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Original Article

Predicting the Response of Neoadjuvant Chemotherapy in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Breast Cancer With Axillary Lymph Node Metastasis by Multigene Assay

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ABSTRACT

Purpose: The GenesWell™ breast cancer test (BCT) is a recently developed multigene assay that predicts the risk of distant recurrence in patients with hormone receptor-positive (HR+) and human epidermal growth factor-2 negative (HER2−) early breast cancer (BC). The ability of this assay to predict the response to neoadjuvant chemotherapy (NACT) has not been established to date.

Methods: Biopsy specimens from HR+/HER2− BC patients with axillary lymph node (LN) metastasis who underwent NACT were analyzed using the BCT score. The modified BCT score was developed and patients classified into high-and low-response groups. A total of 88 patients were available for the BCT score among the 108 eligible patients. The median follow-up duration was 35.9 (7.8–128.5) months.

Results: Among them, 61 (65.1%) had cN1 and 53 (60.2%) had cT1 or cT2 disease. The BCT score was low in 25 (28.4%) patients and high in 63 (71.6%). Among the 50 patients with pathologic complete response or partial response, 41 (82.0%) were in the high BCT score group and 9 (18.0%) were in the low BCT score group. Among the 38 patients with stable or progressive disease, 22 (57.9%) were in the high BCT score group and 16 (42.1%) were in the low BCT score group ($p = 0.025$). Ki-67 before NACT was a significant factor for predicting tumor response ($p = 0.006$; 3.81 [1.50–10.16]). The BCT score showed a significant response to NACT ($p = 0.016$; 4.18 [1.34–14.28]). Distant metastasis-free survival was significantly different between the high- and low-response groups ($p = 0.004$).

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

Conceptualization: Ryu JM, Kim SW; Data curation: Ahn JH, Cho SY; Formal analysis: Lee JH, Ryu JM, Ahn JH, Cho SY, Han J; Methodology: Yu J, Han J; Supervision: Lee SK, Yu J, Chae BJ, Nam SJ, Lee JE; Writing - original draft: Lee JH, Ryu JM.

Conclusion: We demonstrated that the BCT score predicts NACT responsiveness in HR+/HER2− BC with LN metastasis and might help determine whether NACT should be performed. Further studies are required to validate these results.

Keywords: Breast Neoplasms; Genomics; Lymphatic Metastasis; Neoadjuvant Therapy; Recurrence

INTRODUCTION

Over the decades, neoadjuvant chemotherapy (NACT) has become the standard treatment for locally advanced breast cancer (BC) and is a treatment option for many patients with human epidermal growth factor-2 positive (HER2+) and triple-negative early BC [1]. NACT can render previously inoperable BC amenable to surgical resection and has the potential to increase surgical de-escalation in the breast and axilla by downstaging both breast tumors and axillary lymph nodes (LNs) [2]. Pathologic complete response (pCR) is a surrogate marker for improved survival of patients with HER2+, triple-negative, or luminal B subtype BC after NACT [3,4]. In addition, NACT enables the assessment of sensitivity to specific drugs *in vivo* and allows for the development of additional therapeutic strategies according to the NACT response.

In contrast, the benefits of NACT in patients with hormone receptor-positive (HR+)/HER2− BC are limited because of the low rates of pCR observed in these patients [4,5]. Furthermore, the HR+/HER2− subtype is the most common subtype, comprising 60%–70% of all BC cases. This subtype is unresponsive to NACT, and patients have a high risk of delay in surgery for the primary tumor, progression of cancer, and increasing possibilities of tumor cell dissemination. Therefore, appropriate patient selection for NACT is necessary.

