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REVIEW

A nomogram to predict pathologic complete response (pCR) and the value of tumor-infiltrating lymphocytes (TILs) for prediction of response to neoadjuvant chemotherapy (NAC) in breast cancer patients

Hye Won Hwang[1] · Hera Jung[1] · Jiyeon Hyeon[2] · Yeon Hee Park[3] · Jin Seok Ahn[3] · Young-Hyuck Im[3] · Seok Jin Nam[4] · Seok Won Kim[4] · Jeong Eon Lee[4] · Jong-Han Yu[4] · Se Kyung Lee[4] · Misun Choi[5] · Soo Youn Cho[1] · Eun Yoon Cho[1]

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Abstract

Purpose The value of tumor-infiltrating lymphocytes (TILs) for prediction of pathologic complete response (pCR) in breast cancer (BC) patients treated with neoadjuvant chemotherapy (NAC) has received increasing attention. In human epidermal growth factor receptor 2 (HER2)-positive BC, advances in HER2-targeted therapy have not yet clarified the clinical implications of pre-NAC TILs. Likewise, the prognostic role of TILs for long-term survival is not well established.

Methods Pre- and post-NAC TIL levels were evaluated in 248 pair-matched pre-NAC biopsy and post-NAC resection samples, and analyzed for predictive and prognostic significance with other clinicopathologic parameters. Additional 60 pre-NAC biopsy samples of HER2-positive BC treated with a TCHP regimen (docetaxel, carboplatin, and a combination of trastuzumab and pertuzumab) were also assessed.

Results High pre-NAC TILs, clinical nodal stage 0–1 (cN0–1), and negative ER expression were shown to be strong predictive markers for pCR. A nomogram based on these significant clinicopathologic predictors was developed, providing integrated probability of achieving pCR after NAC. The association between high pre-NAC TIL levels and significantly increased pCR rate was also confirmed in HER2-positive BC patients treated with a TCHP regimen. After chemotherapy, increased quantity of post-NAC TILs was shown to have extended BC-specific survival and disease-free survival in univariable and multivariable analyses.

Conclusions High pre-NAC TIL levels were significantly predictive of pCR in BC, and can act as a surrogate marker for predicting therapeutic effects of a TCHP regimen for HER2-positive BC. Post-NAC TILs in residual disease were a new prognostic marker of risk stratification for long-term survival.

Keywords Tumor-infiltrating lymphocytes · Neoadjuvant chemotherapy · Pathologic complete response · Nomogram · TCHP

✉ Soo Youn Cho
sooyoun.cho@samsung.com

✉ Eun Yoon Cho
eunyoon.cho@samsung.com

[1] Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, South Korea

[2] Department of Pathology, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Incheon, South Korea

[3] Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

[4] Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

[5] Division of Forensic Investigation, National Forensic Service Seoul Institute, Seoul, South Korea

Introduction

Antitumor host immune responses, including infiltrating immune cells, play an important role in development and progression of malignant tumors of many types [1–3]. The role of tumor-infiltrating lymphocytes (TILs) in breast cancer (BC) has been significantly explored over the last decades. Previous studies demonstrated the association of high levels of TILs with increased pathologic complete response (pCR) rate in the neoadjuvant setting, and with better long-term survival in the adjuvant setting in certain subtypes of BC [4–9].

As an increasing number of BC patients are treated with neoadjuvant chemotherapy (NAC), identification of a biomarker to predict the probability of pCR in individual cases is a high priority. TILs may be a potent candidate in this regard. Due to uneven distribution of TILs among molecular subtypes, the predictive impact is most robust in triple-negative (TN) subtype and less well established in other subtypes [10, 11]. Moreover, data regarding the prognostic and/or predictive role of post-treatment TIL levels remain limited and inconclusive [12, 13].

In human epidermal growth factor receptor 2 (HER2)-positive BC, therapeutic advances in dual anti-HER2 blockade of trastuzumab and pertuzumab, which are humanized monoclonal antibodies targeting different epitopes of HER2, have substantially improved pCR rate [14–16]. There are few available data regarding whether treatment effect of pertuzumab differed with respect to TIL levels, or on the potential association between pCR rate and treatment (single vs. dual HER2 targeting) regimen.

In this study, we evaluated TILs before and after NAC in a single institute cohort to investigate two key points. First, we tested the hypothesis that higher levels of pre-NAC TILs would be predictive of increased pCR rates, regardless of molecular subtype or NAC regimen for HER2-positive BC. Second, we investigated the prognostic ability of post-treatment TIL levels.

