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Original Research

Predictive and prognostic value of stromal tumour-infiltrating lymphocytes before and after neoadjuvant therapy in triple negative and HER2-positive breast cancer

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Abstract ***Aim:*** Lymphocyte predominant breast cancer (BC) is associated with higher pathological complete response (pCR) rate after neoadjuvant therapy (NAT) and favorable outcome in triple negative breast cancer (TNBC) and HER2+ BC. The predictive and prognostic impact of stromal tumour-infiltrating lymphocytes (TILs) after NAT and the change of TILs before (pre-) and after (post-) NAT are not well studied. We aimed to assess the predictive and prognostic value of pre- and post-NAT TILs, as well as their pharmacodynamics modulation and their change for TNBC and HER2+ BC.
Materials and methods: Two-hundred and nine consecutive patients (n = 80 TNBC, n = 129 HER2+ BC) who received NAT between 2001 and 2009 in a single institution were included.

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lymphocytes

We evaluated the association between pre-NAT TILs and pCR, and the association between pre- and post-NAT TILs, as well as their immunodynamics change with relapse-free survival (RFS) for patients with residual disease (RD).

Results: Low pre-NAT TILs compared to int/high were significantly associated with lower pCR rate (TNBC: 4.0% vs 43.6%; HER2+ BC: 26.0% vs 51.9%). The median follow-up period was 98 months. In TNBC with RD, low pre-NAT TILs showed significant association with shorter RFS (HR = 3.844 [1.190–12.421], p = 0.024) in multivariate analysis. Low post-NAT TILs showed borderline significant association with shorter RFS (HR = 2.836 [0.951–8.457], p = 0.061). The change in TILs was not associated with RFS. In HER2+ BC, low pre-NAT TILs were not associated with RFS.

Conclusion: In TN and HER2+ BCs, low pre-NAT TILs tumours had a low likelihood of achieving pCR. In TNBC with RD, both low pre- and post-NAT TILs were associated with shorter RFS. These results suggest that TILs information should be taken into account when additional therapies may be given in the post-neoadjuvant setting.

1. Introduction

Tumour-infiltrating lymphocytes (TILs) are mononuclear immune cells, which infiltrate tumour tissue and reflect an individual immunological response. Although BC has not been traditionally considered to be immunogenic, the association between the presence of TILs and outcome has been widely investigated in breast cancer (BC) [1]. The extent of TILs and their association with outcome vary in different BC subtypes [2,3]. Overall, TILs are more frequently observed in highly proliferative tumours (e.g. TNBC and HER2+ BC), and, in these tumours, TILs are usually considered to predict better outcome [4,5]. Also, the capability of TILs to predict the benefit from some chemotherapeutic and targeted agents has been investigated. However, the results are still somewhat conflicting [3].

The achievement of pathological complete response (pCR) after NAT correlates with good outcome and may be considered as a surrogate marker for new drugs development [6–8]. In addition to some clinicopathological factors, TILs are associated with high pCR rate and favourable outcome [9–11]. In general, residual disease (RD) after NAT is associated with poor prognosis (7). However, not all patients with RD are going to eventually relapse. It has been reported that the extent of TILs in RD after NAT is associated with better prognosis in patients with TNBC [12]. It is worth mentioning that a high conversion rate from TILs-negative to TILs-positive has been observed from core biopsies before NAT and samples obtained at surgery after NAT(12). A four-gene signature from pre-treatment samples predicts high levels of TILs after NAT and good outcome in patients with TNBC, thus adding prognostic information to a clinicopathological model at diagnosis [13]. The predictive and prognostic impact of TILs on RD after NAT and the change in the extent of TILs before and after NAT deserve further investigation. In this study, we aimed to assess the predictive and prognostic value of TILs before and after NAT and their change over time in early BC.

2. Materials and methods

2.1. Patients

209 consecutive primary BC patients, including 80 TNBC, 129 HER2+ BC patients, who received NAT and underwent surgery between 2001 and 2009 were included in this study. Bilateral breast cancer, multifocal breast cancer, and Stage IV breast cancer at diagnosis were excluded.

For NAT, anthracycline and taxane based regimens were mainly used. Trastuzumab was used for HER2+ BC after its approval for use as NAT. Radiotherapy and endocrine therapy were also given, if indicated. We evaluated all available clinicopathological information, including age, tumour size, nuclear grade, nodal status, response to NAT. The need for written informed consent was waived by the Institutional Review Board because of the retrospective nature of this study. All specimens were collected in accordance to a protocol approved by the Institutional Review Board.