Multigene assays using breast tumor RNA expression profiles have been highly successful as prognostic markers to aid in decision-making for adjuvant chemotherapy. RNA expression signatures from formalin-fixed, paraffin-embedded (FFPE) surgical specimens are now commercially available to assess prognosis in estrogen receptor positive (ER+) BC patients in the early and pN1 stages. Furthermore, pT1-2N0M0 with ER+/HER2− BC combined with low risk multigene panels is expected to be categorized as stage IA according to the National Comprehensive Cancer Network guidelines. Therefore, clinicians are interested in the predictive ability of FFPE breast biopsy specimens in determining NACT response in patients. Several studies have evaluated the ability of various multigene assays on FFPE biopsy specimens before surgery to predict response to NACT. However, few studies have assessed the prediction of NACT response using a multigene assay with FFPE breast biopsy specimens [6-10]. Here, we analyzed the GenesWell™ breast cancer test score (BCT score), a recently developed multigene assay that predicts the risk of distant recurrence in patients with HR+/HER2− early BC, and its ability to predict NACT response in BC patients with axillary LN metastasis using FFPE breast biopsy specimens.

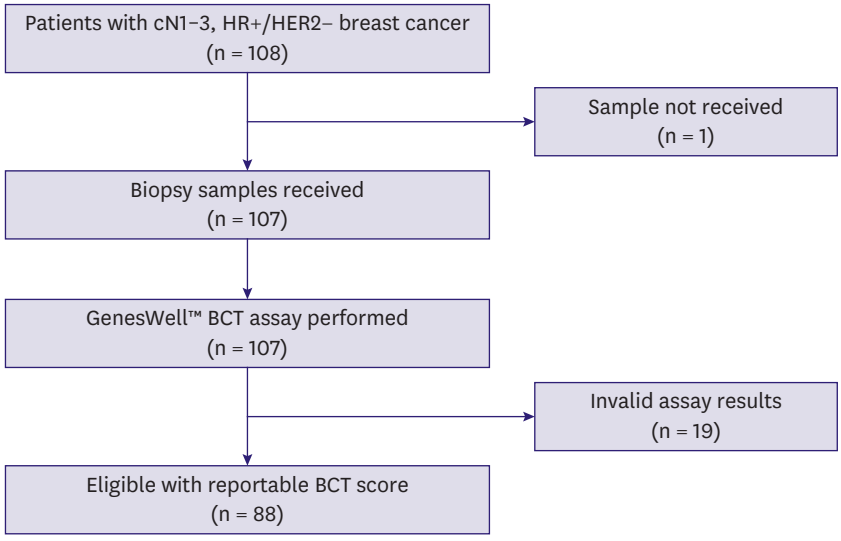

Figure 1. Histogram showing distribution of BCT scores in the all-patients and strongly ER+ groups.
BCT = breast cancer test; ER = estrogen receptor; cN = clinical N staging; HR = hormone receptor; HER = human epidermal growth factor-2.

METHODS

Patient selection

A retrospective review was conducted among cytology-proven HR+/HER2– BC patients with axillary LN metastasis who underwent NACT followed by surgery at Samsung Medical Center between January 2008 and December 2018 (**Figure 1**). We excluded patients with the following: distant metastasis at presentation; lack of immunohistochemistry (IHC) data for ER, progesterone receptor (PgR), and HER2; lack of biopsy slides; insufficient RNA concentration or tumor volume; and inadequate BCT score test.

Data collection

We collected the following variables: age at operation, clinical stage, and pathologic stage according to the 8th edition of the American Joint Committee on Cancer classification [10]; histopathology; nuclear grade; histologic grade; lymphovascular invasion; Ki-67 (pre-NACT and post-NACT); ER, PgR, and HER2 status; and type of adjuvant treatment such as chemotherapy, radiotherapy, and/or hormonal therapy. The Ki-67 labeling index was calculated as follows: nuclear expression was analyzed quantitatively, and at least 1,000 cells were assessed to calculate the labeling index. Pathologists identified the ratio of stained to unstained cells, and when the Ki-67 value is 1+, it was divided on the basis of 25%. Tumor volume was calculated as tumor tissue/total tissue. Tumor response was analyzed using the Response Evaluation Criteria in Solid Tumors [11]. pCR was defined as breast Tis/T0 and N0, partial response (PR) as a ≥ 30% decrease in total tumor lesions by 2 observations not less than 4 weeks apart, stable disease (SD) as neither PR nor progressive disease (PD), and PD as a ≥ 20% increase in the size of one or more measurable lesions or the appearance of new lesions [12]. In terms of tumor response to NACT, we set pCR and PR as positive endpoints because there were only 6 patients with pCR. Node positivity was proven by biopsy (fine-needle aspiration or core-needle biopsy) before NACT. The dates of recurrence and death were collected by reviewing electronic medical charts.