Materials and methods

Patients and samples

A total of 480 cases of BC treated with NAC followed by surgical resection from 2004 to 2013 were retrieved from the computerized records system at Samsung Medical Center, Seoul, Korea. Of these, 248 cases were available for histologic evaluation both in pre-NAC biopsy and post-NAC resection tissue. Besides the cohort group, we collected an additional TCHP group for the comparison of TILs and pCR rate in different NAC regimens, especially in HER2-positive BC. The TCHP group consisted of 60 HER2-positive BC patients preoperatively treated with the TCHP regimen (docetaxel, carboplatin, and a combination of trastuzumab and pertuzumab) from February 2016 to July 2017. To evaluate the molecular subtype classification, the results of immunohistochemistry (IHC) for estrogen receptor (ER), progesterone receptor (PR), and Ki-67 were reviewed. HER2 expression was assessed by IHC and scoring was determined according to the criteria of American Society of Clinical Oncology (ASCO)/College of American Pathologist (CAP) guidelines [17]. Tumors with scores 2+ were further tested by fluorescence in situ hybridization (FISH). IHC for Ki-67 using monoclonal antibody (clone MIB-1, Dako, dilution 1:300) was scored by an automated image analysis system (iSolution DT made by IMT i-Solution Inc.), as the percentage of positively stained tumor cells within the highest proliferative area (hot spot). The level of Ki-67 expression was classified as high versus low with a cut-off point of 20% [18, 19]. Based on the IHC results, tumors were categorized using the St Gallen criteria, as luminal A (ER/PR+, HER2−, and low Ki-67), HER2− luminal B (ER+, HER2−, and either high Ki-67 or PR−), HER2+ luminal B (ER+ and HER2+), HER2+ (ER− and HER2+), or triple-negative (ER/PR− and HER2−) subtypes [20]. ypTN stage was defined according to the American Joint Committee on Cancer [21]. The pCR was defined as complete disappearance of all invasive tumor cells from breast tissue and regional lymph nodes regardless of the presence of residual ductal carcinoma in situ (ypT0/is N0). Determination of residual cancer burden (RCB) was realized according to Symmans [22]. For estimation of RCB score and consequential RCB class, variables including bi-dimensional size and cellularity of the residual tumor bed, and nodal disease burden were entered into the MD Anderson RCB calculator (http://www.mdanderson.org/breastcancer_RCB). Clinicopathologic parameters including age, menopausal status, nuclear grade, histologic grade, histologic type, recurrence, follow-up status, and follow-up period were obtained by a thorough review of clinical records. Study protocols including case selection, slide review, and collection of clinical parameters were approved by the Samsung Medical Center Institutional Review Board.

Evaluation of TILs

We evaluated TILs both in pre-NAC biopsy and post-NAC resection tissue. As post-NAC TILs were assessed in association with residual invasive tumor cells within breast tissue, patients who achieved pCR or had residual tumor cells only in lymph nodes were excluded for evaluation of post-treatment TILs. Stromal TILs were evaluated according to recent

recommendations of the international TILs working group 2014 [23]. Briefly, percentage of stromal areas occupied by all mononuclear cells including lymphocytes and plasma cells was scored as a categorical variable in 10% increments. Intratumoral TILs were also assessed separately, but in most cases these were lower in number and usually parallel to stromal TILs. For that reason, our study focused on stromal TILs as a more reproducible parameter for evaluation, corresponding to current recommendations. Two expert breast pathologists (E.Y.C. and H.W.H.) evaluated TIL levels on hematoxylin and eosin (H&E)-stained sections without additional staining, and the average percentage of each case was obtained. Lymphocyte-predominant breast cancer (LPBC) was defined as tumors that contain > 50% TILs (Fig. 1).

Statistical analysis

Statistical analyses were performed with SPSS Ver.24 software (SPSS Inc., Chicago, IL, USA). In the analysis, we categorized patients into LPBC or non-LPBC groups according to TIL levels before and after NAC. The difference of clinicopathologic parameters between the two groups was evaluated by Pearson's χ^2 with continuity correction or Fisher's exact test. Variables of interest including pre-NAC TIL level, age, menopausal status, tumor size, clinical nodal stage (cN), histologic grade, NAC regimen and cycle number, expression level of ER, PR, HER2, and Ki-67 were assessed to evaluate the predictive value for pCR. Univariable logistic regression model and backward stepwise selection for final multivariable model were conducted. As a more comprehensive predictive tool for pCR, we built a nomogram, given the risk factors in the final multivariable model. Values for each of the model covariates were mapped to points on a scale ranging from 0 to 100, with total points obtained for each model covariate mapped to the probability of pCR associated with that combination of covariate values. The predictive accuracy of the model was assessed by its discrimination and calibration. The area under the receiver operating characteristic curve (AUC) was measured for the model's ability to discriminate between patients with or without pCR. Calibration was also assessed graphically by plotting the relationship between observed outcome frequencies and predicted probabilities.

Two survival end points were evaluated. Disease-free survival (DFS) was defined as time between surgery and recurrence or metastasis, and breast cancer-specific survival (BCSS) as time between initial diagnosis and breast cancer-related death or last follow-up. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated with the Cox proportional hazard regression model. We conducted a multivariable Cox regression analysis including all potential variables significantly associated with survival in each univariable analysis. Kaplan–Meier method was used to estimate DFS and BCSS curves and the log-rank test was performed to compare between groups. All the statistical tests were two-sided, and were regarded as statistically significant when the p value was less than 0.05.

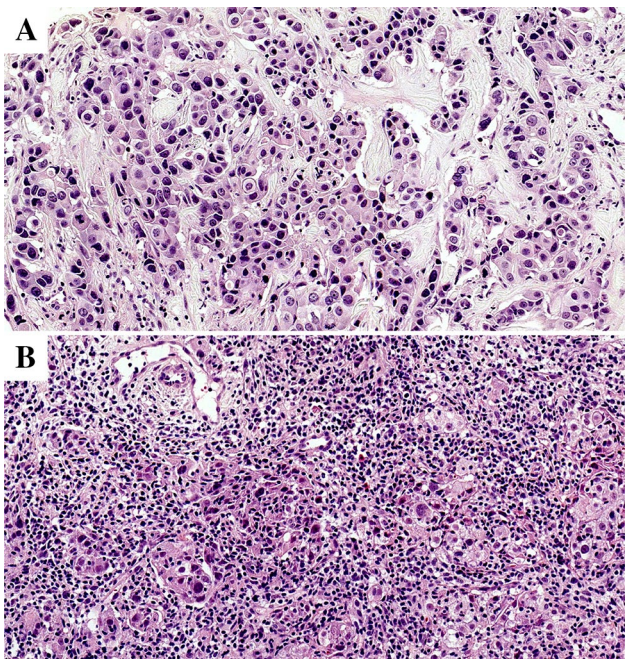

Fig. 1 Representative images of tumor-infiltrating lymphocytes (TILs). Non-lymphocyte-predominant breast cancer (non-LPBC) in **a** (×200) and LPBC in **b** (×200)

