CLINICAL TRIAL

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Only estrogen receptor "positive" is not enough to predict the prognosis of breast cancer

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Abstract

Purpose Beginning in 2018, biomarkers including estrogen receptor (ER) status were incorporated in the 8th AJCC staging system. ER expression levels were not considered in these changes. We hypothesized that the levels of ER expression could affect the prognosis of breast cancer.

Methods A retrospective review was conducted to identify all female patients with invasive breast cancer between 2003 and 2012. ER negative (group I), weakly ER-positive (group II), and strongly ER-positive (group III) were defined as Allred total scores of 0–2, 3–5, and 6–8, respectively. We examined a multigene panel, designated the BCT score, which is a newly developed prognostic model for predicting the risk of a distant metastasis.

Results Among the 4949 patients enrolled in this study, 1310 (26.5%), 361 (7.3%), and 3277 (66.2%) were categorized as group I, II, and III, respectively. Median F/U duration was 57.8 months. Compared to group III, patients in group II were younger, had larger tumors, and were also more likely to have PR-negative tumors, HER-2 amplification, high Ki-67, and high nuclear grade. Between group II and III, there was a significant difference in OS ($P = 0.0764$, 0.909, and 0.010, respectively). After adjusting for additional factors that may affect OS, the HR for OS showed higher in group II than in group III. The baseline median BCT score indicated that lower ER expression was associated with significantly higher BCT score ($P < 0.0001$) and significantly more likely to have high risk group ($P < 0.0001$) relative to higher levels of ER expression group.

Conclusion ER expression levels affect the prognosis of breast cancer. The risk for patients with weakly ER-positive breast cancer should not be underestimated.

Keywords Breast neoplasm · Estrogen receptor · Prognosis

Introduction

Estrogen receptor (ER) status is one of the most important predictive and prognostic biomarkers in breast cancer [1–3]. ER-positive tumors are associated with better survival than ER negative tumors [4–6].

As understanding of the biologic markers ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) and their impacts on prognosis has grown, these biomarkers were incorporated in the eighth edition of the primary tumor, lymph node, and metastasis (TNM) classification of the American Joint Commission of Cancer (AJCC) for breast cancer [7–9]. Patients with hormone receptor-positive and/or HER-2-positive tumors who undergo appropriate targeted therapy can be down-staged according to these guidelines. In addition, for those with pT1 or pT2, pN0, M0, ER-positive, and HER-2-negative cancers combined with a low risk of multigene panels are expected to be categorized as stage IA.

However, the level of ER expression status is not considered in these changes. We hypothesized that the level of ER expression could affect prognosis as well as the risk score from the multigene panel, which predicts distant metastasis. In this study, based on the level of ER expression, we analyzed the characteristics and prognosis of patients, and

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examined the results of a multigene panel that predicts distant metastasis.

Methods

Data collection

A retrospective review was conducted to identify all female patients with invasive breast cancer who underwent surgery at Samsung Medical Center (SMC) between January 2003 and December 2012. We excluded patients who underwent neoadjuvant chemotherapy, had distant metastases or inflammatory breast cancer on presentation, or had other histopathology except for invasive ductal or lobular carcinoma. We also excluded patients who lacked immunohistochemistry (IHC) data (ER, PR, HER-2, and Ki-67) or had short follow-up durations (< 12 months). We collected the following variables: age at operation, family history of breast cancer, type of operation, pathologic stage according to 7th edition of AJCC classification [10], histopathology, nuclear grade (NG), lymphovascular invasion (LVI), ER, PR, HER-2 status, type of adjuvant treatment such as chemotherapy (CTx), radiotherapy (RTx), and anti-endocrine therapy (AET).

The dates of recurrence and death were collected by review of electronic medical charts, and the date of death also was collected from the Korean National Statistical Office database. Overall survival (OS) was defined as the time between the date of operation and the date of death from any cause. Disease-free survival (DFS) was defined as the time between the date of operation and the date of any recurrence because of breast cancer. Distant metastasis was defined as recurrence in any other area not included in locoregional recurrence. Distant metastasis free survival (DMFS) was defined as the time between the date of operation and the date of distant metastasis.

ER, PR, and HER-2 methodology

We used anti-ER (clone 6F11, 1:200 dilution, Novocastra, Bond-Max system) and anti-PgR (clone 16, 1:800 dilution, Novocastra, Bond-Max system) monoclonal antibodies on 10% neutral-buffered formalin fixed and paraffin embedded tissue. Only nuclear (not cytoplasmic) staining was scored.