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$$\begin{aligned}\text{Unscaled BCT score} = {} & 0.63 \times \Delta C_q\_UBE2C + 0.32 \times \Delta C_q\_TOP2A \\ & + 0.13 \times \Delta C_q\_RRM2 + 0.02 \times \Delta C_q\_FOXM1 + 0.04 \times \Delta C_q\_MKI67 \\ & - 0.42 \times \Delta C_q\_BTN3A2 + 1.47\end{aligned}$$

The unscaled BCT score was then re-scaled from 0 to 10 as follows:

$$\text{BCT score} = 0.8 \times \text{unscaled BCT score} - 13.71$$

Figure 2. Consort flow diagram of eligible patient selection for BCT score analysis.
BCT = breast cancer test.

BCT score assay

The BCT score criteria were obtained from a previous study. A modified BCT score was developed as follows and classified as high or low according to predicted NACT response (**Figure 2**). Among the 16 candidate genes in FFPE tissues from BC patients measured byquantitative reverse transcription polymerase chain reaction, the expression levels of 6 prognostic genes (UBE2C, TOP2A, RRM2, FOXM1, MKI67, and BTN3A2) were selected to develop a novel prognostic algorithm to predict the response of NACT in the HR+/HER2− subtype. A detailed description of the identification and selection of prognostic genes has been described previously [13]. Some modifications were made to the existing algorithm because clinical information on tumor size and nodal status was applied, which is not suitable for the NACT setting. The cut-off of the BCT score was set to 4, the same as in the previous paper, as the point where the sum of sensitivity and specificity reaches a maximum.

Statistical analyses

Patient characteristics were compared using independent *t*-tests for continuous variables and χ^2 or Fisher's exact test for categorical variables. Values are reported as mean ± standard deviation or median with ranges. Patients with missing or unknown data were excluded from the analysis using the Cox model. All tests were 2-sided, and statistical significance was set at $p < 0.05$. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, USA) and R3.4.0 (R Foundation, Vienna, Austria; http://www.R-project.org).

Ethics approval and consent to participate

The requirement for informed consent was waived due to the low risk posed by this study. The study adhered to the ethical tenets of the Declaration of Helsinki. This study was approved by the Institutional Review Board (IRB) of Samsung Medical Center in Korea (IRB No. 2018-12-096).

RESULTS

Patient selection

A total of 88 patients were available for BCT scores among the 108 eligible patients (**Figure 1**). The median follow-up duration was 35.9 months (7.8–128.5). Accordingly, 63 (71.6%) patients were assigned to the high BCT score group, and 25 (28.4%) patients were assigned to the low BCT score group (**Table 1**). Among these, 61 (65.1%) had cN1 and 53 (60.2%) had cT1 or cT2 mutations. Among the patients who displayed pCR or PR, 41 of 63 patients (65.1%) had high BCT scores and 9 out of 25 patients (36.0%) had low BCT scores. Among the patients with SD or PD, 22 out of 63 (34.9%) patients had high BCT scores and 16 out of 25 patients (64.0%) had low BCT scores, which were significantly different between the 2 groups ($p = 0.025$). Ki-67, a proliferative marker, was divided into high- and low-response groups according to the

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Table 1. Demographics and baseline characteristics of all patients