Results

Patient characteristics

The review of pre-NAC TILs was available for 248 patients in the cohort group. Post-NAC TIL levels were evaluated in 204 patients except 44 patients who achieved pCR ($n = 37$) or had residual tumor cells in lymph nodes only ($n = 7$). The median age was 44 years (range 23–71 years), and 76% ($n = 188$) were premenopausal. Clinically, most patients received a taxane-based regimen (61%, $n = 151$) as neoadjuvant treatment. Overall pCR rate in response to NAC was 15% ($n = 37$).

HER2-positive BC accounted for 25% ($n = 62$) of the cohort group, consisting of 22 hormone receptor (HR)-positive (corresponding to HER2+ luminal B subtype) and 40 HR-negative (corresponding to HER2+ subtype) BC patients. With standard cytotoxic agents, 20 patients received trastuzumab, while 42 patients had no anti-HER2 therapy.

Median age of the TCHP group ($n=60$) was 45 years (range 28–59 years), and 80% ($n=48$) were premenopausal. Of these, 50% were HR positive, and 50% were HR negative.

Associations between pre-NAC TILs and clinicopathologic parameters

The median value of pre-NAC TILs was 14.6%, and 23% ($n=57$) were classified as pre-NAC LPBC in the overall cohort group. Statistical group analysis for clinicopathologic variables was performed between LPBC and non-LPBC patients (Table 1). Higher pre-NAC TIL levels were significantly associated with more aggressive clinicopathologic characteristics, such as higher clinical T (cT) stage ($p=0.02$), histologic grade ($p<0.001$), and Ki-67 index ($p<0.001$). No obvious differences were observed in age, menopausal status, cN stage, or histologic type.

Predictive value of pre-NAC TILs and nomogram for predicting pCR

Consistent with findings in previous studies, high pre-NAC TILs were shown to be a strong predictive marker for favorable outcomes after NAC [4–6]. By comparison, 44.9% of pre-NAC LPBC patients had a pCR, as did 6.3% of non-LPBC patients ($p<0.001$). Notably, high TIL levels were associated with a 25.9% (95% CI 17.4–38.6%) increase in pCR rate compared with low TIL levels ($p<0.001$; $\chi^2=37.71$).

The predictive role of a pre-NAC LPBC phenotype for excellent response to NAC was also demonstrated in residual disease after treatment, including significantly smaller residual tumor size (ypT, $p<0.001$) and negative nodal metastasis (ypN, $p=0.017$). Moreover, a significantly higher proportion of patients in the pre-NAC LPBC group yielded lower RCBs in comparison with non-LPBC group (53% vs. 19%, $p<0.001$).

For further comprehensive prediction of pCR, we constructed a nomogram based on the significant and predefined predictors. Univariable logistic regression analysis showed that the proportion of patients with pCR increased with high pre-NAC TILs, old age, high histologic grade, high Ki-67 expression, cN0–1, and small tumor size (Table 2). A taxane-based regimen, >4 cycles of NAC, additional administration of trastuzumab, negative ER or PR expression, and HER2 positivity were also associated with pCR. We identified novel association of high pre-NAC TILs, cN0–1, and negative ER expression with increased probability of achieving pCR in a successive multivariable logistic regression model. Figure 2a illustrates the nomogram to calculate the probability of achieving pCR. In the nomogram, each variable is assigned to a point ranging from 0 to 100, and the predicted probability of pCR can be read in two steps: (i) Draw a vertical line for each variable (pre-NAC TILs, cN, ER) to the axis termed 'Points' at the top of the figure. The points assigned for the value of each variable can be read where the vertical line crosses the 'Points' axis. (ii) Add the three-point scores determined at step 1 and find the sum score on the axis termed 'Total Points.' Determine the predicted value of pCR by drawing a vertical line from the sum score on the 'Total Points' axis down to the axis termed 'Predicted Value.' The estimated probability of achieving pCR can be read where the vertical line crosses with the 'Predicted Value' axis (Fig. 2b).

Association of TILs with pCR rate based on NAC regimen in HER2-positive BC

There were no significant differences in median value of pre-NAC TILs, or pre-NAC LPBC rate based on type of anti-HER2 therapy, specifically no anti-HER2 blockade (13.8% for median pre-NAC TIL levels, 24% for pre-NAC LPBC rate), trastuzumab only (27.5%, 45%), or TCHP (27.7%, 45%).

pCR was achieved in 73% of HER2-positive BC patients treated with TCHP, compared with 50% given trastuzumab only and 14% with no anti-HER2 therapy. Consistent with previous randomized controlled trials, the benefit of dual HER2 blockade in improved pCR rate has been noted in this study [14–16].

Our study confirmed the association between high pre-NAC TILs and significantly increased pCR rate in HER2-positive BC (OR 6.49; 95% CI 2.83–14.88; $p<0.001$). No association between pre-NAC TILs and pCR rate according to type of NAC was detected. Our findings show that high pre-NAC TILs in HER2-positive BC are strongly associated with increased pCR probability in patients treated with NAC plus trastuzumab and pertuzumab either alone (50%) or in combination (73%). High pre-NAC TILs were also associated with a significant decrease in RCB class (OR 2.42; 95% CI 1.29–4.56; $p=0.002$),

In the TCHP group, we found no significant difference in the proportion of pre-NAC LPBC by HR expression. Thirteen patients (43%) of HR-negative tumors ($n=30$) were classified as pre-NAC LPBC, as was 14 (47%) of HR-negative tumors ($n=30$). More patients with HR-negative tumors achieved pCR than those with HR-positive tumors ($n=26$ vs. 18; OR 4.33; 95% CI 1.20–15.61; $p=0.039$).

