it is costly to perform a double-check on all patients [17]. Therefore, it is important to determine which patients would benefit from a double-check.

In this study, breast cancer patients were divided into groups based on whether the CNB and SS results for tumor subtype were concordant or discordant, and the clinicopathological characteristics of each group were compared to determine which clinicopathological characteristics were associated with a change in breast cancer subtype between CNB and SS.

METHODS

Data collection

We collected information from the medical records of patients who underwent breast cancer surgery at Pusan National University Yangsan Hospital between April 2009 and June 2018 ($n = 1,353$). Clinical information obtained included age, body mass index (BMI), menopausal status, tumor-node-metastasis stage, history of neoadjuvant chemotherapy (NAC), the size, number, histological type, and histological grade of tumors, levels of CEA and CA15-3, and tumor expression of ER, PR, and HER2 from both CNB and SS samples. Patients with missing data or with previous NAC were excluded. Also, we excluded patients diagnosed with other histological types such as ductal carcinoma *in situ*, lobular carcinoma *in situ*, mucinous, metaplastic, papillary, etc. A total of 620 patients met our inclusion criteria.

Tissue processing and immunohistochemical staining for ER, PR, and HER2

Preoperative CNB specimen was fixed by 10% formalin for 2 hours. Postoperative SS was serially sectioned into thin slices and fixed by 10% formalin for at least 5 hours. CNB was submitted entirely and SS was submitted at least 2 representative sections of tumor for tissue processing (HistoCore PELORIS 3 Premium Tissue Processing System, Leica Biosystems, IL, USA).

Serial 4- μ m-thick paraffin-embedded sections from CNB and SS were stained for ER (SP1, Ventana Medical System, Tucson, AZ, USA), PR (1E2, Ventana Medical System, Tucson, AZ, USA) and HER2/neu (4B5, Ventana Medical Systems) by using an automated immune stainer (BenchMark XT; Ventana Medical System). Silver *in situ* hybridization was performed using HER2/CEP17 dual-probe (Ventana Medical Systems) through an automated stainer (BenchMark XT; Ventana Medical System).

Cutoff values for Hormone receptors (HR; ER or PR), and HER2

The expression status of tumors for ER, PR, and HER2 was analyzed by IHC using commercially available antibodies against these proteins (Roche/Ventana Corp., Tucson, AZ, USA). IHC staining with anti-ER and anti-PR antibodies was carried out using suitable positive and negative controls. A positive result was defined as staining of $\geq 1\%$ of tumor cells. A negative result was defined as staining of $< 1\%$ of tumor cells [18].

The results of HER2/neu IHC were scored semi-quantitatively on a scale of 0, 1+, 2+, and 3+. Scores of 0 and 1+ are considered negative, 2+ is considered indeterminate, and 3+ is considered strongly positive; the likelihood of response to anti-HER2 treatment increases with increasing score. A positive test for HER2 was defined as either an IHC score of 3+ or an

IHC score of 2+ combined with demonstration by silver *in situ* hybridization of amplification of the gene encoding HER2. The test was performed according to the recommendations of the American Society of Clinical Oncology/College of American Pathologists for HER2 testing in breast cancers [19].

Classification of subtypes

Breast cancer molecular subtypes were classified based on the results of IHC as follows: HR+/HER2+, HR+/HER2-, HR-/HER2+, HR-/HER2- [4,20-22].

Classification of obesity

Height and weight are the simplest and most commonly used measures of obesity. We used BMI defined as weight in kilograms divided by height in meters squared (kg/m^2).

We categorized BMI based on World Health Organization Western Pacific Region criteria, which define underweight as BMI $<18.5 \text{ kg}/\text{m}^2$, normal weight as $18.5\text{--}22.9 \text{ kg}/\text{m}^2$, overweight as $\geq 23.0\text{--}24.9 \text{ kg}/\text{m}^2$, and obesity as $\geq 25.0 \text{ kg}/\text{m}^2$ [23].