Characteristics	All patients	BCT score		*p*-value
		High	Low	
No. of patients	88	63	25	
cT				0.788
1 or 2	53 (60.2)	39 (61.9)	14 (56.0)	
3 or 4	35 (39.8)	24 (38.1)	11 (44.0)	
cN				0.308
1	61 (69.3)	41 (65.1)	20 (80.0)	
2 or 3	26 (29.5)	21 (33.3)	5 (20.0)	
NA	1 (1.1)	1 (1.6)	0 (0.0)	
Response				0.025
pCR or PR	50 (56.8)	41 (65.1)	9 (36.0)	
SD or PD	38 (43.2)	22 (34.9)	16 (64.0)	
RCB class				1.000
0 or I	15 (17.0)	11 (17.5)	4 (16.0)	
II or III	73 (83.0)	52 (82.5)	21 (84.0)	
Ki-67*				0.030
1+	33 (37.5)	19 (30.2)	14 (56.0)	
> 1+	54 (61.4)	44 (69.8)	10 (40.0)	
NA	1 (1.1)	0 (0.0)	1 (4.0)	
Grade after NACT				0.238
0 or 1	18 (20.5)	11 (17.5)	7 (28.0)	
2 or 3	67 (76.1)	51 (80.9)	16 (64.0)	
NA	3 (3.4)	1 (1.6)	2 (8.0)	
pT after NACT				0.160
0–2	67 (76.1)	51 (81.0)	16 (64.0)	
3 or 4	21 (23.9)	12 (19.0)	9 (36.0)	
pN after NACT				0.636
0	20 (22.7)	16 (25.4)	4 (16.0)	
1	32 (36.4)	22 (34.9)	10 (40.0)	
2 or 3	36 (40.9)	25 (39.7)	11 (44.0)	

Values are presented as number (%).
BCT = breast cancer test; cT = clinical T staging; cN = clinical N staging; pCR = pathologic complete response; PR = partial response; SD = stable disease; PD = progressive disease; RCB = residual cancer burden; NACT = neoadjuvant chemotherapy; pT = pathologic T staging; pN = pathologic N staging; NA = not available.
*Ki-67 index measurement methods are as follows. 1+ were divided on the basis of 25% of nuclear expression in the ratio of stained and unstained cells quantitatively. See "Data collection" in the article for details.

BCT score based on the 1+ score ($p = 0.030$). No significant differences were observed in the clinical, ypT, and N stages and grades after NACT between the high and low BCT score groups.

We divided the patients into subgroups comprised of strongly ER+ patients with an Allred score of > 5 except for weakly ER+ patients, and analyzed the same clinicopathologic parameters (**Table 2**). Significant differences in tumor response and Ki-67 expression were observed between the BCT score groups, similar to the analysis of all patients. When plotting the BCT score distribution as a histogram, no difference was found in the distribution of the BCT scores between the 2 groups, and the distribution of pCR or PR was high with a BCT score of ≥ 4 (**Figure 3**).

The predictive value of the BCT score for NACT response was indicated for all patients, especially in strongly ER+ patients (**Table 3**). The sensitivity and specificity were higher in the strongly ER+ group than in the all-patient group (sensitivity: 86.4% vs. 82.0%, specificity: 45.7% vs. 42.1%, respectively). The positive predictive value (PPV) and negative predictive value (NPV) were also higher in the strongly ER+ group than in the all-patient group (PPV, 66.7% vs. 65.1%; NPV, 72.7% vs. 64.0%, respectively). This result demonstrates that the BCT score predicts tumor response in strongly ER+ patients.

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Table 2. Demographics and baseline characteristics of strongly ER+ patients