Prognostic value of post-NAC TILs

After chemotherapy, the median TIL percentage fell to 10.2%. TIL levels showed a decrease in 44% ($n=89$), no change in 39% ($n=80$), and an increase in 17% ($n=35$). Post-NAC LPBC comprised 12% of patients with residual tumors. No association was observed between post-NAC

Table 1 Association between pre-/post-NAC TILs and clinicopathologic parameters

Variables	Pre-NAC				Post-NAC			
	All, *n* (%)	Non-LPBC, *n* (%)	LPBC, *n* (%)	*p* value	All, *n* (%)	Non-LPBC, *n* (%)	LPBC, *n* (%)	*p* value
	248	191 (77)	57 (23)		204	180 (88)	24 (12)	
Age (years)								
< 50	178 (72)	137 (72)	41 (72)		151 (74)	131 (73)	20 (83)	
≥ 50	70 (28)	54 (28)	16 (28)	0.56	53 (26)	49 (27)	4 (17)	0.33
Menopausal status								
Pre	188 (76)	147 (77)	41 (72)		158 (77.5)	137 (76)	21 (87.5)	
Post	60 (24)	44 (23)	16 (28)	0.48	46 (22.5)	43 (24)	3 (12.5)	0.3
Clinical T stage								
cT1–2	102 (41)	71 (37)	31 (54)		75 (37)	64 (36)	11 (46)	
cT3–4	146 (59)	120 (63)	26 (46)	0.02	129 (63)	116 (64)	13 (54)	0.37
Clinical N stage								
cN0–1	54 (22)	39 (20)	15 (26)		40 (20)	35 (19)	5 (21)	
cN2–3	194 (78)	152 (80)	42 (74)	0.36	164 (80)	145 (81)	19 (79)	0.79
Histologic type								
Ductal	229 (92)	174 (91)	55 (96.5)		185 (91)	164 (91)	21 (87.5)	
Others	19 (8)	17 (9)	2 (3.5)	0.26	19 (9)	16 (9)	3 (12.5)	0.47
Histologic grade								
1–2	116 (47)	105 (55)	11 (19)		109 (53)	99 (55)	10 (42)	
3	132 (53)	86 (45)	46 (81)	< 0.001	95 (47)	81 (45)	14 (58)	0.28
Pre-NAC Ki-67								
Low	66 (29)	63 (36)	3 (6)		62 (34)	56 (34)	6 (29)	
High	161 (71)	114 (64)	47 (94)	< 0.001	122 (66)	107 (66)	15 (71)	0.81
Molecular subtype								
Lu A	45 (18)	42 (22)	3 (5)		43 (21)	41 (23)	2 (8)	
HER2− Lu B	64 (26)	55 (29)	9 (16)		60 (29)	58 (32)	2 (8)	
HER2+ Lu B	22 (9)	16 (8)	6 (10)		20 (10)	15 (8)	5 (21)	
HER2+	40 (16)	27 (14)	13 (23)		25 (12)	20 (11)	5 (21)	
TN	77 (31)	51 (27)	26 (46)	0.002	56 (28)	46 (26)	10 (42)	0.01
NAC regimen								
Anthracycline based	97 (39)	79 (41)	18 (32)		87 (43)	75 (42)	12 (50)	
Taxane based	151 (61)	112 (59)	39 (68)	0.22	117 (57)	105 (58)	12 (50)	0.51
NAC cycle								
≤ 4	90 (36)	71 (37)	19 (33)		79 (39)	69 (38)	10 (42)	
> 4	158 (64)	120 (63)	38 (67)	0.64	125 (61)	111 (62)	14 (58)	0.83
pCR								
Yes	37 (15)	12 (6)	25 (44)					
No	211 (85)	179 (94)	32 (56)	< 0.001				
RCB class								
0–1	66 (27)	36 (19)	30 (53)		26 (13)	12 (7)	14 (58)	
2–3	182 (73)	155 (81)	27 (47)	< 0.001	178 (87)	168 (93)	10 (42)	< 0.001
Residual tumor size								
≤ 2 cm	123 (50)	78 (41)	45 (79)		80 (39)	61 (34)	19 (79)	
> 2 cm	125 (50)	113 (59)	12 (21)	< 0.001	124 (61)	119 (66)	5 (21)	< 0.001
Nodal status								
Neg	94 (38)	65 (34)	29 (51)		137 (67)	115 (64)	22 (92)	
Pos	154 (62)	126 (66)	28 (49)	0.03	67 (33)	65 (36)	2 (8)	0.005
Post-NAC Ki-67								
Low	116 (59)	105 (64)	11 (32)		113 (58.5)	104 (59)	9 (50)	
High	82 (41)	59 (36)	24 (68)	0.001	80 (41.5)	71 (41)	9 (50)	0.46

TILs tumor-infiltrating lymphocytes, *NAC* neoadjuvant chemotherapy, *LPBC* lymphocyte-predominant breast cancer, *Lu A* luminal A, *Lu B* luminal B, *TN* triple negative, *pCR* pathologic complete response

Table 2 Univariable and multivariable analyses for predictive factors of pathologic complete response (pCR)

