Predictive value of tumor-infiltrating lymphocytes for pathological response after neoadjuvant chemotherapy in breast cancer patients with axillary lymph node metastasis

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Abstract

Purpose We investigated the role of tumor-infiltrating lymphocytes (TILs) in pretreatment primary breast cancer to predict pathological response to neoadjuvant chemotherapy (NAC) in patients with clinical node-positive disease (cN+).

Methods The subjects of this study were 60 patients with cN+, who received NAC followed by breast surgery with axillary lymph node dissection (ALND). We conducted a semi-quantitative assessment of TILs in pretreatment primary tumors and their association with clinicopathological factors and axillary lymph node metastasis .

Results We observed a higher number of TILs in tumors with negative hormone receptors, positive human epidermal growth factor receptor 2, or high Ki67. TILs were associated with a favorable response to NAC in primary tumors. The rate of axillary pathologic complete response (Ax-pCR) was significantly higher in patients with a high number of TILs than in patients with a low number of TILs (72.0% versus 17.1%, $p < 0.001$). In multivariable analysis, a high number of TILs was a significant predictor of Ax-pCR as well as of pCR of the primary tnmor after NAC. Importantly, all patients with HER2 positive tumors in the high TILs group showed Ax-pCR on ALND.

Conclusion TILs in pretreatment primary breast cancer had the potential to predict therapeutic efficacy of NAC in patients with clinical node-positive disease.

Introduction

Neoadjuvant chemotherapy (NAC) is used widely in the treatment of invasive breast cancer. NAC has several potential advantages. First, it may reduce the risk of distant recurrence after breast surgery. Second, it may downstage the tumor; thereby, resulting in a better cosmetic outcome. Third, it may provide useful treatment response information. Another possible advantage of NAC is that it may eliminate axillary lymph node metastasis in patients with limited clinical node-positive disease (cN+), in whom unnecessary axillary lymph node dissection (ALND) should be avoided. However, ALND has been the standard surgical approach for patients with cN+, regardless of the response to NAC because accutate identification of axillary pathological complete response (Ax-pCR) to NAC is difficult.

Previous studies show that approximately 40–60% of patients with initial cN+ ultimately convert to a negative nodal status after NAC [1, 2]. In clinical tumor subtypes, a high rate of Ax-pCR has been achieved for human epidermal growth factor receptor 2-enriched breast cancer (HER2BC) or triple-negative breast cancer (TNBC) [3, 4]. Furthermore, various factors have been reported to predict axillary status. The generally accepted predictors are tumor size, lymphovascular invasion, histological grade, radiological response, the age of the patient, , and histological analysis performed by sentinel lymph node biopsy (SLNB) [5]. Other reported predictors are serum biomarkers like insulin-like growth factor 1 (IGF1) and vascular endothelial growth factor (VEGF) . Circulating IGF1 and VEGF levels may predict lymph

node metastasis and help in the decision to avoid ALND for patients with early-stage breast cancer [6]. However, the optimal predictor of Ax-pCR has not yet been established.

The efficacy of NAC can be influenced by the host immune system in breast cancer and other cancers [7, 8]. Previous studies have demonstrated the importance of tumor immunity in inducing the effects of chemotherapy [9, 10]. In fact, the number of tumor-infiltrating lymphocytes (TILs) has been identified as a useful novel biomarker for the therapeutic efficacy of NAC [9, 10]. However, to the best of our knowledge, no studies have addressed the association of pretreatment TILs with Ax-pCR after NAC. We hypothesized that unnecessary ALND can be avoided by using TILs as a predictive marker for Ax-pCR. We conducte this study to investigate the status of TILs in pretreatment primary breast cancer in association with pathological response to NAC in the axillary lymph nodes of patients with cN+.

Methods

Patient background

Between January, 2011 and December, 2018, 629 patients with clinical stage 1–3 breast cancer underwent breast cancer surgery at the Nara Medical University. Breast cancer was confirmed histologically by core needle biopsy (CNB) or vacuum-assisted biopsy (VAB) and staged with systemic imaging studies including ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI). TNM staging was evaluated according to the seventh edi-

tion of the American Committee on cancer staging [11]. According to the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki67, the breast cancers were categorized into the following immunophenotypes: luminal A (ER + and/or PgR + , HER2 − , Ki67<20%), luminal B/HER2- (ER+and/or PgR+, HER2 − , Ki67≥20%), luminal B/HER2+(ER+and/or PgR + , any Ki-67, HER2 +), HER2BC (HER2-enriched breast cancer; ER−, PgR−, and HER2+), and TNBC (negative for ER, PgR, and HER2) [12]. In this study, luminal A and luminal B/HER2- types were classified as hormone receptor-positive breast cancer (HRBC). Luminal B/HER2 + type was classified as hormone receptor- positive and HER2-enriched breast cancer (HR/HER2BC). Therefore, tumors were classified into four intrinsic sub- types: HRBC, HR/HER2BC, HER2BC, and TNBC.