Characteristics	Strongly ER+ patients	BCT score		*p*-value
		High	Low	
No. of patients	79	57	22	
cT				0.610
1 or 2	45 (57.0)	34 (59.6)	11 (50.0)	
3 or 4	34 (43.0)	23 (40.4)	11 (50.0)	
cN				0.182
1	58 (73.4)	39 (68.4)	19 (86.4)	
2 or 3	21 (26.6)	18 (31.6)	3 (13.6)	
NA	0 (0.0)	0 (0.0)	0 (0.0)	
Response				0.004
pCR or PR	44 (55.7)	38 (66.7)	6 (27.3)	
SD or PD	35 (44.3)	19 (33.3)	16 (72.7)	
RCB class				0.169
0 or I	11 (13.9)	10 (17.5)	1 (4.5)	
II or III	68 (86.1)	47 (82.5)	21 (95.5)	
Ki-67*				0.044
1+	32 (40.5)	19 (33.3)	13 (59.1)	
> 1+	46 (58.2)	38 (66.7)	8 (36.4)	
NA	1 (1.3)	0 (0.0)	1 (4.5)	
Grade after NAC				0.543
0 or 1	17 (21.5)	11 (19.3)	6 (27.3)	
2 or 3	62 (78.5)	46 (80.7)	16 (72.7)	
NA	0 (0.0)	0 (0.0)	0 (0.0)	
pT after NAC				0.132
0–2	58 (73.4)	45 (78.9)	13 (59.1)	
3 or 4	21 (26.6)	12 (21.1)	9 (40.9)	
pN after NAC				0.162
0	14 (17.7)	13 (22.8)	1 (4.5)	
1	30 (38.0)	20 (35.1)	10 (45.5)	
2 or 3	35 (44.3)	24 (42.1)	11 (50.0)	

Values are presented as number (%).
ER = estrogen receptor; BCT = breast cancer test; cT = clinical T staging; cN = clinical N staging; pCR = pathologic complete response; PR = partial response; SD = stable disease; PD = progressive disease; RCB = residual cancer burden; NACT = neoadjuvant chemotherapy; pT = pathologic T staging; pN = pathologic N staging; NA = not available.
*Ki-67 index measurement methods are as follows. 1+ were divided on the basis of 25% of nuclear expression in the ratio of stained and unstained cells quantitatively. See "Data collection" in the article for details.

The BCT scores and clinicopathological parameters were analyzed using univariate and multivariate logistic regression analyses to identify factors related to tumor response (**Table 4**). cT1/2 and cN1 did not show a significant NACT response ($p = 0.090$; odds ratio [95% confidence interval], 0.47 [0.19–1.12] and $p = 0.617$; 1.27 [0.50–3.32]). A high molecular score and more than 1+ score of Ki-67 showed a significant positive correlation with tumor response ($p = 0.015$; 3.31 [1.28–9.02] and $p = 0.009$; 3.35 [1.38–8.46]) in all patient groups and in the strongly ER+ group ($p = 0.003$; 5.33 [1.87–16.96], $p = 0.006$; 3.81 [1.50–10.16]). A more than 1+ score of Ki-67 was a marginally significant factor ($p = 0.054$; 2.74 [0.99–7.79]), and BCT score was the only significant factor related to tumor response in the multivariate analysis ($p = 0.016$; 4.18 [1.34–14.28]).

DISCUSSION

This study investigated prediction of the responsiveness of tumors to NACT, and identified factors related to NACT response using the BCT score in HR+/HER2− BC with metastatic LN. We demonstrated that BCT score with pCR or PR results could predict high-response to NACT in HR+/HER2− BC with metastatic LN.

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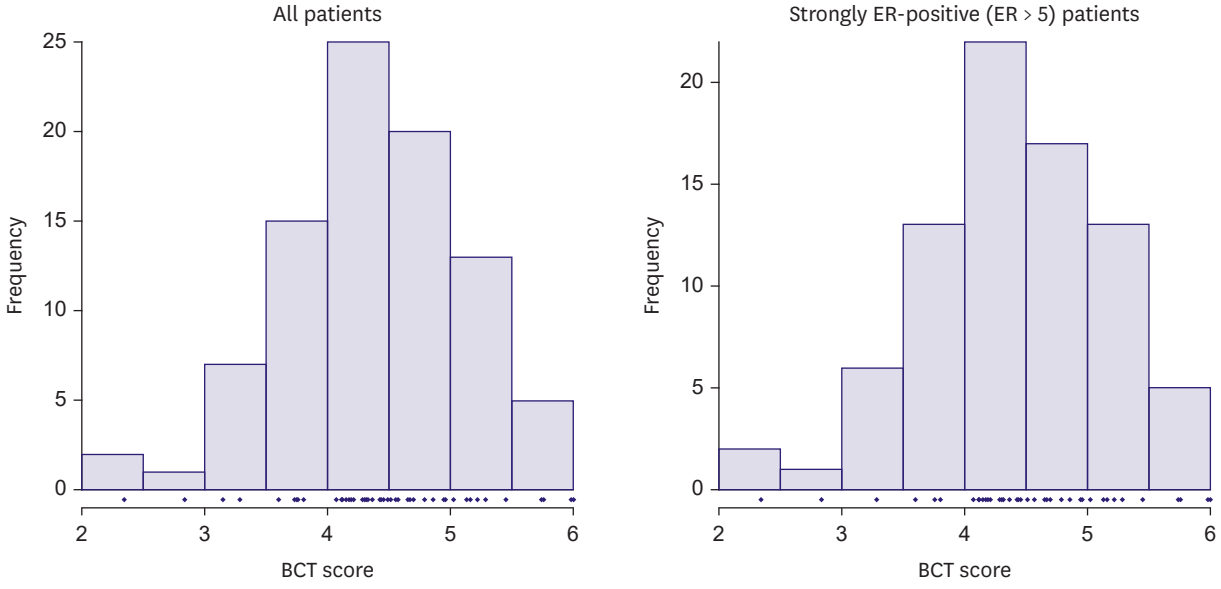