Variables	Univariable analysis			Multivariable analysis		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Pre-NAC TILs: per 10% increment	6.42	3.27–12.58	< 0.001	3.38	1.60–7.13	0.001
Age: ≥ 50 versus < 50	1.35	0.70–2.60	0.378			
Menopause: post versus pre	1.06	0.52–2.14	0.881			
Histologic grade: 3 versus 1–2	3.30	1.45–7.52	0.005	1.08	0.43–2.76	0.867
Ki-67: high versus low	4.09	1.44–11.66	0.002	4.05	1.28–12.85	0.067
Nodal status: cN0–1 versus cN2–3	2.39	1.21–4.72	0.012	2.93	1.41–6.05	0.004
Tumor size: ≤ 4 cm versus > 4 cm	2.24	1.16–4.33	0.016	2.76	1.36–5.62	0.19
NAC regimen: Taxane versus Anthracycline	3.87	1.62–9.25	0.002	1.46	0.21–10.33	0.704
Trastuzumab: with versus no	2.14	0.78–5.94	0.142			
NAC cycle: > 4 versus ≤ 4	4.49	2.00–10.11	< 0.001	1.97	0.94–4.14	0.074
ER: − versus +	4.76	2.07–10.93	< 0.001	3.40	1.14–10.13	0.028
PR: − versus +	4.51	1.60–12.75	0.004	1.67	0.44–6.34	0.45
HER2: + versus −	1.90	0.99–3.65	0.053			

TILs tumor-infiltrating lymphocytes, *NAC* neoadjuvant chemotherapy, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *OR* odds ratio, *CI* confidence interval

TILs and baseline pre-treatment clinicopathologic variables (Table 1). In residual disease, increased quantity of post-NAC TILs was significantly associated with smaller residual tumor size ($p < 0.001$), negative nodal status ($p = 0.005$), and lower RCBs ($p < 0.001$), representing a prognostic role of host antitumor immunity after treatment. Therefore, we hold the hypothesis that post-NAC TIL levels can predict long-term outcomes in the neoadjuvant setting. For further confirmation, univariable and multivariable survival analyses were conducted. Median follow-up was 60.1 months. Five-year BCSS rate was 45.6%, and 5-year DFS rate was 38.3%. The presence of high post-NAC TIL levels has been shown to have significantly extended BCSS and DFS in univariable analysis (Table 3; Fig. 3a, b). Lower pre- and post-NAC Ki-67 expression, achievement of pCR, lower RCB class, and absence of metastatic lymph nodes after NAC were other parameters associated with better BCSS. Aforementioned variables and smaller residual tumor size were also statistically significant factors of better DFS. When the significant variables were entered as potential regression candidates, only high post-NAC TILs and low Ki-67 index were significant predictors of BCSS and DFS in the multivariable model.

We further compared long-term survival of post-LPBC and non-post-LPBC patients with patients who achieved pCR (Fig. 3c, d). Of interest, patients who did not achieve pCR and had high post-NAC TIL levels had slightly poorer BCSS and DFS than pCR group, but significantly better survivals than non-pCR and non-post-LPBC group. Patients who did not achieve pCR and had low levels of post-NAC TILs had the poorest survival.

Association between pre- and post-NAC TILs and molecular subtypes

The proportion of pre-NAC LPBC differed among molecular subtypes, with highest in TN (46%) and lowest in luminal A subtype (5%) (Table 1). This distribution was statistically significant ($p = 0.002$), and consistent with findings in previous studies. We also found significant differences between molecular subtypes in post-NAC LPBC rate, which was highest in TN (42%), lowest in luminal A and HER2− luminal B (8%).

Patients who achieved pCR were significantly more likely to be TN (41%) or HER2+ (38%) subtypes than HER2- luminal B (11%), HER2+ luminal B (5%), or luminal A (5%) subtypes. By pre-NAC TIL value, the pre-NAC LPBC phenotype was significantly associated with pCR in the HER2+ subtype (OR 5.60; 95% CI 1.33–23.62; $p = 0.031$) (Table 4). The pCR rate was also significantly higher in LPBC patients across TN (OR 5.75; 95% CI 1.71–19.38; $p = 0.005$) and HER2− luminal B (OR 27.00; 95% CI 2.41–302.19; $p = 0.007$) subtypes. The odds ratio of pCR increased by 3.91 (95% CI 3.50–4.48) per 10% increment in pre-NAC TILs in HER2+ subtype, 2.63 (95% CI 1.91–3.42) in TN subtype, and 1.82 (CI 1.62–2.07) in HER2-luminal B subtype. No correlation between high pre-NAC TIL level and pCR was identified in luminal A or HER2+ luminal B due to lack of achieving pCR in non-LPBC patients of these subtypes.

In survival analysis, all subtypes demonstrated a tendency for extended BCSS and DFS in pre-NAC LPBC in comparison with non-LPBC, but only BCSS of the TN subtype was statistically significant.