Cutoff values for CEA and CA15-3

Various cutoff values for CEA and CA15-3 have been used in breast cancer screening, including for CEA 2.5, 4, 5, and 6 ng/ml and for CA15-3 30 or 50 U/mL [24-28]. We selected the most commonly used cutoff values of $\geq 5 \text{ ng}/\text{mL}$ for CEA and $\geq 30 \text{ U}/\text{mL}$ for CA15-3. These values have been used in more than 30 studies and have been shown to have a specificity $>80\%$ and a sensitivity $>70\%$ [29,30].

Statistical methods

Clinicopathological factors including age, obesity, histological grade of tumor, preoperative CEA, preoperative CA15-3, T stage, N stage, and menopausal status were assessed to determine whether they were associated with subtype change using chi-square or Fisher exact tests in univariate analysis. Factors that were significantly associated with subtype change in the univariate analysis were included in a multivariate analysis using logistic regression. All tests of significance were two-sided, and a P-value of <0.05 was considered significant. Statistical analysis was carried out using IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA).

Ethical approval

Approval was granted by the Institutional Review Board of Pusan National University (IRB No. 05-2018-166). Patient records and information were anonymized and identification was removed before analysis to protect personal information. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or

Table 1. Clinical and pathological characteristics of breast cancer patients evaluated by double-checking of core needle biopsy and surgical specimen (n = 620)

Characteristic	No. (%)
Age (yr)	
<50	236 (38.1)
≥ 50	384 (61.9)
Body mass index (kg/m^2)	
<18.5 (underweight)	14 (2.3)
18.5–22.9 (normal)	220 (35.5)
23–24.9 (overweight)	157 (25.3)
≥ 25 (obese I)	191 (30.8)
≥ 30 (obese II)	38 (6.1)
Grade	
1	86 (13.9)
2	302 (48.7)
3	232 (37.4)
T stage	
pT1	287 (46.3)
pT2	287 (46.3)
\geq pT3	46 (7.4)
N stage	
pN0	392 (63.2)
pN1	161 (26.0)
pN2	41 (6.6)
pN3	26 (4.2)
Preoperative CEA	
<5	572 (92.3)
≥ 5	48 (7.2)
Preoperative CA 15-3	
<30	609 (98.2)
≥ 30	11 (1.8)
Menopause status	
Premenopausal	290 (46.8)
Postmenopausal	325 (53.2)
CNB subtype	
HR+ / HER2+	416 (67.1)
HR+ / HER2-	73 (11.8)
HR- / HER2+	54 (8.7)
HR- / HER2-	77 (12.4)
SS subtype	
HR+ / HER2+	401 (64.7)
HR+ / HER2-	73 (11.8)
HR- / HER2+	66 (10.6)
HR- / HER2-	80 (12.9)
Histological type	
IDC	597 (96.3)
ILC	23 (3.7)

CNB, core needle biopsy; HR, hormone receptors (ER or PR); HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

comparable ethical standards.

RESULTS

The clinicopathological characteristics of 620 breast cancer

Table 2. Subtype change and additional treatments according to SS or CNB

The change of subtypes			Additional treatment plan according to SS	Additional treatment plan according to CNB	No. (%)
CNB	→	SS			
HR+ HER2+	→	HR+ HER2-	-	Anti-HER2	2 (4.4)
		HR- HER2+	-	HTx.	12 (26.7)
HR+ HER2-	→	HR+ HER2+	Anti-HER2	-	10 (22.2)
		HR- HER2+	Anti-HER2	HTx.	1 (2.2)
		HR- HER2-	-	HTx.	9 (20.0)
HR- HER2+	→	HR+ HER2+	HTx.	-	3 (6.7)
		HR- HER2-	-	Anti-HER2	1 (2.2)
HR- HER2-	→	HR+ HER2+	HTx. + Anti-HER2	-	1 (2.2)
		HR+ HER2-	HTx.	-	3 (6.7)
		HR- HER2+	Anti-HER2	-	3 (6.7)
Total			21 (46.7)	25 (55.6)	45 (100)

SS, surgical specimen; CNB, core needle biopsy; HR, hormone receptors; HER2, human epidermal growth receptor-2; HTx., hormone therapy.

patients who were evaluated by a double-check of both CNB and SS are summarized in Table 1. The median age of these patients was 52 years (range, 23–93 years), and the median tumor size was 2.3 cm (range, 0.1–12.5 cm). The proportions of patients who had HR+/HER2+, HR+/HER2-, HR-/HER2+, HR-/HER2- subtypes on CNB were 67.1%, 11.8%, 8.7%, and 12.4%, respectively, while the proportions of patients with HR+/HER2+, HR+/HER2-, HR-/HER2+, HR-/HER2- subtypes on SS were 64.7%, 11.8%, 10.6%, and 12.9%, respectively.