2.2. Pathological assessment

ER and progesterone receptor (PR) expression were measured by immunohistochemical staining (IHC) and scored by the Allred method [14]. Allred scores are based on the proportion and intensity of tumour cells. The thresholds for ER and PR positivity were set at 3 or greater. HER2 expression was assessed by IHC and its score was calculated according to the American Society of Clinical Oncology/College of American Pathologists guidelines [15]. Positive status was defined 3 + score by IHC or HER2 amplification by fluorescent in situ

hybridisation. pCR was defined as the absence of residual invasive tumour cells in the breast and axillary lymph nodes (ypT0/is, ypN0).

2.3. Assessment of TILs

TILs were assessed on pre-treatment biopsy samples (pre-NAT TILs) for all patients and on surgical specimens (post-NAT TILs) for patients who did not achieve pCR. TILs were evaluated on haematoxylin and eosin (H&E) sections originally sampled from each invasive cancer, following the recommendation of the international TILs Working Group [2]. The area for TILs evaluation was defined as within the borders of the invasive tumours, and immune infiltrates in adjacent normal tissue. All stromal mononuclear cells that did not directly contact carcinoma cells were scored as TILs. TILs were categorised as Low; 0–9%, Intermediate (Int); 10–49%, and High (LPBC; ≧50%), respectively. Change in TILs was calculated as the difference from post-NAT TILs counts to pre-NAT TILs counts and categorised into increase higher than 10%, stable less or equal to 10%, decrease higher than 10%. For analysis of prognosis, change in TILs was classified into two groups: increase (+1～+99), decrease (−99–0) according to their differences. Two experienced pathologists who were blinded to clinical outcomes reviewed and evaluated TILs.

2.4. Statistical methods

The Chi-square test was used for comparisons of continuous variables. A change in TILs level was assessed using a paired-t test. Relapse-free survival (RFS) was defined as the period from diagnosis to locoregional recurrence, distant metastasis, or death from any cause. The follow-up period was set within 120 months. Patients who did not experience such events were censored at the date of the latest follow-up. Survival curves were drawn using the Kaplan–Meier method and the log-rank test was applied for comparisons of survival curves. Hazard ratio was examined by Cox proportion hazards model. All statistical analyses were performed using SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). All *p*-value were two-sided and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Patient characteristics

Baseline characteristics and treatment are summarised in Table 1. Median age was 52 years (range 27–75 years). Of the 129 HER2+ BC patients, 91 (70.5%) received trastuzumab. 25 TNBC patients (31.3%), 54 HER2+ BC patients (41.9%) achieved pCR.

Table 1
Baseline characteristics (n = 209).

Characteristic	TNBC (n = 80)	HER2+ BC (n = 129)
Age (years)		
Median (range)	52 (27–75)	52 (28–74)
≦50	35 (43.8%)	54 (41.9%)
> 50	45 (56.2%)	75 (58.1%)
Nuclear grade		
1	10 (12.5%)	32 (24.8%)
2	21 (26.3%)	47 (36.4%)
3	49 (61.3%)	50 (38.8%)
Pre-NAT TILs		
Low	25 (31.3%)	50 (38.8%)
Intermediate/high	55 (68.7%)	79 (61.2%)
Post-NAT TILs (in RD)		
Low	23/55 (41.8%)	50/75 (66.7%)
Intermediate/high	32/55 (58.2%)	25/75 (33.3%)
Tumour size (post-NAT)		
ypT0/ypTis	26 (32.6%)	56 (43.4%)
ypT1	31 (38.8%)	44 (34.1%)
ypT2	22 (27.5%)	23 (17.8%)
ypT3	1 (1.3%)	4 (3.1%)
ypT4	0	2 (1.6%)
Lymph node status (post-NAT)		
Positive	14 (17.5%)	24 (18.6%)
Negative	66 (82.5%)	105 (81.4%)
Response to NAT		
pCR	25 (31.3%)	54 (41.9%)
RD	55 (68.7%)	75 (58.1%)
Recurrence		
Yes	23 (28.7%)	21 (16.3%)
No	57 (71.3%)	108 (83.7%)

TNBC, triple negative breast cancer; BC, breast cancer; NAT, neoadjuvant therapy; TILs, tumour-infiltrating lymphocytes; RD, residual disease; pCR, pathological complete response.