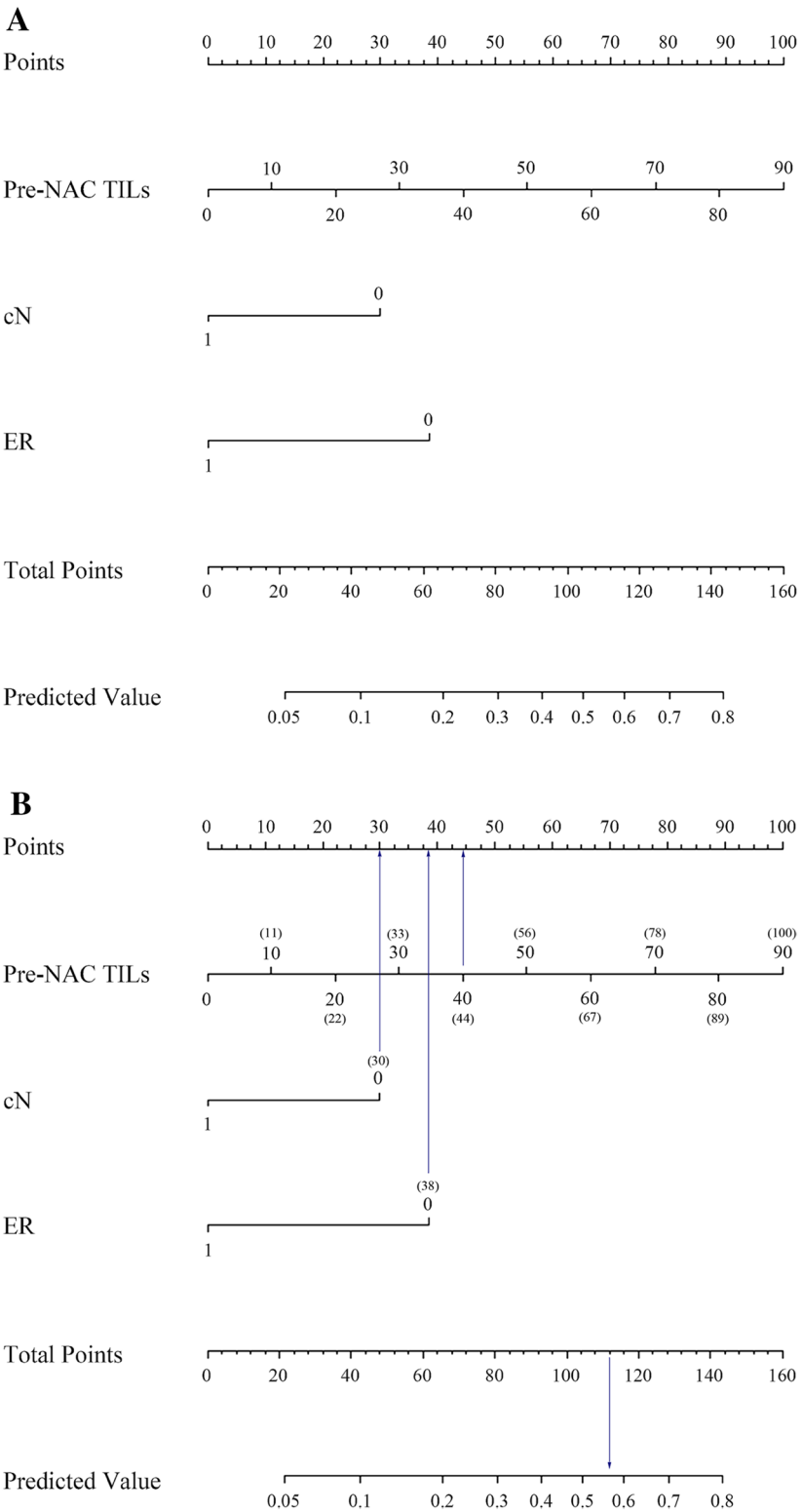

Fig. 2 **a** Nomogram to predict the probability of pathologic complete response (pCR). *TILs* tumor-infiltrating lymphocytes, *NAC* neoadjuvant chemotherapy, *cN* clinical N stage, *ER* estrogen receptor. **b** A patient of triple-negative breast cancer with cN0 stage and 40% of pre-NAC TILs would have a total of 112 points (44 for 40% pre-NAC TILs, 30 for cN0, and 38 for ER negativity). The predictive value of pCR after NAC for this patient is 57%

Discussion

NAC has been increasingly used in the management of both locally advanced and earlier operable stage BC. NAC is mainly used to decrease extent of the tumor but it also provides a prognostic value, as pCR serves as a predictor for long-term survival [24].

Recent preclinical studies demonstrated that standard neoadjuvant cytotoxic agents, including anthracyclines and taxanes, can trigger an antitumor host immune

Table 3 Factors associated with prognosis in univariable and multivariable analyses

Variables	Univariable analysis			Multivariable analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Breast cancer-specific survival						
Pre-LPBC versus non-LPBC	1.23	0.64–2.36	0.53			
Post-LPBC versus non-LPBC	2.38	0.85–6.66	0.04	6.57	0.87–19.57	0.005
Age: ≥ 50 versus < 50	1.55	0.77–3.10	0.22			
Menopause: post versus pre	1.06	0.54–2.08	0.86			
cT stage: cT1–2 versus cT3–4	1.7	0.91–3.16	0.09			
cN stage: cN0–1 versus cN2–3	1.49	0.70–3.18	0.3			
Histologic grade: 1–2 versus 3	1.78	0.99–3.19	0.053			
Pre Ki-67: low versus high	2.67	1.19–5.99	0.018	1.90	0.80–4.48	0.144
NAC regimen: Taxane versus Anthracycline	0.9	0.50–1.61	0.72			
NAC cycle: > 4 versus ≤ 4	0.76	0.42–1.38	0.37			
pCR versus non-pCR	4.15	1.01–17.08	0.049	4.62	2.95–7.26	0.97
RCB class: 0–1 versus 2–3	5.66	1.76–18.22	0.004	2.06	0.27–15.95	0.49
Residual tumor size: ≤ 2 cm versus > 2 cm	1.67	0.94–2.99	0.082			
Nodal status: Neg versus Pos	2.32	1.16–4.65	0.018	1.49	0.64–3.45	0.354
Post KI-67: low versus high	4.66	2.43–8.94	< 0.001	4.59	2.23–9.46	< 0.001
Disease-free survival						
Pre-LPBC versus non-LPBC	1.26	0.72–2.20	0.43			
Post-LPBC versus non-LPBC	1.99	0.91–4.35	0.044	2.24	0.81–5.48	0.025
Age: ≥ 50 versus < 50	1.24	0.75–2.06	0.41			
Menopause: post versus pre	0.9	0.55–1.48	0.68			
cT stage: cT1–2 versus cT3–4	1.6	0.99–2.56	0.054			
cN stage: cN0–1 versus cN2–3	1.8	0.95–3.40	0.07			
Histologic grade: 1–2 versus 3	1.33	0.85–2.08	0.21			
Pre Ki-67: low versus high	2.36	1.24–4.49	0.009	2.15	1.10–4.19	0.091
NAC regimen: Taxane versus Anthracycline	0.66	0.41–1.06	0.086			
NAC cycle: > 4 versus ≤ 4	0.66	0.40–1.06	0.085			
pCR versus non-pCR	3.7	0.35–10.12	0.011	2.57	1.75–3.78	0.971
RCB class: 0–1 versus 2–3	3.76	1.81–7.81	< 0.001	1.82	0.42–7.86	0.424
Residual tumor size: ≤ 2 cm versus > 2 cm	1.94	1.23–3.08	0.005	1.21	0.68–2.14	0.524
Nodal status: Neg versus Pos	2.42	1.41–4.13	0.001	1.68	0.82–3.46	0.155
Post KI-67: low versus high	2.65	1.64–4.28	< 0.001	2.45	1.43–4.21	0.001