ER and PR expression are measured primarily by IHC. Any staining of 1% of cells or more is considered positive for ER and PR [6]. We used the combined report with the Allred score interpretation system including intensity score (0–3) and proportion score (0–5) [11, 12]. The ER-negative (group I) was defined as having a total score (TS) of 0 and 2, weakly ER-positive (group II) was defined as a TS of 3–5, and the strongly ER-positive (group III) was defined as a TS of 6–8.

For Her-2 staining, we used anti-HER2 (4B5, Ventana, BenchMark XT) monoclonal antibody on 10% formalin fixed and paraffin-embedded tissue. Only the membrane staining intensity and pattern were evaluated using the new recommendations of the 2013 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guideline [13]. Circumferential membrane staining that is complete, intense, and within $> 10\%$ of tumor cells results in a score of "3 +". Circumferential membrane staining that is incomplete and/or weak/moderate and within $> 10\%$ of tumor cells or complete and circumferential membrane staining that is intense, and within $\geq 10\%$ of tumor cells is scored 2 +. Incomplete membrane staining that is faint/barely perceptible and within $> 10\%$ of tumor cells is scored 1 +. When no staining is observed or membrane staining is incomplete and is faint/barely perceptible and within $\geq 10\%$ of tumor cells the assigned score is 0. A positive test is defined as staining resulting in a 3 + score. The score of 2 + is interpreted as equivocal. Additionally, a negative test is defined as staining assigned a 0/1 + score. We considered positive results to be "amplification," and equivocal and negative tests were considered to be "not amplification."

Multigene panels

We examined a newly developed prognostic model (BCT score) for predicting the risk of distant metastasis. The BCT score assay is a molecular prognostic signature used to predict the risk of distant metastasis in patients with pN0-N1, HR+/HER2− breast cancer [14]. Among patients who underwent curative resection of a primary breast tumor at the SMC, the BCT scores were retrospectively obtained from 386 patients with pN0-N1, HR+/HER2- breast cancer. The BCT score assay was performed as previously described [14]. In brief, the BCT score assay is based on the quantification of six prognostic genes (UBE2C, TOP2A, RRM2, FOXM1, and MKI67) and three reference genes (CTBP1, CUL1, and UBQLN1) in FFPE tissue sections. All FFPE samples were collected at the time of surgical resection. Total RNA was isolated from the FFPE samples using a Tissue Preparation System device (Siemens AG, Munich, Germany), and qRT-PCR was performed using a QuantiFast Multiplex RT-PCR Kit (Qiagen, Hilden, Germany) and a LightCycler 480 system (Roche Applied Science, Mannheim, Germany). Relative expression of each prognostic gene was calculated as difference between target Cq value and average Cq value of three reference genes.

The BCT score was derived from relative expression values for the six prognostic genes and two clinical variables [tumor size (cm), and pN status (0 or 1)] and assessed on a scale from 0 to 10. The patients were categorized as being in either the high risk or low risk group according to a pre-specified cutoff BCT score of 4. Patients were classified as

low risk for distant metastasis if BCT score was <4, whereas patients with BCT score ≥ 4 were stratified as high risk. To assess different recurrence risk by ER status, the BCT scores were sub-classified according to levels of ER expression.