The tumor subtyping of 45 patients (7.3%) in this study differed between the preoperative CNB and the postoperative SS. There were 14 cases (31.1%) that the subtypes changed from HR+/HER2+ to other types. There were 20 cases (44.4%) that the subtypes changed HR+/HER2- to other types. 4 (8.9%) were changed from HR-/HER2+ to others. Seven (15.6%) were changed from HR-/HER2- to other types. The most common of these was the change from HR+/HER2+ to HR-/HER2+ (26.7%). The change from HR+/HER2- to HR+/HER2+ (22.2%), HR+/HER2- to HR-/HER2- (20.0%) was the next most common (Table 2).

The results of univariate analysis of factors associated with subtype change are presented in Table 3. Histological grade, CEA \geq 5 ng/mL, and T stage were significantly associated with subtype change. The likelihood of subtype change increased significantly with increasing histological grade ($P < 0.001$; odds ratio [OR], 3.693; 95% confidence interval [CI], 1.941–7.025).

Patients with preoperative CEA \geq 5 ng/mL were significantly more likely to have subtype change than patients with CEA $<$ 5 ng/mL ($P = 0.042$; OR, 2.399; 95% CI, 1.009–5.707). The higher the T stage, the more likely the subtype was changed ($P = 0.015$; OR, 2.241; 95% CI, 1.152–4.357).

However, age, BMI, CA15-3 level, N stage, and menopausal status were not significantly associated with subtype change.

In the multivariate analysis (Table 4), subtype changes were more common in high-grade breast cancer ($P < 0.001$; OR, 1.077; 95% CI, 1.031–1.113). CEA \geq 5 ng/mL was a significant predictor of subtype change ($P = 0.032$; OR, 2.658; 95% CI, 1.088–6.490).

DISCUSSION

When adjuvant treatment was based on the results of the double-check, 21 (46.7%), 25 (55.6%) treatment were changed from CNB, SS only. 7 (16.6%), 15 patients (33.3%) had hormone or anti-HER2 treatment added from CNB only. 22 (48.9%), 3 patients (6.7%) additionally had hormone or anti-HER2 treatment from SS only.

Thus, additional treatments that would not have been used based on the results of SS alone were implemented based on the results of the CNB, resulting in benefit for many patients. However, the double-check of both CNB and SS has the disadvantage of redundant costs. Therefore, it would be preferable to be able to select those patients who could benefit

Table 3. Univariate analysis of factors associated with subtype change

Variable	Subtype changed (n = 45) (% of subgroup)	Subtype unchanged (n = 575) (% of subgroup)	OR (95% CI)	P-value
Age (yr)				0.967
<50	17 (7.2)	219 (92.8)		
≥50	28 (7.3)	356 (92.7)		
Body mass index				0.578
Underweight <18.5	1 (7.1)	13 (92.9)		
Normal 18.5–22.9	20 (9.1)	200 (90.9)		
Overweight 23–24.9	9 (5.7)	148 (94.3)		
Obese I 25	14 (7.3)	177 (92.7)		
Obese II 30	1 (2.6)	37 (97.4)		
Tumor grade				<0.001
1	5 (5.8)	81 (94.2)		
2	10 (3.3)	292 (96.7)		
3	30 (16.8)	202 (87.1)		
G1 + G2	15 (3.9)	373 (96.1)	3.693 (1.941–7.025)	<0.001
G3	30 (12.9)	202 (87.1)		
Histology				0.584
IDC	44 (7.4)	553 (92.6)		
ILC	1 (4.3)	22 (95.7)		
Preoperative CEA			2.399 (1.009–5.707)	0.042
<5	38 (6.6)	534 (93.4)		
≥5	7 (14.6)	41 (85.4)		
Preoperative CA15-3				0.813
<30	44 (7.2)	565 (92.8)		
≥30	1 (9.1)	10 (90.9)		
pT stage				0.049
pT1	13 (4.5)	274 (95.5)		
pT2	27 (9.4)	260 (45.2)		
pT3	5 (10.9)	41 (89.1)		
T1	13 (4.5)	274 (95.5)	2.241 (1.152–4.357)	0.015
T2 + T3	32 (9.6)	301 (90.4)		
pN stage				0.274
0	27 (6.9)	365 (93.1)		
1	11 (6.8)	150 (93.2)		
2	6 (14.6)	35 (85.4)		
3	1 (3.8)	25 (96.2)		
Menopause				0.745
Premenopausal	20 (6.9)	270 (93.1)		
Postmenopausal	25 (7.6)	305 (92.4)		