LPBC lymphocyte-predominant breast cancer, *NAC* neoadjuvant chemotherapy, *pCR* pathologic complete response, *HR* hazard ratio, *CI* confidence interval

response by inducing tumor-specific cytotoxic T cells. In HER2-positive BC, dual anti-HER2-targeted therapy combined with cytotoxic agents has become a standard NAC regimen, with the benefit partly from antibody-dependent cell-mediated cytotoxicity (ADCC). However, as the mechanisms of how TCHP regimens influence tumor microenvironment are not yet clarified, predictive or prognostic role of TILs remains unknown in this setting. This was the first goal of our study.

Secondly, previous investigations have focused on the prognostic effect of TILs mainly in the TN subtype, due to it having the highest rate of LPBC [9, 25]. Luminal types have been considered as less immunogenic, lymphocyte-depleted tumors as compared to other subtypes, questioning their surrogacy for pCR or long-term survival. However, accumulated data suggest a possible association of tumor biology with immunity and inflammation in these subtypes [26, 27]. Though a majority of previous studies have regarded luminal types altogether as HR-positive, luminal A and luminal B have been revealed as distinct molecular subtypes, with specific oncogenic drivers [28, 29]. The luminal B subtype can be further divided according to HER2 amplification status. As the overexpression of HER2 itself has an immunogenicity and triggers the immune system, the tumor-immune