Statistical analysis

Patient characteristics were compared using Kruskal–Wallis test or the analysis of variance (ANOVA) test for continuous variables and the Chi square or Fisher's exact test for categorical variables. Values are reported as mean ± standard deviation (SD) or median with ranges. Kaplan–Meier curves with corresponding results of log-rank tests were constructed for DFS, DMFS, and OS. Univariate and multivariate analyses for OS were conducted with a Cox regression and proportional hazard model to estimate the hazard ratio (HR) and 95% confidence interval (CI). Patients with any missing or unknown data were excluded from the analysis using the Cox model. All tests were two sided, and $P < 0.05$ was considered significant. All statistical analyses used SAS version 9.4 (SAS Institute, Cary, NC, USA) and R3.4.0 (Vienna, Austria; http://www.R-project.org). The need for informed consent was waived because of the low risk posed by this investigation. This study adhered to the ethical tenets of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of SMC in Seoul, Korea (IRB No.: 2017-06-130).
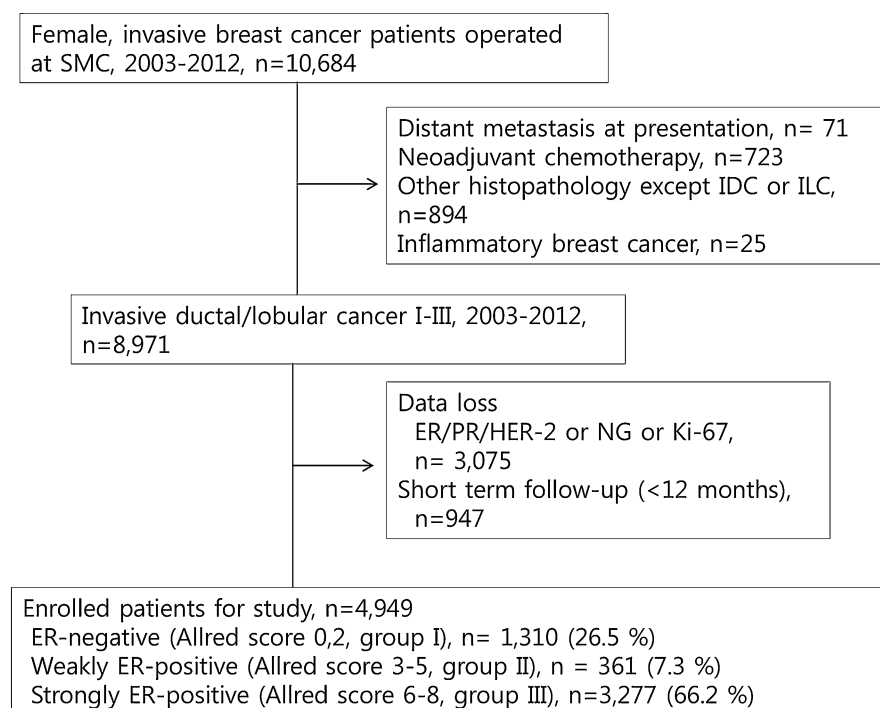
Results

A schematic diagram of patient selection is shown in Fig. 1. Overall, 4949 patients were enrolled in this study. Among them, 1310 (26.5%) were categorized in the ER-negative group (group I; Allred score 0, 2), 361 (7.3%) were categorized in the weakly ER-positive group (group II; Allred score 3–5), and 3277 (66.2%) were categorized in the strongly ER-positive group (group III; Allred score 6–8).

Patient characteristics

The clinicopathologic characteristics of patients in the three groups are shown in Table 1. The mean age was 48.6 ± 9.2 and the median follow-up duration was 57.8 (range 12.0–136.4) months. Compared with group III, patients in group II were younger (mean age, 46.5 vs. 48.4, $P < 0.0001$), and had larger tumors (T2/3 44.3% vs. 35.4%, $P = 0.008$). They were also more likely to have tumors that were PR-negative (28.5% vs. 5.6%, $P < 0.0001$), weakly PR-positive (31.0% vs. 14.8%, $P < 0.0001$), HER-2-amplified (40.4% vs. 10.6%, $P < 0.0001$), high Ki-67 (64.0% vs. 38.6%, $P < 0.0001$), and high NG (57.6% vs. 21.8%, $P < 0.0001$). Compared with patients in group II, patients in group I had larger tumors (T2/3 47.1% vs. 44.3%, $P = 0.043$), and were more likely be younger than 35 years

Fig. 1 Schematic diagram for patients selection

Female, invasive breast cancer patients operated at SMC, 2003-2012, n=10,684

Distant metastasis at presentation, n= 71
Neoadjuvant chemotherapy, n=723
Other histopathology except IDC or ILC, n=894
Inflammatory breast cancer, n=25

Invasive ductal/lobular cancer I-III, 2003-2012, n=8,971

Data loss
  ER/PR/HER-2 or NG or Ki-67,
  n= 3,075
Short term follow-up (<12 months),
  n=947

Enrolled patients for study, n=4,949
 ER-negative (Allred score 0,2, group I), n= 1,310 (26.5 %)
 Weakly ER-positive (Allred score 3-5, group II), n = 361 (7.3 %)
 Strongly ER-positive (Allred score 6-8, group III), n=3,277 (66.2 %)

SMC, Samsung Medical Center; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, Progesterone receptor; NG, nuclear grade