A total of 65 patients with cN + were enrolled, but five were excluded because of incomplete pathological and clinical data (Fig. 1). We diagnosed cN + by US and MRI. By using dynamic contrast-enhanced MRI, the morphology of the lymph nodes was evaluated as either normal or abnormal: normal if the margins were well defined with a central, fatty hilum; and abnormal if the central fat was replaced or the margins were ill defined [13]. A short-axis diameter exceeding 10 mm or a round rather than oval shape (ratio of longest to shortest axes less than 1.5) was considered indicative of a metastatic lymph node [14, 15]. Diagnosis by US was based on real-time images. We evaluated the morpho- logical characteristics of the axillary lymph nodes, classifying them as suspicious for malignancy by diffuse cortical thickening > 3

mm, focal nodular thickening, multilobulated cortical thickening, or absence of the fatty hilum [16].

Lymph nodes with abnormal image findings were examined by fine needle aspiration cytology or CNB for the pathological diagnosis of metastasis. However, pathological diagnosis was omitted if multiple lymph nodes were fused or clinically evident that they were firmly fixed to the surroundings when metastasis was diagnosed by MRI and US. In this study, cN + was pathologically confirmed in 26 patients before NAC. All 60 patients with cN + received NAC, as the anthracycline regimen with epirubicin or doxorubicin and cyclophosphamide combined with a taxane-based regimen in 55. Patients with HER2BC also received targeted therapy with trastuzumab. Two patients with luminal B subtype received docetaxel combined with carboplatin regimen chemotherapy. For patients in poor physical condition or with heart disease, the docetaxel and cyclophosphamide regimen was chosen to minimize the toxicity of chemotherapy. Imaging examinations, including mammography, US, MRI, and CT, were performed to assess the effect of NAC on the primary tumor and axillary lymph node metastases. Therapeutic anti-tumor effects were evaluated according to the Response Evaluation Criteria for Solid Tumors [17]. Patients underwent mastectomy or breast-conserving surgery following NAC. We did not perform SLNB in these patients, all of whom received ALND. All pathology and histopathology analyses were performed using standard procedures for postoperative tissue to evaluate the pathological complete response (pCR) following the National Surgical Adjuvant Breast and Bowel Project B-18 protocol [18]. A pCR

was defined as no histological evidence of the infiltration of tumor cells in the breast and axillary tissues, including ductal carcinoma in situ.

Evaluation of TILs

We evaluated the TILs in primary tumors from biopsy specimens obtained by CNB or VAB. We measured the percent- age of area occupied by lymphocytes on the hematoxylin and eosin (H&E)-stained tumor section at the time of breast cancer diagnosis. Stromal TILs were evaluated according to recent recommendations of the International TILs Working Group 2014 [19]. Proportional scores were defined as 3, 2, 1, and 0 if the area of the stroma region with lymphoplasmacytic infiltration around the invasive tumor cell nests was > 50% (Fig. 2a), > 10–50% (Fig. 2b), ≤ 10% (Fig. 2c), and absent (Fig. 2d), respectively, according to a previous report [20]. Tumors with scores of 2 or 3 were in the high TILs group and those with scores of 0 or 1 were in the low TILs group [5, 20]. Two expert breast pathologists blindly evaluated TIL levels on hematoxylin and eosin-stained sections without any additional staining.

Statistical analysis

Categorical variables were compared using the Chi squared test (or Fisher's exact test when necessary). Logistic regression models were used to identify the predictors for pCR and Ax-pCR. Significance was set at $p < 0.05$ and statistical analysis was conducted using the JMP software package (SAS, Tokyo, Japan).

Results

Patient characteristics

The median age of the patients was 57 (range, 31–73) years. Before NAC, the median size of the 60 tumors was 40 (range, 13–150) mm, with 31 classified as HRBC, 5 as HR/ HER2BC, 12 as HER2BC, and 12 as TNBC. There were 25 patients (41.7%) in the high TILs group and 35 (58.3%) in the low TILs group at the time of breast cancer diagnosis. After NAC, clinical complete response (CR) of the primary tumor was observed in seven patients, partial response (PR) in 38 patients, stable disease (SD) in 12 patients, and progressive disease (PD) in three patients. We identified pCR in 13 patients (21.7%). In the evaluation of axillary lymph nodes, clinical CR was observed in 40 patients (66.7%); however, pathological examination revealed axillary metastasis on ALND in 36 of the 60 patients (60.0%; Table 1).