Figure 3. Development of modified BCT score model using molecular data.
BCT = breast cancer test; ER = estrogen receptor.

Table 3. Predictive value of the breast cancer test score with all and strongly ER+ in each patient

Parameters	pCR or PR	SD or PD	NPV or PPV
All patients (n = 88)			
Low	9	16	NPV 64.0% (42.5%–82.0%)
High	41	22	PPV 65.1% (52.0%–76.7%)
	Sensitivity 82.0% (68.6%–91.4%)	Specificity 42.1% (26.3%–59.2%)	
Strongly ER+ patients (n = 79)			
Low	6	16	NPV 72.7% (49.8%–89.3%)
High	38	19	PPV 66.7% (52.9%–78.6%)
	Sensitivity 86.4% (72.7%–94.8%)	Specificity 45.7% (28.8%–63.4%)	

ER = estrogen receptor; pCR = pathologic complete response; PR = partial response; SD = stable disease; PD = progressive disease; NPV = negative predictive value; PPV = positive predictive value.

Table 4. Univariate and multivariate logistic regression analyses with the BCT score and clinicopathologic parameters with all-patients and strongly ER+ groups

Parameters	Univariate		Multivariate	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
All patients (n = 88)				
BCT score (Low vs. High)	3.31 (1.28–9.02)	0.015	2.62 (0.93–7.65)	0.070
cT stage (cT1 or 2 vs. cT3 or 4)	0.47 (0.19–1.12)	0.090	0.54 (0.20–1.45)	0.223
cN stage (cN1 vs. cN2 or 3)	1.27 (0.50–3.32)	0.617	1.19 (0.42–3.46)	0.743
Ki-67 (1+ vs. > 1+)	3.35 (1.38–8.46)	0.009	2.60 (0.99–0.97)	0.054
Strongly ER+ patients (n = 79)				
BCT score (Low vs. High)	5.33 (1.87–16.96)	0.003	4.18 (1.34–14.28)	0.016
cT stage (cT1 or 2 vs. cT3 or 4)	0.44 (0.17–1.07)	0.074	0.58 (0.20–1.65)	0.303
cN stage (cN1 vs. cN2 or 3)	1.08 (0.40–3.03)	0.876	0.89 (0.28–2.93)	0.847
Ki-67 (1+ vs. > 1+)	3.81 (1.50–10.16)	0.006	2.74 (0.99–7.79)	0.054

BCT = breast cancer test; cT = clinical T staging; cN = clinical N staging; ER = estrogen receptor; OR = odds ratio; CI = confidence interval.