Table 1 Baseline characteristics according to the level of ER status

	ER-negative, n (%) Group I	Weakly ER-positive, n (%) Group II	Strongly ER-positive, n (%) Group III	P value	
				Group I versus II	Group II versus III
Number, %	1310 (26.5)	361 (7.3)	3277 (66.2)		
Mean age, ±SD	49.5±9.9	46.5±8.1	48.4±9.0	< 0.0001	< 0.0001
Age				<0.0001	0.039
≤35	114 (8.7)	21 (5.8)	137 (4.2)		
35–55	858 (65.5)	286 (79.2)	2489 (76.0)		
≥56	338 (25.8)	54 (15.0)	651 (19.9)		
Menopausal status				<0.0001	0.394
Premenopause	613 (46.8)	114 (31.6)	1113 (34.0)		
Postmenopause	683 (52.1)	244 (67.6)	2152 (65.7)		
Unknown	14 (1.1)	3 (0.8)	12 (0.4)		
Family history				0.991	0.180
Yes	134 (10.2)	37 (10.3)	268 (8.2)		
No	1176 (89.8)	324 (89.8)	3006 (66.7)		
Unknown	0 (0.0)	0 (0.0)	3 (0.1)		
Bilaterality				0.181	0.647
Unilateral	1288 (98.3)	351 (97.2)	3199 (97.6)		
Bilateral	22 (1.7)	10 (2.8)	78 (2.4)		
Histopathology				<0.0001	0.510
IDC	1300 (99.2)	345 (95.6)	3120 (95.2)		
ILC	10 (0.8)	16 (4.5)	157 (4.8)		
Multiplicity				0.938	0.056
Yes	248 (18.9)	69 (19.1)	773 (23.6)		
No	1062 (81.1)	292 (80.9)	2504 (76.4)		
PR status				<0.0001	<0.0001
PR-negative	1262 (96.3)	103 (28.5)	182 (5.6)		
Weakly PR-positive	40 (3.0)	112 (31.0)	486 (14.8)		
Strongly PR-positive	8 (0.7)	146 (40.4)	2609 (79.6)		
HER-2 status				0.913	<0.0001
Amplification	534 (40.8)	146 (40.4)	348 (10.6)		
Not amplification	776 (59.2)	215 (59.6)	2929 (89.4)		
Ki-67				<0.0001	<0.0001
≤20.0%	197 (15.0)	130 (36.0)	2013 (61.4)		
>20.0%	1113 (85.0)	231 (64.0)	1264 (38.6)		
Nuclear grade				<0.0001	<0.0001
Low	18 (1.4)	30 (8.3)	738 (22.5)		
Intermediate	245 (18.7)	123 (34.1)	1826 (55.7)		
High	1047 (79.9)	208 (57.6)	713 (21.8)		
LVI				0.065	0.303
Yes	386 (29.5)	125 (34.6)	1047 (32.0)		
No	916 (69.9)	235 (65.1)	2220 (67.7)		
Unknown	8 (0.6)	1 (0.3)	10 (0.3)		
T stage				0.043	0.008
T1	693 (52.9)	201 (55.7)	2117 (64.6)		
T2	567 (43.3)	139 (38.5)	1013 (30.9)		
T3	50 (3.8)	21 (5.8)	147 (4.5)		
N stage				0.991	0.295
N0	830 (63.4)	229 (63.4)	1929 (58.9)		
N1	322 (24.6)	89 (24.7)	963 (29.4)		

Table 1 (continued)

	ER-negative, n (%) Group I	Weakly ER-positive, n (%) Group II	Strongly ER-positive, n (%) Group III	P value	
				Group I versus II	Group II versus III
N2	95 (7.3)	27 (7.5)	247 (7.5)		
N3	63 (4.8)	16 (4.4)	138 (4.2)		
Pathologic stage				0.721	0.319
Stage I	526 (40.2)	153 (42.4)	1519 (46.4)		
Stage II	610 (46.6)	160 (44.3)	1327 (40.5)		
Stage III	174 (13.3)	48 (13.3)	431 (13.2)		

SD standard deviation, *ER* estrogen receptor, *PR* progesterone receptor; *HER-2* human epidermal growth factor-2, *LVI* lymphovascular invasion

old (8.7% vs. 5.8%, $P < 0.0001$), and to have tumors that were PR-negative (96.3% vs. 28.5%, $P < 0.0001$), high Ki-67 (85.0% vs. 64.0%, $P < 0.0001$), and high NG (79.9% vs. 57.6%, $P < 0.0001$).

Treatment characteristics

The types of surgery, adjuvant treatment, and clinical outcomes among the three groups are summarized in Table 2. In total, 3453 (69.8%) patients underwent

Table 2 Treatment characteristics and clinical course according to the level of ER status