Correlations between clinicopathological features and TILs

We analyzed correlations of various clinicopathological features with TILs (Table 2). The rate of tumors without ER was significantly higher in the high TILs group than in the low TILs group. There were more tumors with HER2 and high Ki67 expression in the high TILs group than in the low TILs group and more HER2BC and TNBC tumors in the high TILs group than in the low group. Interestingly, pCR of the primary tumor was seen after NAC in 12 of the 25 (48.0%) high TILs group patients, but in only 1 of the 35 (2.9%) low TILs group patients.

Although there was no significant correlation between axillary clinical CR and TILs status, Ax-pCR was higher in the high TILs group than in the low group. Ax-pCR was seen after NAC in 18 of the 25 (72.0%) high TILs group patients, but in only 6 of the 35 (17.1%) low TILs group patients. There was no correlation between other clinicopathological factors and TILs.

Correlations between clinicopathological features and pathological axillary lymph nodes metastasis

We evaluated the correlation of clinicopathological features with metastasis in the axillary lymph nodes obtained by ALND (Table 2). Ax-pCR was observed more frequently in patients with T ($\leq$ 40 mm) and negative ER or HER2 primary tumors than in those with other tumors. Furthermore, both the clinical and pathological response of the primary tumor were positively associated with Ax-pCR. However, there was no correlation between clinical and pathological CR of the axillary lymph nodes. Of the 40 patients with a complete CR of the axillary lymph nodes after NAC, 21 (52.5%) had metastases. There were fewer axillary lymph node metastases in the high TIL group than in the low TIL group ($p < 0.001$).

Analysis of predictive factors for pCR of the primarytumor

HER2BC and a high number of TILs were significantly associated with the pCR of the primary tumor (Table 3). As no patient with an HRBC tumor showed pCR, these patients were excluded

from the analysis. Multivariate analysis also indicated that both HER2BC intrinsic subtype and a high number of TILs were significant independent predictors of pCR after NAC.

Analysis of predictive factors for Ax-pCR

We analyzed the predictors for Ax-pCR and found HRBC, HER2BC, and TILs status to be significantly associated with Ax-pCR (Table 4). Multivariate analysis revealed that only a high number of TILs was a significant independent predictor of Ax-pCR.

Conditions for predicting negative lymph node metastasis after NAC

Finally, we investigated the potential of TILs in the primary tumor before NAC to predict Ax-pCR. While 6 of 35 patients (17.1%) in the low TIL group had no axillary metastasis, 18 of 25 (72.0%) in the high TIL group had no axillary metastasis (Fig. 3). This difference was significant ($p < 0.001$). In particular, all patients with HER2 tumors in the high TIL group, regardless of hormone receptor status, were negative for metastasis on ALND.

Discussion

Early diagnostic modalities and effective treatments such as molecular-targeted therapy have improved the prognosis of breast cancer patients remarkably over the last few decades [21, 22]. As the survival rate associated with breast cancer increases, many patients suffer from long-term sequelae related to treatment, including fatigue, peripheral neuropathy, musculoskeletal

symptoms, osteoporosis, absence of menstrual periods, and menopausal symptoms. Lymphedema is also one of the most common side effects after breast cancer treatment. It is well recognized that ALND and adjuvant radiation therapy are major risk factors [23]. Clinical manifestations of lymphedema vary and include swelling, pain, discomfort, reduced joint dexterity, as well as increased infection risk [24]. Patients with lymphedema may also experience chronic and progressive swelling, recurrent skin infections, and low self-esteem and compromised quality of life [25]. Since ALND is a major risk for lymphedema, it should be avoided if feasible. SLNB is recommended for axillary staging for patients with early-stage or invasive breast cancer without clinically or pathologically positive lymph nodes [26]. It is known that patients undergoing SLNB have a low incidence of lymphedema since the procedure was developed to reduce the risk. Performing SLNB after NAC can identify the status of the axillary lymph nodes at the time of breast cancer surgery, whereby the axillary lymph node preservation rate might be improved. However, SLNB false-negative rates are higher after NAC in patients with positive axillary nodes at initial presentation than in patients with cN0. Before 2012, retrospective evaluations reported SLNB false-negative rates of more than 20% [27]. The prospective studies evaluated women with positive lymph nodes before NAC who had experienced clinical CR and who underwent SLNB and axillary dissection [28–30]. The resulting overall false-negative rates were 12.6–14.2%. The reason for this is that chemotherapy causes fibrosis, fat necrosis, and granulation tissue formation, which alters lymphatic

drainage patterns [31, 32]. To date, no factors or measures have been established to eliminate unnecessary ALND after NAC in patients with cN+.