Although NACT has been established as a standard treatment option for HER2+ and triple-negative BC (TNBC) subtypes, many oncologists still have difficulty determining NACT application in HR+ BC because of the low rate of pCR and the limited benefit of NACT for HR+ BC [4]. In this study, 11 of 63 patients who underwent only sentinel LN biopsy without axillary LN dissection were in the BCT high-score group and 7 of 25 patients in the BCT

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low-score group (**Appendix 1**). As the application of ACOSOG Z0011 and AMAROS to axillary treatment has expanded and the pCR rate in response to NACT has increased in the HER2 and TNBC subtypes, axillary de-escalation is increasing in patients with positive axillary LN [14-16]. Ironically, although luminal type BCs show favorable biological characteristics compared with the HER2 and TNBC subtypes, axillary LN dissection continues in terms of axillary treatment for the luminal subtype [17]. Therefore, many efforts are being made to predict the responsiveness to NACT in HR+ BC using various modalities, such as multigene assay, clinicopathologic scale, and Ki-67 labeling index.

Recently, attempts have been made to predict the responsiveness to NACT of HR+/HER2− BC using multigene assays. The BCT score was developed to predict the risk of distant metastasis and responsiveness to chemotherapy using 5 proliferation-related genes, one immune response-related gene, and clinical information, such as tumor size and nodal status [13,18]. Through this study, concordant results were obtained when comparing the predictive power of pCR with existing gene tests in predicting responsiveness to NACT (**Table 5**) [9, 10, 19-25].

The BCT score is an assay validated for Asian patients, and this study assessed prediction of the NACT effect in Asian patients, whereas other assays have been validated in Western postmenopausal women with BC. Conventional genetic tests reflect only the genetic information, analyzing the activity of genes that can influence the likelihood of cancer growth and response to chemotherapy. However, the BCT score reflects clinical information, such as tumor size and nodal status, as well as genetic information, such as proliferation-related genes and immune response-related genes. In addition, few studies have used FFPE samples obtained from core needle biopsies. In this study, the response to NACT was predicted using FFPE samples in ER+ luminal cancers. However, compared to the existing assays, the lack of randomized controlled trials in assessing this method is insufficient.

Table 5. Comparison table of multigene assay for predicting neoadjuvant chemotherapy responsiveness in hormone receptor-positive breast cancer

References	Multigene assay	No. of sample	pCR	pCR or PR	Age (mean)	cT1 or 2	cT3+	cN0	cN+	Tumor grade 1	Tumor grade 2/3	Ki-67 > 1+	OR (95% CI)
Pease et al. (2019) [9]	Oncotype Dx®	989	42 (4.3)	NA	54.6	882 (89.2)	107 (10.8)	757 (76.5)	232 (23.5)	123 (12.4)	866 (87.6)	NA	4.87 (2.01–11.82)
Pardo et al. (2021) [19]	Oncotype Dx®	158	10 (6.3)	NA	NA	158 (100)	0 (0)	0 (0)	158 (100)	105 (66.5)	53 (33.5)	NA	3.16 (1.06–9.45)
Sella et al. (2021) [20]	Oncotype Dx®	76	10 (13.2)	NA	35.9	51 (67.1)	25 (32.9)	20 (26.3)	56 (73.7)	4 (5.3)	71 (93.4)	NA	4.80 (0.95–24.34)
Chang et al. (2008) [25]	Oncotype Dx®	72	2 (2.8)	NA	49.0	N/A	N/A	63 (87.5)	7 (9.7)	2 (2.8)	70 (97.2)	NA	5.0 (1.3–6.0)
Gianni et al. (2005) [10]	Oncotype Dx®	82	21 (25.6)	NA	53.1	53 (64.6)	29 (35.4)	28 (34.1)	54 (65.9)	2 (2.4)	80 (97.6)	NA	NA (RS was positively a/w the likelihood of pCR)
Ohara et al. (2019) [21]	Prosigna™ (PAM50)	124	12 (9.7)	NA	51.3	98 (79.0)	26 (21.0)	41 (33.1)	83 (66.9)	25 (20.2)	99 (79.8)	59 (47.6)	6.98 (1.17–133.97)
Bertucci et al. (2014) [22]	EndoPredict®	553	64 (11.6)	NA	49.0	40 (7.2)	512 (92.6)	183 (33.1)	336 (60.8)	47 (8.50)	464 (83.9)	NA	1.13 (1.04–1.24)
Dubsky et al. (2020) [23]	EndoPredict®	134	NA	NA	NA	116 (86.6)	18 (13.4)	69 (51.5)	63 (47.0)	1 (0.7)	121 (90.3)	NA	1.44 (1.20–1.74)
Mathieu et al. (2012) [24]	Breast Cancer Index℠	150	22 (14.7)	NA	51.0	97 (64.7)	53 (35.3)	70 (46.7)	76 (50.7)	16 (10.7)	132 (88.0)	NA	26.25 (3.19–216.24)
Present study	GenesWell™ BCT	88	6 (6.8)	50 (56.8)	NA	53 (60.2)	35 (39.8)	0 (0)	87 (98.9)	18 (20.5)	67 (76.1)	54 (61.4)	4.18 (1.34–14.28)