3.2. Predictive value of TILs before NAT

25/80 (31.3%) TNBC, 50/129 (38.8%) HER2+ BC had low pre-NAT TILs. For TNBC, pCR rates were 4.0% (1/25) in low pre-NAT TILs and 43.6% (24/55) in int/high pre-NAT TILs on biopsy specimen ($p < 0.001$) (Table 2). 13 out of 50 (26.0%) low pre-NAT TILs group and 41 out of 79 (51.9%) int/high pre-NAT TILs group among HER2+ BC patients achieved pCR (26.0%) ($p = 0.004$). For each subtype, low pre-NAT TILs were significantly associated with low pCR rate as compared to int/high pre-NAT TILs.

Table 2
Association of TILs with pCR rate in patients with TNBC and HER2-positive breast cancer.

TNBC	Non-pCR	pCR	p value
TILs low	24 (96.0%)	1 (4.0%)	< 0.001
TILs intermediate/high	31 (56.4%)	24 (43.6%)	
HER2+ BC			
TILs low	37 (74.0%)	13 (26.0%)	0.004
TILs intermediate/high	38 (48.1%)	41 (51.9%)	

pCR, pathological complete response; TNBC, triple negative breast cancer; TILs, tumour-infiltrating lymphocytes; BC, breast cancer.

3.3. Prognostic value of TILs before and after NAT

23/55 (41.8%) TNBC and 50/75 (66.7%) HER2+ BC had low post-NAT TILs. At a median follow-up of 98 months (range: 2–120 months), 23 TNBC (28.7%) and 21 HER2+ BC (16.3%) relapsed (Table 1).

For TNBC, low pre-NAT TILs were significantly associated with shorter RFS period as compared to int/high pre-NAT TILs ($p < 0.001$, Fig. 1a). In patients with RD, both pre- and post-NAT low TILs were significantly associated with shorter RFS period as compared to int/high TILs ($p = 0.001$, and 0.003 respectively, Fig. 1b and c). For HER2+ BC, low pre-NAT TILs were not associated with RFS ($p = 0.385$) (Fig. 2a). In patients with RD, both pre- and post-NAT low TILs were not associated with RFS ($p = 0.937$, and 0.441, respectively) (Fig. 2b and c).

3.4. Change in TILs level after NAT in patients with RD

Among TNBC, TILs level was increased in 7 (12.7%) patients, did not change in 39 patients (70.9%), and was decreased in 9 patients (16.4%) ($p = 0.430$) (Fig. 3a). Among HER2+ BC patients, 3 (4.0%) had increased TILs level, 57 (76.0%) had no change, and 15 (20.0%) had decreased TILs level ($p < 0.001$) (Fig. 3b).

In TNBC patients with RD, change in TILs was not significantly associated with RFS (increase; 30.9%, decrease; 69.1%, $p = 0.163$, Fig. 4). Among low pre-NAT TILs group, low post-NAT TILs had a trend towards shorter RFS period as compared to int/high post-NAT TILs ($p = 0.110$) (Fig. 5a). In contrast, in int/high pre-NAT TILs group, low post-NAT TILs were not significantly associated with shorter RFS period ($p = 0.246$) (Fig. 5b). For HER2+ BC, there was no statistical difference with respect to RFS because of low incidence of increased TILs level.

3.5. Univariate and multivariate analysis for RFS in patients with RD

In univariate analysis, low pre- and post-NAT TILs were significantly associated with shorter RFS period for patients with TNBC and RD (Table 3). The risk of