In this study, we investigated the potential role of TILs in primary breast cancer before NAC in patients with cN + . First, a higher number of TILs was observed in tumors with negative hormone receptors, high Ki67, and HER2BC, suggesting that TILs in primary tumors before treatment may correlate with high malignancy of the breast cancer. In contrast, TILs were associated with the response to NAC. Patients with more TILs had a higher rate of pathological CR of both the primary tumors and axillary lymph nodes than those with fewer TILs. Importantly, multivariate analysis demonstrated that the TIL status was of significant value in predicting the complete response to NAC of both

the primary tumors and axillary lymph nodes. Data indicated that TILs in pretreatment primary tumors might be a good marker for NAC response. These findings were partly consistent with those of previous studies [33–36]. Second, the pretreatment evaluation of TILs in primary tumors may provide useful information about axillary lymph node positivity after NAC. Our data showed that the rate of Ax-pCR in patients with a higher number of TILs in primary tumors before NAC was significantly higher than that in patients with lower TILs (72.0% versus 17.1%, $p < 0.001$). Moreover, axillary metastasis was not found after NAC in any patients with HER2 tumors and a high number of TILs. A recent study using the large-scale national cancer database found that lymph node metastasis was rare in patients with cN0 HER2BC or TNBC with pCR of the primary tumor [37]. However, that study also found that patients with cN + ,

regardless of the hormone or HER2 status had consider- able lymph node metastasis with a 10–40% pathological positivity even if they had pCR of the primary tumor. In the present study, 60% of cN + patients had axillary metastasis on ALND. Furthermore, 1 of 13 pCRs of the primary tumor had axillary metastasis. Taken together, pCR of the primary tumor was not always associated with axillary metastasis, especially in patients with cN + . However, based on our data, we may consider omitting ALND in patients with HER2 and a high number of TILs in the pretreatment primary tumor. This strategy may be feasible and desirable, since the procedure is simple and less invasive for patients with cN + . Before introducing this treatment protocol, careful evaluation in a larger clinical setting will be needed. Moreover, SLNB should be considered to minimize the risk of recurrence and secure curability for patients with cN + . This should be evaluated simultaneously in future clinical studies.

Recent studies have shown breast cancer to be an immunogenic tumor [38], and TNBC and HER2BC are now considered subtypes with high immunoreactivity [39, 40]. Our data might be consistent with those studies. Furthermore, there are many studies evaluating the scoring of TILs as prognostic factors and effective predictors. Although there are few reports on its application in clinical practice, our study further highlights the potential of TILs to predict therapeutic efficacy, especially in a NAC setting. Immunotherapy such as targeting programmed death-1 or cytotoxic T lymphocyte-associated protein 4 is now used widely for various clinical malignancies. The combination of immunotherapy and conventional chemotherapy may enhance therapeutic efficacy; thereby inducing Ax-pCR, even in patients

with cN + , especially when a high number of TILs is identified in the pretreatment primary tumor.

The present study had several limitations. First, it was limited by its retrospective design and small number of sample cases. Second, the NAC treatment protocol varied, although recommended regimens were used according to guidelines. To the best of our knowledge, this is the first report to address the potential role of TILs in pretreatment primary tumors to predict Ax-pCR on ALND after NAC. For a definitive recommendation, a larger prospective study is needed to validate the results.

In conclusion, TILs in primary breast tumors are potential predictive markers for the therapeutic efficacy of NAC in patients with cN + . ALND after NAC may be omitted for patients with both the HER2 subtype and a high concentration of TILs.

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Compliance with ethical standards

Conflict of interest We have no conflicts of interest to declare with respect to the research, authorship, and publication of this article.

Ethics statement This study was conducted at the Nara Medical University, Nara, Japan. Sufficient explanation was provided, and written, informed consent was obtained from all study subjects for their involvement in this study and for the storage and use of their data. The study protocol was approved by the ethics committee of the Nara Medical University (No. 2386) in accordance with the Declaration of Helsinki.