OR, odds ratio; CI, confidence interval.

Table 4. Multivariate analysis of factors associated with subtype change

Variable	P-value	OR	95% CI
Grade (G1/G2 vs. G3)	<0.001	1.077	1.031–1.113
Tumor size (pT1 vs. pT2/T3)	-	-	-
CEA ≥5 ng/mL	0.032	2.658	1.088–6.490

OR, odds ratio; CI, confidence interval.

from the double-check.

Our study showed that clinicopathological factors, including

the CEA level, tumor size, and histological grade, were associated with the likelihood of a change in molecular subtype of breast cancer between preoperative CNB and postoperative SS. Patients with CEA ≥ 5 ng/mL were significantly more likely to have a change in molecular subtype than those with CEA < 5 ng/mL (14.6% vs. 6.6%). Patients with high-grade tumor (≥3) were more likely to change subtype than those with moderate and low-grade tumors (12.9% vs. 3.9%). Although multivariate analysis did not show a significant association, we also noted that the larger the size of the primary tumor, the more likely it was to change subtype (T1 vs. T2 + T3 = 4.5% vs. 9.6%).

CEA, which belongs to a family of cell surface glycoproteins, is the most widely used tumor marker in clinical practice. It is a marker for colorectal, gastrointestinal, lung, and breast cancer [27]. Elevated CEA in breast cancer is known to be associated with metastatic disease. Preoperative CEA measurements correlate with pathological tumor stage and circulating levels of CEA in breast cancer patients and are directly dependent on the size of both primary and metastatic tumors [28]. In our study, we found that high CEA, and high histological grade were associated with subtype changes between preoperative CNB and postoperative SS. Thus, it can be assumed that advanced breast cancer can further change the subtype.

This study had some limitations. First, because it was a single-center retrospective study, there might have been selective bias in the results, which are then difficult to generalize to other populations. Second, many patients underwent surgery at our hospital after being diagnosed with breast cancer at another hospital. When CNB was performed at another hospital, we did not repeat it. However, slides obtained from other hospitals were reexamined by our pathologists, who reported the biopsy results. Slides from other hospitals might have had technical problems with sample collection and fixation that we could not control. Third, we exclude unusual histological groups such as ductal carcinoma *in situ*, lobular carcinoma *in situ*, mucinous, metaplastic, papillary, etc. So, we do not know about the association of CEA with the unusual groups. To study the effect of CEA on unusual group, it is necessary to collect more data from the unusual histological groups.

In conclusion, double-checking of CNB and SS samples

for evaluation of molecular subtype is necessary to plan adjuvant therapy in breast cancer. Depending on the results of the double-check, the patients may benefit from additional treatment. However, because of its cost-inefficiency, this double-check cannot be performed for all patients. It would be desirable to be able to select those patients who would benefit more from double-checking of their tumor molecular subtype.

The results of the present study indicate that patients with moderate- to high-grade tumors or CEA ≥ 5 ng/mL are most likely to undergo subtype changes and to require examination of the molecular subtype of their tumor by preoperative CNB.

Patients with preoperative CEA ≥ 5 ng/mL are required a double-check to determine the molecular subtype of breast cancer. When high-grade breast cancer is found on postoperative histologic examination, the results of IHC should be reevaluated through tissue obtained from a preoperative CNB.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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