	ER-negative, n (%) Group I, n = 1310 (26.5)	Weakly ER-positive, n (%) Group II, n = 361 (7.3)	Strongly ER-positive, n (%) Group III, n = 3277 (66.2)	P value	
				Group I versus II	Group II versus III
Breast surgery				0.028	< 0.0001
BCS	876 (66.9)	219 (60.7)	2358 (72.0)		
TM	434 (33.1)	142 (39.3)	919 (28.0)		
Axillary surgery				0.011	0.049
SLNB	627 (47.9)	186 (51.5)	1697 (51.8)		
ALND	611 (46.6)	143 (39.6)	1393 (42.5)		
No operation	72 (5.5)	32 (8.9)	187 (5.7)		
Anti-hormonal therapy				< 0.0001	0.0009
Yes	11 (0.8)	350 (97.0)	3240 (98.9)		
No	1201 (91.7)	7 (1.9)	16 (1.3)		
Unknown	98 (7.5)	4 (1.1)	21 (0.6)		
Chemotherapy				< 0.0001	< 0.0001
Yes	1105 (84.4)	284 (78.7)	2257 (68.9)		
No	197 (15.0)	75 (20.8)	1013 (30.9)		
Unknown	8 (0.6)	2 (0.6)	7 (0.2)		
Radiotherapy				< 0.0001	< 0.0001
Yes	969 (74.0)	249 (69.0)	2581 (78.8)		
No	331 (25.3)	109 (30.2)	681 (20.8)		
Unknown	10 (0.8)	3 (0.8)	15 (0.5)		
Recurrence				0.0001	0.316
Yes	233 (17.8)	34 (9.4)	259 (7.9)		
No	1077 (82.2)	327 (90.6)	3018 (92.1)		
Distant metastasis				< 0.0001	0.611
Yes	117 (8.9)	21 (5.8)	170 (5.2)		
No	1193 (91.1)	340 (94.2)	3107 (94.8)		
Expire				< 0.0001	0.001
Yes	92 (7.0)	17 (4.7)	65 (2.0)		
No	1218 (93.0)	344 (95.3)	3212 (98.0)		

breast conserving surgery (BCS) and 2510 (50.7%) patients underwent sentinel lymph node biopsy (SLNB). Compared to patients in group III, fewer patients in group II underwent anti-hormonal therapy (**97.0% vs. 98.9%**, $P = 0.0009$), BCS (60.7% vs. 68.3%, $P < 0.0001$), adjuvant radiotherapy (RTx) (69.0% vs. 78.8%, $P < 0.0001$), and more patients underwent adjuvant chemotherapy (CTx) (78.8% vs. 68.9%, $P < 0.0001$). Compared with patients in group I, fewer patients in group II underwent CTx (84.4% vs. 78.7%, $P < 0.0001$) and RTx (74.0% vs. 69.0%, $P < 0.0001$).

Oncologic outcome

The clinical outcomes, including recurrence, distant metastasis, and death, are summarized in Table 2. Patients in group II were significantly more likely to die than those in group III (4.7% vs. 2.0%; $P = 0.001$). There was no significant difference in recurrence between patients in groups II and III (9.4% vs. 7.9%; $P = 0.316$).