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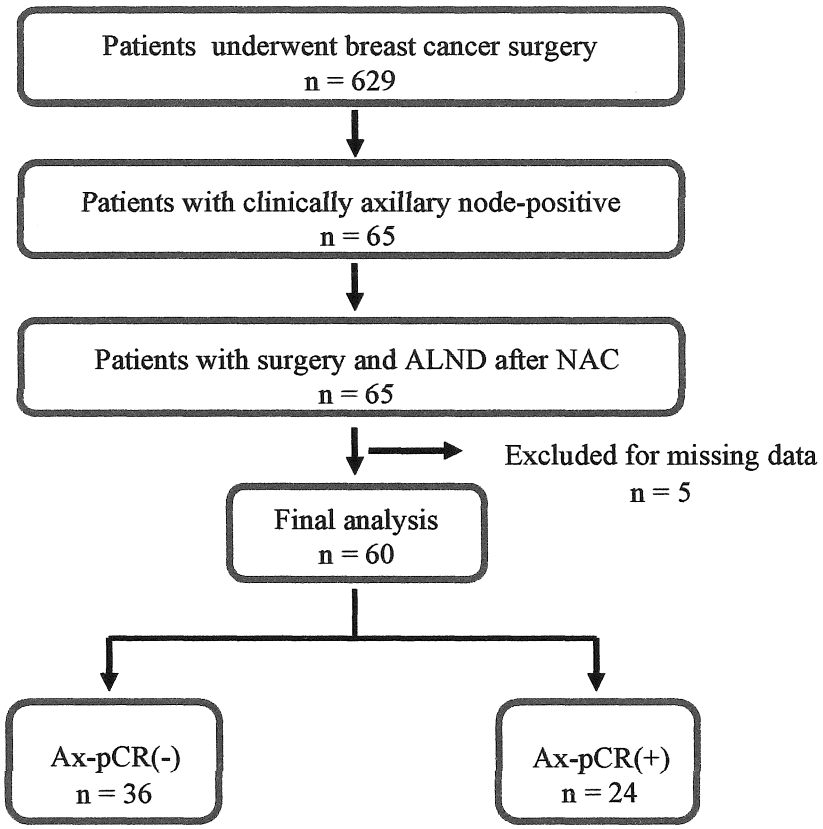
Figure Legends

Fig. 1 Flow diagram. Axillary lymph node dissection (ALND) was performed after neoadjuvant chemotherapy (NAC) in 65 patients with clinically axillary lymph node metastasis evaluated before NAC. The final analysis included 60 patients. Ax LN axillary lymph node metastasis

Fig. 2 Histopathological evaluation of tumor-infiltrating lymphocytes (TILs). TILs were evaluated in biopsy specimens of the primary tumor prior to treatment by measuring the percentage of area occupied by lymphocytes on the hematoxylin and eosin (H&E)-stained tumor section at the time of breast cancer diagnosis. The area of the stroma region with lymphoplasmacytic infiltration was > 50%, ≥ 10–50%, < 10%, or absent, and the corresponding score assigned was 3 (a), 2 (b), 1 (c), or 0 (d), respectively

Fig. 3 Association of tumor subtypes and axillary lymph node metastasis after neoadjuvant chemotherapy (NAC) was done according to the tumor- infiltrating lymphocytes (TILs) status. All patients with HER2- positive tumors in the high TILs group showed Ax-pCR on axillary lymph node dissection. HRBC hormone receptor-positive breast cancer, HR/HER2BC hormone receptor-positive and human epidermal growth factor receptor 2-enriched breast cancer, HER2BC HER2-enriched breast cancer, TNBC triple- negative breast cancer, Ax-pCR axillary pathological complete response