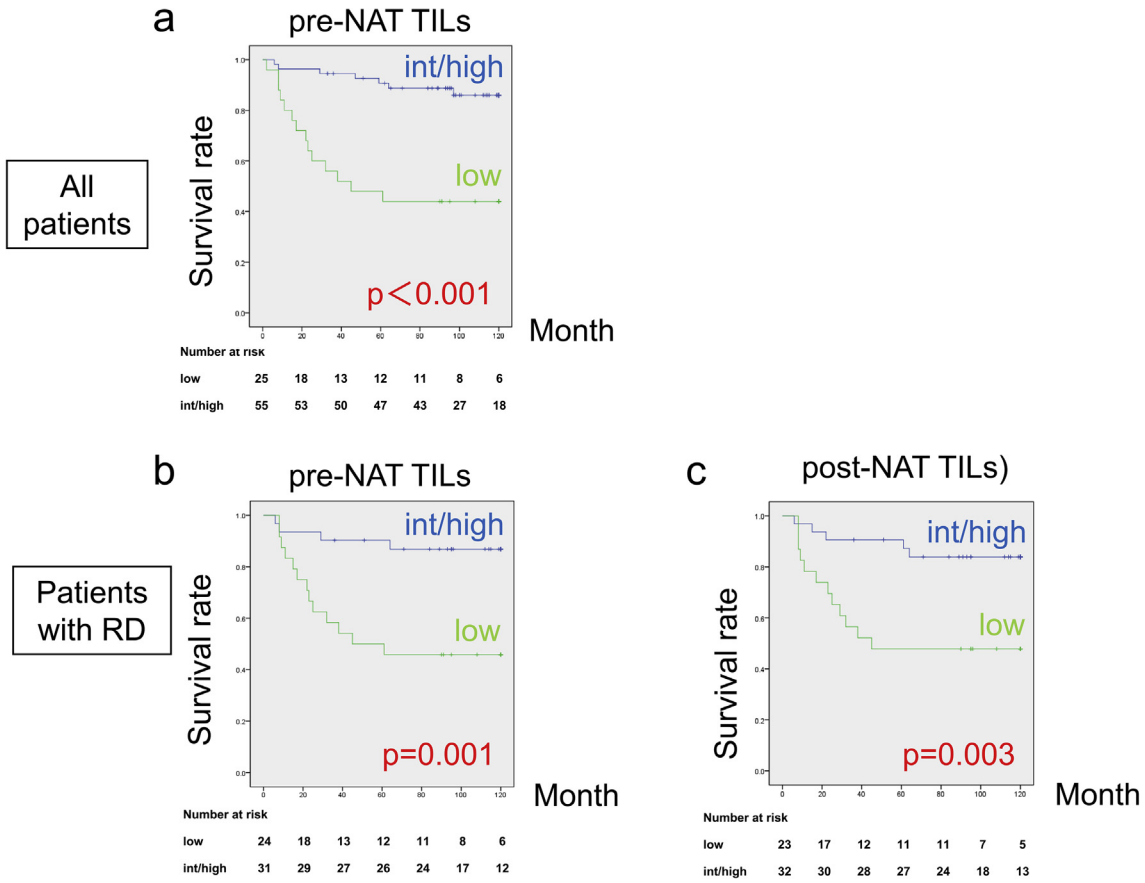

Fig. 1. The association of TILs with RFS in TNBC patients: (a) low pre-NAT TILs were significantly associated with a shorter RFS period as compared to int/high pre-NAT TILs in 80 TNBC patients ($p < 0.001$), (b) pre-NAT low TILs were significantly associated with shorter RFS period as compared to int/high TILs in patients with RD ($p = 0.001$) and (c) post-NAT low TILs were significantly associated with shorter RFS period as compared to int/high TILs in patients with RD ($p = 0.003$). TILs, tumour-infiltrating lymphocytes; RFS, relapse-free survival; NAT, neoadjuvant therapy; TNBC, triple negative breast cancer; RD, residual disease.