Between groups II and III, there were no significant difference in DFS and DMFS, but there was a significant difference in OS ($P = 0.0764$, 0.909, and 0.010, Fig. 2). Patients in group III were more likely to have bone metastasis, low Ki-67, and > 3-year distant metastasis-free interval (DMFI) than
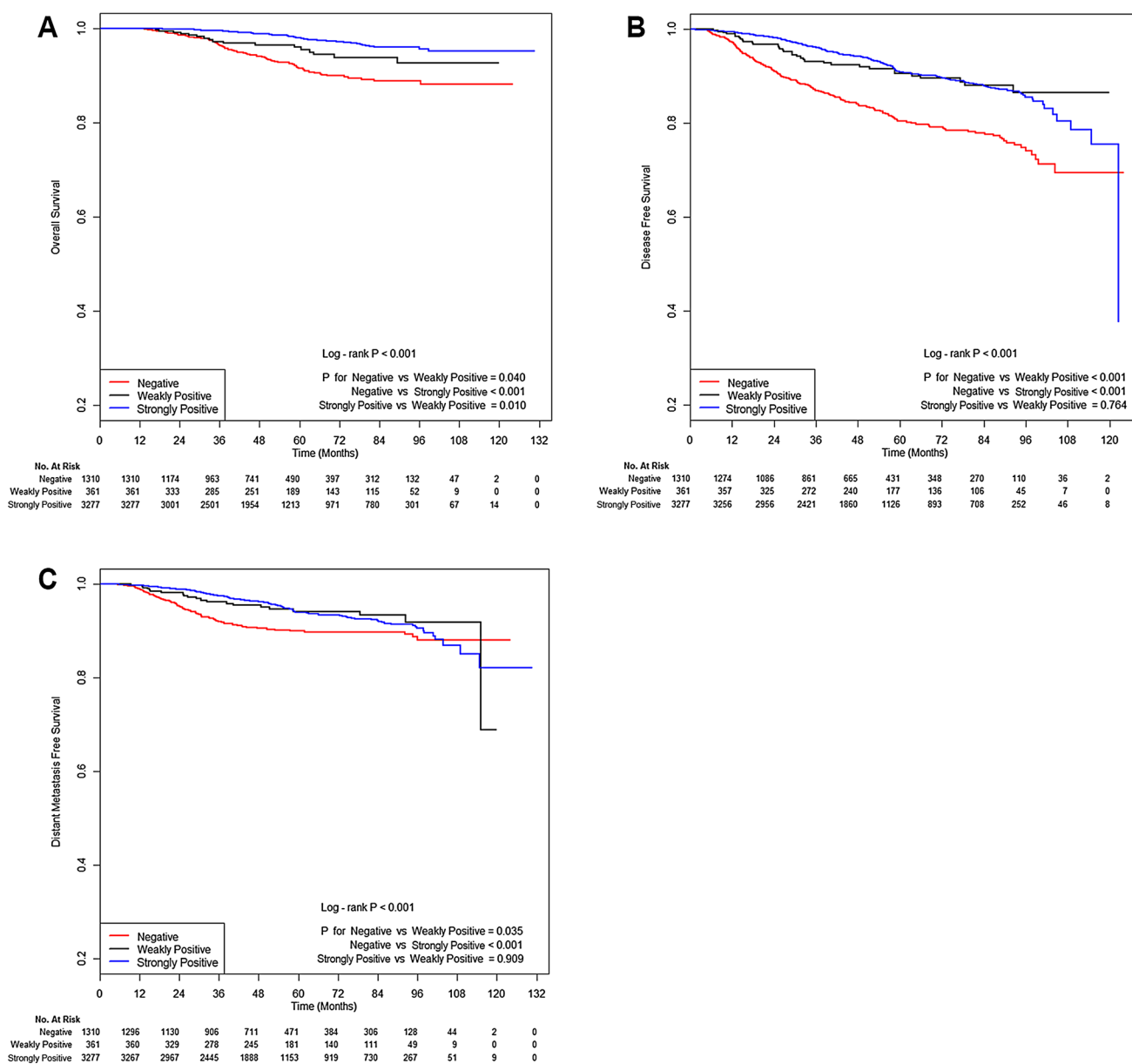

Fig. 2 The Kaplan–Meier survival curves for OS, DFS, and DMFS

patients in groups I and II (Table 3). In the univariate analysis, patients in group II were shown to have significantly higher HRs for OS than patients in group III (HR 2.051; 95% CI 1.202–3.500; Table 4). After additional adjustments for pathologic stage, NG, LVI, Ki-67, PR, and HER-2 status, the HR for OS was higher in group II than in group III (group II: HR 1.773; 95% CI 1.002–3.137, group I: HR 1.868; 95% CI 1.002–3.481, Table 5).

BCT score according to the level of ER status

We examined BCT scores as an independent cohort in SMC. The baseline median BCT score showed that low ER expression was associated with a significantly higher risk score (4.87 vs. 4.63 vs. 3.54; $P < 0.0001$) and patients with this score were significantly more likely be in a high risk group (79.2% vs. 72.3% vs. 39.0%; $P < 0.0001$) compared to those with higher levels of ER expression (Fig. 3).

Discussion

Compared with the strongly ER-positive group, the weakly ER-positive group showed more unfavorable characteristics including: younger age, larger tumor size, and were more likely to be PR negative, have HER-2 amplification, higher

Table 3 Descriptive characteristics of patients with De Novo distant metastasis

Total	ER negative, n (%) N = 117	Weakly ER-positive, n (%) N = 21	Strongly ER-positive, n (%) N = 170
Metastasis site			
Bone	7 (6.0)	2 (9.5)	56 (32.9)
Lung/Pleura	21 (17.9)	6 (28.6)	34 (20.0)
Liver	6 (5.1)	3 (14.3)	12 (7.1)
Brain	8 (6.8)	1 (4.8)	1 (0.6)
Lymph node	4 (3.4)	0 (0)	7 (4.1)
Other sites or combination	71 (60.7)	9 (42.9)	60 (35.3)
DMFI			
≥ 3 years	32 (27.4)	10 (47.6)	110 (64.7)
< 3 years	85 (70.1)	11 (52.3)	60 (35.3)
Stage			
Stage I	19 (16.2)	6 (28.6)	18 (10.6)
Stage II	59 (50.4)	8 (38.1)	77 (45.2)
Stage III	39 (33.3)	7 (33.3)	75 (44.1)
Ki-67			
< 20%	19 (16.2)	5 (23.8)	62 (36.5)
≥ 20%	98 (84.8)	16 (76.2)	108 (63.5)