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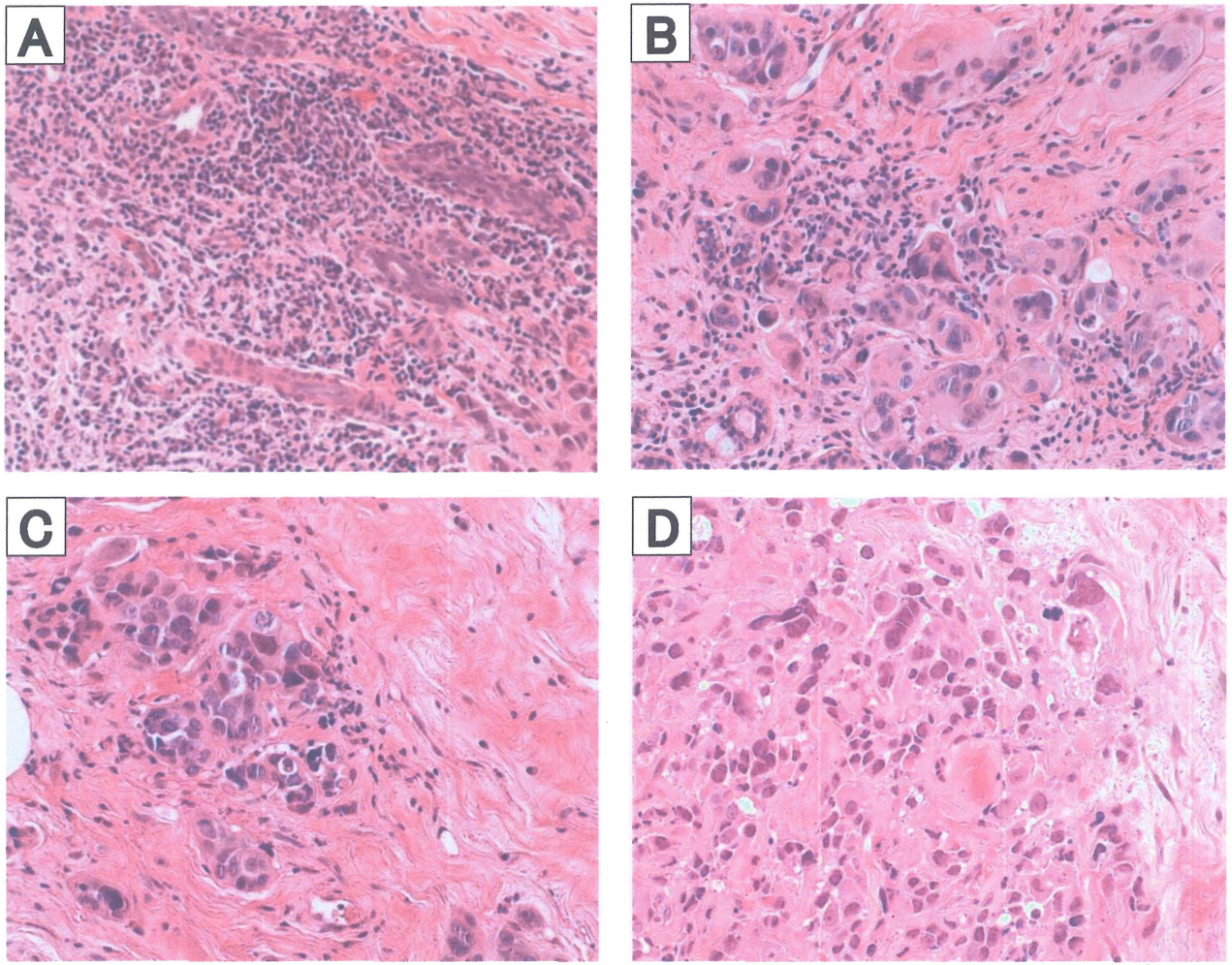

Fig. 2 Histopathological evaluation of TILs. TILs were evaluated on biopsy specimens of the primary tumor prior to treatment by measuring the percentage of area occupied by lymphocytes on the hematoxylin and eosin (H&E)-stained tumor section at the time of breast cancer diagnosis. The area of the stroma region with lymphoplasmacytic infiltration was >50%, ≥10–50%, <10%, or absent, and the corresponding score assigned was 3 (A), 2 (B), 1 (C), or 0 (D), respectively. *TILs* Tumor-infiltrating lymphocytes