Values are presented as number (%).
pCR = pathologic complete response; cT = clinical T staging; cN = clinical N staging; RS = Recurrence Score; a/w = associated with, NA = not applicable; OR = odds ratio; CI = confidence interval.

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According to the latest American Society of Clinical Oncology/College of American Pathologists guidelines, only 1%–10% of ER expression assessed by IHC is divided by low positive ER [26]. Low ER has a property similar to basal-like gene expression profiles as shown in the TNBC subtype; thus, it is emerging as an important prognostic factor for NACT [27,28]. Moreover, in the case of the strongly ER+ group in this study, the predictive power for pCR and PR was higher in the high BCT score group than in the all-patient group. In other words, the response to NACT was high even in the low BCT score group in the case of weakly ER+ patients. Therefore, our results demonstrate that BCT score clearly predicts tumor response and is an independent factor for predicting tumor response to NACT, especially in strongly ER+ patients.

Ki-67 is a proliferation marker in BC and a well-known prognostic factor for neoadjuvant treatment. As Ki-67 values in patients with pCR were high in triple-negative or HR+, HER2− BC, a more favorable prognosis was obtained in pCR patients [29]. Although Ki-67 was marginally significant in the multivariate study, when determining NACT response, Ki-67 might be more important in strongly ER+ patients (**Table 4**).

Residual breast cancer burden (RCB) is a new independent risk factor that improves the prediction of distant relapse after NACT compared with the currently used risk factors [30]. We also analyzed RCB, wherein most patients were classified as RCB class II or III, and there were no significant differences in RCB class between the high and low BCT groups. As the results show, patients with strong ER positivity do not respond well to NACT, but the response to NACT might be better in patients with low ER, so we expect prospective studies to be able to solve this discrepancy.

Although this study was a retrospective, single-center study with a small sample size, it is significant as it predicted the NACT response from a core biopsy sample using the BCT score for the first time. In addition, efforts have been made to predict the responsiveness to neoadjuvant endocrine therapy (NET) using multigene assays [23,31]. Further studies are needed to prove the predictive potential of the BCT score for response to NET in Asian patients with BC because many multigene assays have been developed with a focus on Western populations [18,32]. Although the number of patients with pCR was so small that we could not analyze the BCT score as a tool to determine surgical de-escalation, the BCT score might be a helpful gene test for determining the surgical treatment plan after NACT if larger populations are included and further prospective studies are conducted.

In conclusion, we demonstrated that BCT score predicts NACT responsiveness in HR+/HER2− BC with LN metastasis. The BCT score might be an early surrogate of prognostic signatures for predicting the response to NACT in HR+/HER2− BC with LN metastasis. Therefore, the BCT score may be a helpful tool for predicting NACT responsiveness in HR+/HER2− BC with LN metastasis. Further validation using the BCT score and prospective studies are needed to increase the accuracy of NACT response prediction.

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Appendix 1. Types of axillary surgery for each breast cancer test risk group

Characteristics	SLNB	ALND	Total
High	11	52	63
Low	7	18	25
Total	18	70	88

SLNB = sentinel lymph node biopsy; ALND = axillary lymph node dissection.

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