ER estrogen receptor, *DMFS* distant metastasis free interval

Table 4 Univariate analysis for overall survival according to the levels of ER status

	HR for OS (95% CI)	P value
ER expression		< 0.0001
ER-negative (Group I)	3.588 (2.612, 4.930)	< 0.0001
Weakly ER-positive (Group II)	2.051 (1.202, 3.500)	< 0.0001
Strongly ER-positive (Group III) (ref)		
Pathologic stage		< 0.0001
Stage I		
Stage II	2.407 (1.584, 3.658)	< 0.0001
Stage III	7.012 (4.584, 10.724)	< 0.0001
Nuclear grade		< 0.0001
Low		
Intermediate	3.345 (1.438, 7.780)	0.005
High	7.372 (3.244, 16.751)	< 0.0001
Lymphovascular invasion		
Yes	2.982 (2.207, 4.030)	< 0.0001
No (ref)		
Ki-67		
≤ 20.0%	0.333 (0.233, 0.475)	< 0.0001
> 20.0% (ref)		
PR expression		
Positive	0.299 (0.221, 0.404)	< 0.0001
Negative (ref)		
HER-2 status		
Amplification	1.079 (0.753, 1.547)	0.677
Not amplification (ref)		

ER estrogen receptor, *PR* progesterone receptor, *HER-2* human epidermal growth factor-2

Ki-67, and higher NG. DFS and DMFS were not significantly different between groups II and III, but patients in the strongly ER-positive group were more likely to have bone metastasis and a more than 3-year DMFI. The weakly ER-positive group had significantly worse OS than the strongly ER-positive group and had significantly higher HR for OS than the strongly ER-positive group after adjusting for factors that could affect OS. The median BCT risk score was significantly higher and patients were more likely to be in the high risk group compared to patients with higher levels of ER expression.

With the increased understanding of how biomarkers affect the prognosis of breast cancer, most ER-positive tumors have been down-staged in the 8th AJCC TNM staging. Since the Oncotype DxR (Genomic Health, Redwood City, CA) recurrence score (RS) assay was shown to be useful in predicting the benefits of adjuvant CTx, many early breast cancer patients with the luminal subtype don't have to undergo CTx [15, 16]. Several algorithms for predicting the Oncotype DxR RS have been developed using clinicopathologic data. However, none of these algorithms take into

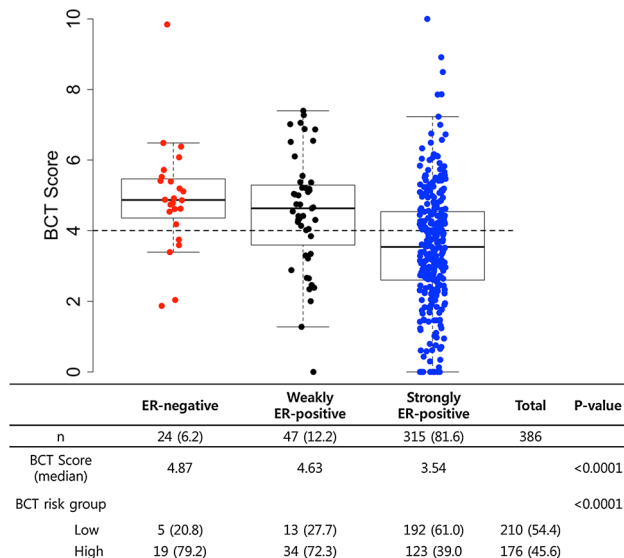**Table 5** Multivariate analysis for overall survival according to the levels of ER status

ER expression	Number (%)	Expire, N (%)	HR (95% CI)[a]	HR (95% CI)[b]	HR (95% CI)[c]
ER-negative	1310(26.5)	92(7.0)	3.617 (2.630, 4.973)	2.943 (2.019, 4.291)	1.868 (1.002, 3.481)
Weakly ER positive	361(7.3)	17(4.7)	2.035 (1.192, 3.472)	1.757 (1.015, 3.044)	1.773 (1.002, 3.137)
Strongly ER-positive (ref)	3277(66.2)	65(2.0)			

ER estrogen receptor, *HR* hazard ratio, *CI* confidence interval, *ref* reference

[a]Adjusted for Stage

[b]Adjusted for Stage, nuclear grade, lymphovascular invasion, Ki-67

[c]Adjusted for Stage, nuclear grade, lymphovascular invasion, Ki-67, progesterone receptor, HER-2 status