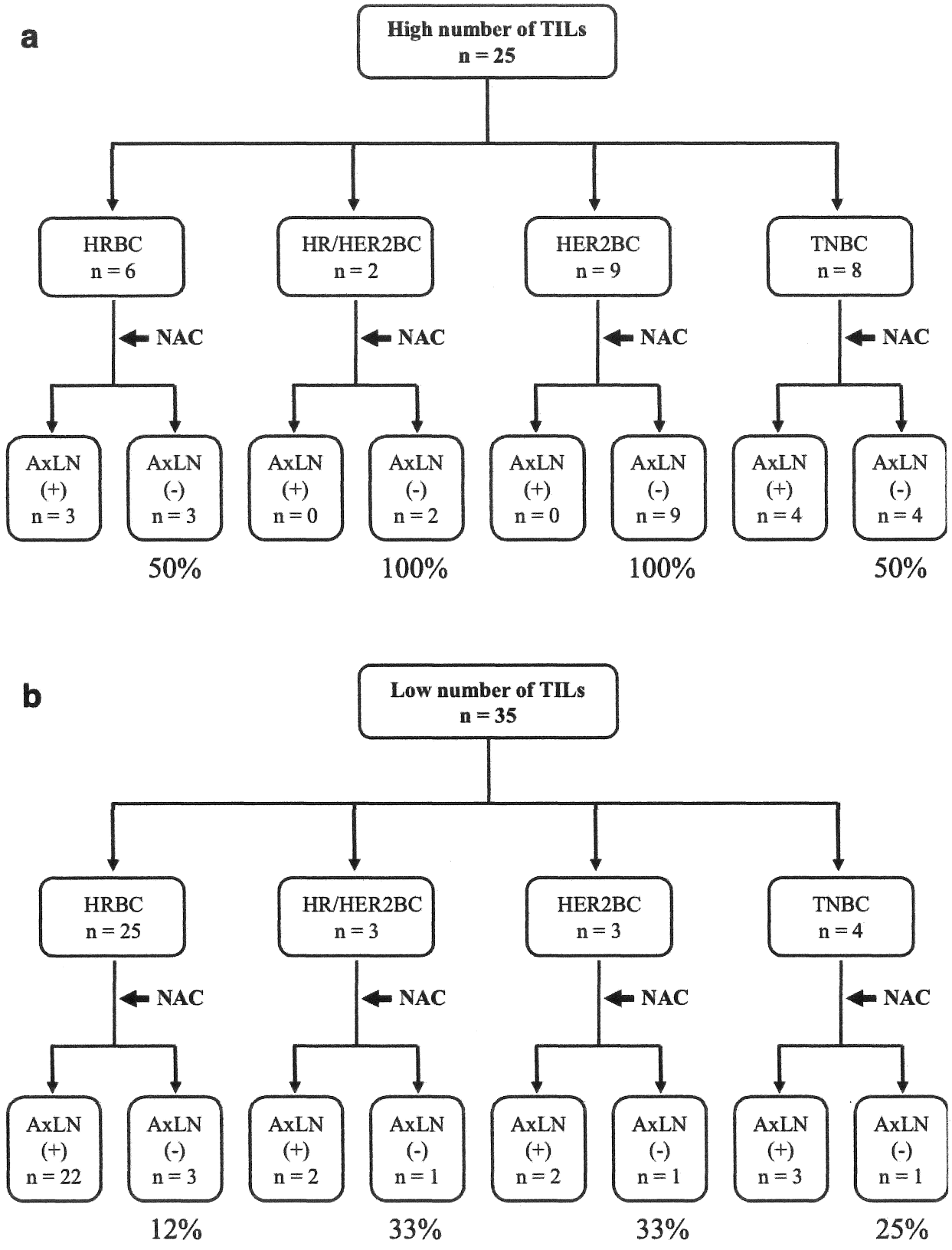

Fig. 3 Association of tumor subtypes and axillary lymph node metastasis after neoadjuvant chemotherapy (NAC) was done according to the tumor- infiltrating lymphocytes (TILs) status. All patients with HER2-positive tumors in the high TILs group showed Ax-pCR on axillary lymph node dissection. HRBC hormone receptor-positive breast cancer, HR/HER2BC hormone receptor-positive and human epidermal growth factor receptor 2-enriched breast cancer, HER2BC HER2-enriched breast cancer, TNBC triple- negative breast cancer, Ax-pCR axillary pathological complete response

Table 1 Statistical data of 60 patients at the time of surgery

Parameters (n = 60)	Number of patients (%)
Age (years old)	57 (31 - 73)
Tumor size (mm)	40 (13 - 150)
Estrogen receptor	
positive	36 (60.0)
negative	24 (40.0)
HER2	
positive	17 (28.3)
negative	43 (71.7)
Ki67	
< 20 %	18 (30.0)
≥ 20 %	42 (70.0)
Intrinsic subtype	
HRBC	31 (51.7)
HR/HER2BC	5 (8.3)
HER2BC	12 (20.0)
TNBC	12 (20.0)
TILs	
high	25 (41.7)
low	35 (58.3)
Clinical complete response of primary tumor	
cCR	7 (11.7)
non-cCR	53 (88.3)
Pathological complete response of primary tumor	
pCR	13 (21.7)
non-pCR	47 (78.3)
Clinical complete response of axillary lymph nodes	
Ax-cCR	40 (66.7)
non-Ax-cCR	20 (33.3)
Pathological complete response of axillary lymph nodes	
Ax-pCR	24 (40.0)
non-Ax-pCR	36 (60.0)

HER2 human epidermal growth factor receptor 2, *HRBC* hormone receptor-positive breast cancer, *HR/HER2BC* hormone receptor-positive and HER2-enriched breast cancer, *HER2BC* HER2-enriched breast cancer, *TNBC* triple negative breast cancer, *TILs* Tumor-infiltrating lymphocytes, *cCR* clinical complete response, *pCR* pathological complete response, *Ax-cCR* axillary clinical complete response *Ax-pCR* axillary pathological complete response

Table 2 Correlations between clinicopathological features and the number of TILs or axillary lymph node metastasis