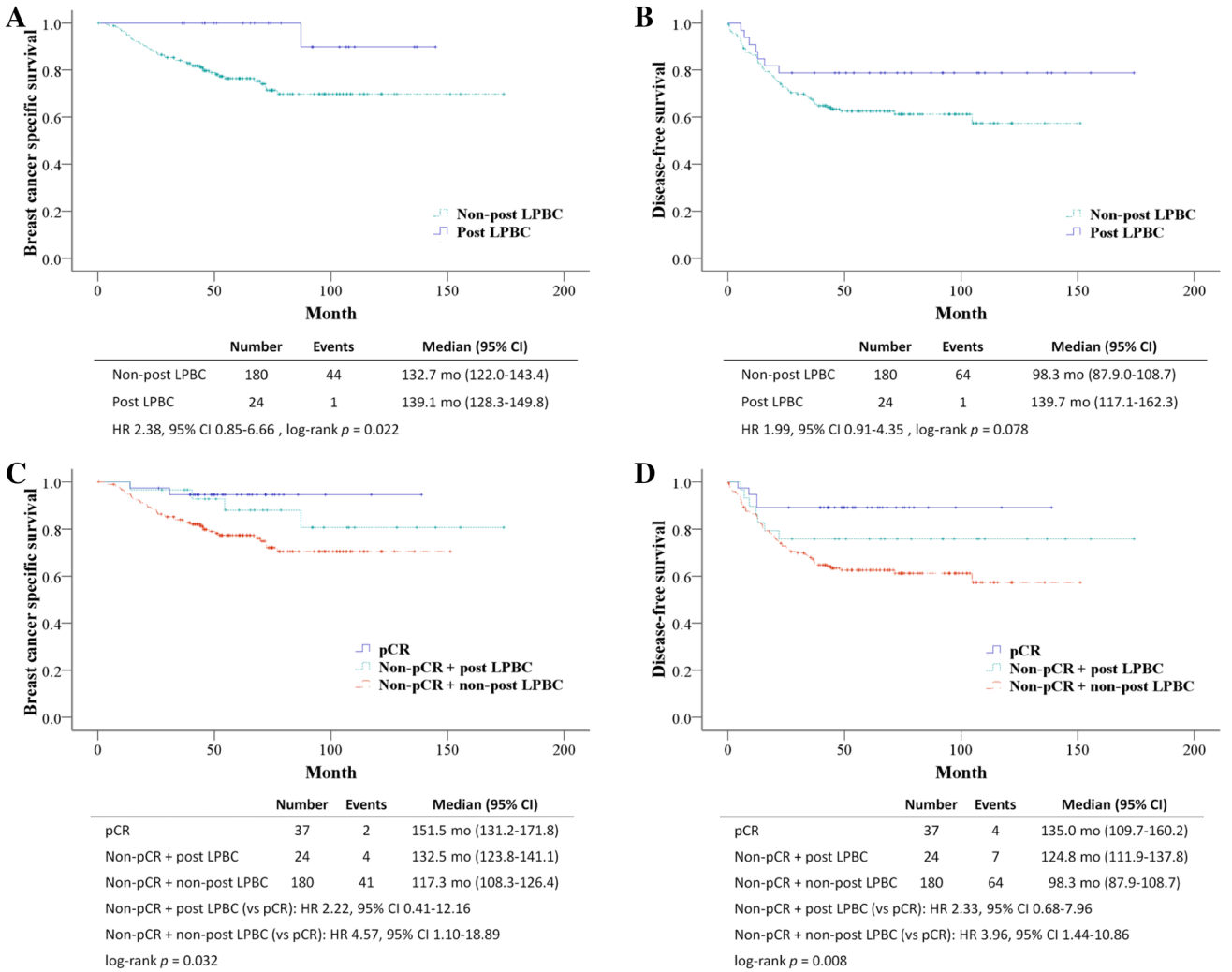

Fig. 3 Kaplan–Meier curves of breast cancer-specific survival and disease-free survival stratified by post-NAC TIL levels (**a, b**), and in comparison with pCR group (**c, d**). *LPBC* lymphocyte-predominant breast cancer, *pCR* pathologic complete response, *HR* hazard ratio, *CI* confidence interval

Table 4 Pathologic complete response (pCR, $n=37$) rate according to molecular subtypes

	pCR, *n* (%)			
	Overall	LPBC ($n=25$)	non-LPBC ($n=12$)	*p* value
Luminal A ($n=45$)	2	2 (100%)	0 (0%)	0.003
HER2-Luminal B ($n=64$)	4	3 (75%)	1 (25%)	0.007
HER2+ Luminal B (*n*-22)	2	2 (100%)	0 (0%)	0.065
HER2+ ($n=40$)	14	8 (57.1%)	6 (42.9%)	0.031
Triple negative ($n=77$)	15	10 (66.7%)	5 (33.3%)	0.005

LPBC lymphocyte-predominant breast cancer, *HER2* human epidermal growth factor receptor 2

interface between the two luminal B subtypes may be quite different.

Finally, few studies have investigated TILs after NAC, and the results have been controversial regarding the prognostic significance of post-treatment TILs [12, 13]. The correlation between high levels of post-treatment TILs and lower residual tumor burden or better outcome was found in the TN subtype only.

In this study, 248 pair-matched pre-treatment biopsy and post-treatment resection specimens provided new insights into pre- and post- NAC TIL levels in different molecular subtypes and NAC regimens.

The results of pre-NAC TILs confirmed their predictive value for pCR in the overall cohort group, especially standing out in the TN, HER2+, and HER2− luminal B subtypes. Although absence of cases achieving pCR limits the conclusion for luminal A and HER2+ luminal B subtypes, our findings imply possible predictive role of pre-NAC TILs in these subtypes. Our results demonstrated that besides high pre-NAC TILs, cN0–1 stage and low ER expression levels were other significant factors for predicting pCR. On the basis of these results, we created a nomogram to predict the probability of pCR. This nomogram provides the estimated probability of achieving pCR after the planned NAC using clinicopathologic features. There have been other well-designed nomograms predicting pCR after NAC in the literature, but to the best of our knowledge, our study is the first to generate a nomogram providing a detailed probability associated with TILs. Although we used a calibration plot to assess agreement between the predicted and observed probabilities, we did not use a bootstrap method to validate the model internally or externally. Further research focused on validation will extend the generalized use of the nomogram.

Of note, strong association between high TILs and increased pCR rate was also observed in the TCHP group of HER2-positive BC, validating pre-NAC TIL levels as a persistent biomarker for response to current anti-HER2 treatment. Hence, our findings support the hypothesis that the pre-treatment host immune response may enhance the ability of chemotherapeutic agents to eliminate cancer cells regardless of molecular subtype, especially for HER2-positive BC with a NAC regimen. Our data also suggest pre-NAC TILs as a potential stratification parameter for newly targeted drug development in future clinical trials. Short duration of follow-up in patients of the TCHP group is a limitation in this study. As pertuzumab was just approved in 2013, we did not obtain enough events to analyze long-term efficacy of TILs. Future studies in larger cohorts with long-term follow-up are warranted.

Lastly, we demonstrated the prognostic impact of post-NAC TILs using univariable and multivariable analyses. The TN subtype especially had statistically significant correlation between post-NAC TILs and BCSS, but other subtypes also showed improved survival in post-NAC LPBC patients. Thus, our data suggest that post-NAC TILs should be considered as a stratification biomarker with which to identify a subset of patients most likely to benefit from NAC. In the present study, patients with both residual tumor burden and low post-NAC TILs presented a 32% 5-year BCSS. Therefore, this subset of patients could be considered to have a poorer response to chemotherapy.

In conclusion, this study reveals the predictive and prognostic value of pre- and post-NAC TILs. These findings apply not only to the well-established TN and HER2+ subtypes, but luminal A and B also. Pre-NAC TIL levels demonstrated a strong predictive impact for pCR, as well as a discriminative ability to identify a subgroup of patients characterized by particularly strong response to chemotherapy in any of the molecular subtypes. Additionally, findings suggest that post-NAC TILs could be a surrogate for long-term treatment efficacy in the neoadjuvant setting.

Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval 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 comparable ethical standards. For this type of study, formal consent is not required.

Informed consent Informed consent was obtained from all individual participants included in the study.

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