	ER-negative	Weakly ER-positive	Strongly ER-positive	Total	P-value
n	24 (6.2)	47 (12.2)	315 (81.6)	386	
BCT Score (median)	4.87	4.63	3.54		<0.0001
BCT risk group					<0.0001
Low	5 (20.8)	13 (27.7)	192 (61.0)	210 (54.4)	
High	19 (79.2)	34 (72.3)	123 (39.0)	176 (45.6)	

Fig. 3 BCT score according to the levels of ER status

consideration levels of the ER expression [17–21], despite several studies reporting that RS increases as ER expression decreases (RS 0–17, 18–30, and > 30; mean ER Allred score 7.78, 7.27, and 5.80) [20]. This trend could result in the underestimation of the risk for patients with weakly ER-positive tumors. In our study, we observed higher median BCT scores were more likely to result in a higher risk compared to higher levels of ER expression.

Furthermore, tumors with ER positivity of 1–9% on IHC have been shown to be ER-negative by mRNA gene expression; this could result in the misclassification of tumor subtype [22–24]. Iwamoto et al. [23] reported on 25 patients who were identified as being ER-positive with 1–9% staining by IHC. Among these patients, 19 (76.0%) were reported to be ER negative based on mRNA expression. They reported that tumors with 1–9% positivity by IHC result from a testing artifact, and CTx as well as AET is recommended for this group. Deyarmin et al. [24] also reported that 88.0% of weakly ER-positive tumors identified by IHC were classified as basal-like or HER-2

enriched subtype, and only 10.0% of those patients were classified as having the luminal A subtype by the intrinsic molecular subtype.

Lastly, in concurrent with our results, several studies have reported that ER expression levels can affect clinical outcomes [25–30]. Zhang et al. [25] analyzed the clinico-pathological features in 1700 consecutive invasive breast cancer patients based on four subgroups based on ER expression levels: ER < 1%, 1–10%, 11–70% and > 70%. As the ER expression level lessened, there were significantly more unfavorable clinicopathological features, such as Nottingham grade, NG, and PR, and significantly worse survival in DFS. Bouchard-Fortier et al. [27] evaluated the effect of AET according to ER expression levels. They identified 2221 breast cancer patients who had been ER tested using a ligand-based assay (LBA) between 1976 and 1995. They subgrouped patients by ER expression level: 0–3, 4–9, 10–19, 20–49, and 50 fmol/mg or more cytosolic protein. Among the 661 patients treated with AET, 20-year BCSS were 41%, 41%, 77%, 68%, and 61% respectively. In the adjusted analysis for age, body mass index, tumor size, axillary lymph node involvement, surgery, RTx, and CTx, levels of ER expression were associated with a HR for lowered breast cancer mortality: 1.00 (reference), 0.59 ($P = 0.09$), 0.19 ($P < 0.0001$), 0.26 ($P < 0.0001$), and 0.31 ($P < 0.0001$), respectively.

Several studies have addressed some factors associated with survival among breast cancer patients with distant metastasis, and the preferred distant metastasis site by molecular subtype and have found that ER-positive tumors are more likely to result in bone metastases while ER-negative tumors are more likely to result in visceral or brain metastases [31]. Commonly, hormone receptor negative, high Ki-67 level, metastasis sites such as brain and liver, more than two organs metastases, and short duration of DMFI were independent predictors of poor OS after distant metastasis [32–34]. Our study demonstrated that strongly ER-positive tumors are more likely to result in bone metastasis, have lower Ki-67 and result in long periods of DMFI, compared to weakly ER-positive tumors with de novo distant metastasis. These differences may be associated with the

differences in OS despite DFS and DMFS not being significantly different between groups II and III.

There are a few limitations associated with this study. First, there was no centralized IHC testing in our study. This could possibly have resulted in subjectivity in the interpretation of results, which could, accordingly, have resulted in different categorization. Second, we defined patients as having weakly ER-positive tumors when the Allred score was 3–5. Total score was consistent with the intensity score and proportion score. However, since 2010, ER positivity has been defined as having more than 1% of cells stained positive for the estrogen receptor. Consequently, some patients could have been misclassified. Third, we retrospectively collected data at a single institute and treatment was not assigned in a randomized method. Lastly, the follow-up duration was 57.8 months which is a relative short follow-up period.

In conclusion, weakly ER-positive tumors resulted in worse OS than strongly ER-positive tumors and this higher HR for OS after adjusting for other factors could affect prognosis. Weakly ER-positive tumors had significantly higher BCT scores and were more likely to be found in the high risk group compare strongly ER-positive tumors. We should not underestimate the risk for patients with weakly ER-positive tumors.

Compliance with ethical standards

Conflict of interest JK and BK are employees of Gencurix. The other authors have no competing interests to declare.

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