Parameters	TILs		*p* value	Axillary lymph node		*p* value
	High (n = 25)	Low (n = 35)		Ax-pCR (n = 24)	non Ax-pCR (n = 36)	
Age						
≤ 57	12	18		12	19	
> 57	13	17	1.000	12	17	1.000
Tumor size (mm)						
≤ 40	17	18		18	17	
> 40	8	17	0.289	6	19	0.038
Estrogen receptor						
Positive	8	28		9	27	
Negative	17	7	<0.001	15	9	0.007
HER2						
Positive	11	6		13	4	
Negative	14	29	0.040	11	32	<0.001
Ki67						
< 20 %	3	15		5	13	
≥ 20 %	22	20	0.012	19	23	0.258
Intrinsic subtype						
HRBC	6	25		6	25	
Non-HRBC	19	10	0.001	18	11	0.001
HR/HER2BC	2	3		3	2	
Non-HR/HER2BC	23	32	1.000	21	34	0.380
HR/HER2BC	2	3		3	2	
Non-HR/HER2BC	23	32	1.000	21	34	0.380
HER2BC	9	3		10	2	
Non-HER2BC	16	32	0.019	14	34	<0.001
TNBC	8	4		5	7	
Non-TNBC	17	31	0.099	19	29	1.000
Clinical complete response						
cCR	5	2		6	1	
Non-cCR	20	33	0.117	18	35	0.013
Pathological complete response						
pCR	12	1		12	1	
Non-pCR	13	34	< 0.001	12	35	<0.001
Clinical complete response of axillary lymph nodes						
Ax-cCR	18	22		19	21	
non Ax-cCR	7	13	0.581	5	15	0.161
Pathological complete response of axillary lymph nodes						
Ax-pCR	18	6		-	-	
non Ax-pCR	7	29	< 0.001	-	-	-
TILs						
High	-	-		18	7	
Low	-	-	-	6	29	<0.001

TILs Tumor-infiltrating lymphocytes, *Ax-pCR* axillary pathological complete response, *HER2* human epidermal growth factor receptor 2, *HRBC* hormone receptor-positive breast cancer, *HR/HER2BC* hormone receptor-positive and HER2-enriched breast cancer, *HER2BC* HER2-enriched breast cancer, *TNBC* triple negative breast cancer, *cCR* clinical complete response, *pCR* pathological complete response, *Ax-cCR* axillary clinical complete response

Table 3 Univariate and multivariate analysis of factors predicting pCR of the primary tumor after NAC

	Univariate		Multivariate	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Tumor size (≤ 40)	4.84 (0.966 - 24.260)	0.055		
Ki67 (≥ 20 %)	1.563 (0.374 - 6.520)	0.540		
HRBC	-	-		
HR/HER2BC	2.667 (0.396 - 17.959)	0.314		
HER2BC	17.2 (3.777 - 78.326)	< 0.001	12.742 (2.013 - 80.656)	0.007
TNBC	1.267 (0.2887 - 5.5687)	0.754		
TILs (high)	31.385 (3.701 - 266.157)	0.002	24.799 (2.536 - 242.474)	0.006

pCR pathological complete response, *CI* confidence interval, *HRBC* hormone receptor-positive breast cancer, *HER2* human epidermal growth factor receptor 2, *HR/HER2BC* hormone receptor-positive and HER2-enriched breast cancer, *HER2BC* HER2-enriched breast cancer, *TNBC* triple negative breast cancer, *TILs* Tumor-infiltrating lymphocytes,

Table 4 Univariate and multivariate analysis of factors predicting Ax-pCR after NAC

	Univariate		Multivariate	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Tumor size (≤ 40)	3 (0.968 - 9.302)	0.057		
Ki67 (≥ 20 %)	2.148 (0.649 - 7.110)	0.211		
HRBC	0.147 (0.046 - 0.470)	0.001	0.542 (0.120 - 2.447)	0.426
HR/HER2BC	2.429 (0.374 - 15.758)	0.352		
HER2BC	12.143 (2.354 - 62.649)	0.003	5.683 (0.779 - 41.471)	0.087
TNBC	1.090 (0.302 - 3.942)	0.895		
TILs (high)	12.429 (3.601 - 42.902)	< 0.001	8.153 (2.062 - 32.233)	0.003

Ax-pCR axillary pathological complete response, *CI* confidence interval, *HRBC* hormone receptor-positive breast cancer, *HER2* human epidermal growth factor receptor 2, *HR/HER2BC* hormone receptor-positive and HER2-enriched breast cancer, *HER2BC* HER2-enriched breast cancer, *TNBC* triple negative breast cancer, *TILs* Tumor-infiltrating lymphocytes