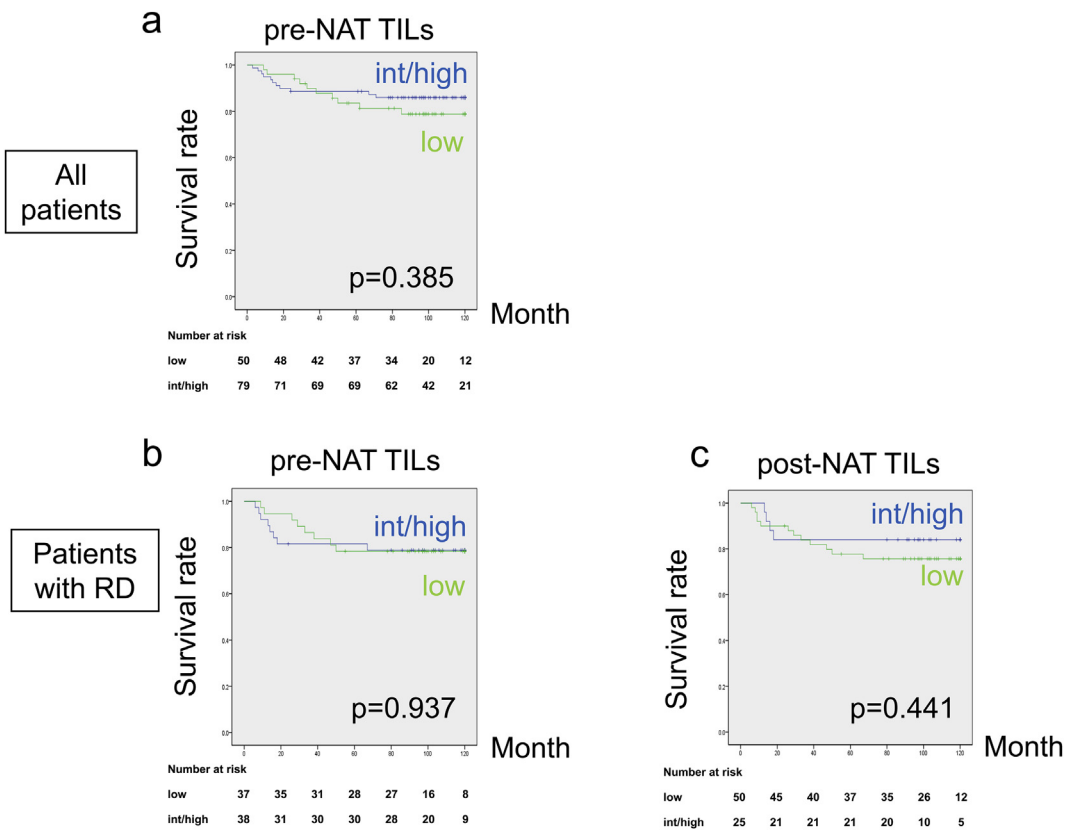

Fig. 2. The association of TILs with RFS in TNBC patients: (a) low pre-NAT TILs were not associated with RFS as compared to int/high pre-NAT TILs in 129 HER2+ BC patients (p = 0.385), (b) pre-NAT low TILs were not associated with RFS in 75 HER2+ BC patients with RD (p = 0.937) and (c) post-NAT low TILs were not associated with RFS in 75 HER2+ BC patients with RD (p = 0.441). TILs, tumour-infiltrating lymphocytes; RFS, relapse-free survival; NAT, neoadjuvant therapy; TNBC, triple negative breast cancer; RD, residual disease; BC, breast cancer.

recurrence was higher in low pre-NAT TILs (HR = 5.333 [1.731–16.427], p = 0.004), and in low post-NAT TILs (HR = 4.271 [1.498–12.173], p = 0.007). Change in TILs, age, and nodal status were not significantly associated with risk of recurrence. Age and pathological nodal status were not associated with RFS. In multivariate analysis including both pre- and post-NAT TILs, only pre-NAT TILs retained significance (HR = 3.844 [1.190–12.421], p = 0.024). Low post-NAT TILs showed a borderline significant association with shorter RFS period (HR = 2.836 [0.951–8.457], p = 0.061), suggesting that both pre- and post-NAT TILs might provide independent prognostic information. For HER2+ BC, these clinicopathological factors including TILs were not associated with RFS.

4. Discussion

In TNBC and HER2+ BC, low TILs were associated with low pCR rate. Low pre-NAT TILs showed significant association with shorter RFS period and low post-NAT TILs showed a similar trend in TNBC. Post-NAT TILs, in addition to pre-NAT TILs, provide independent prognostic information that might help identifying those patients who might require additional therapies.

The predictive value of low pre-NAT TILs was concordant with previous studies [10,11,16,17]. We also analysed RD to assess the prognostic impact of post-NAT TILs. Among TNBC with RD, both pre- and post-NAT low TILs were independently associated with poor prognosis. TILs evaluated in the RD after exposure to chemotherapy could carry additional prognostic information because they may partly reflect the reaction of the immune microenvironment to chemotherapy [18]. Our results are superimposable to those reported by another publication in which the presence of TILs in RD after NAT was associated with better prognosis in TNBC patients [19]. In HER2+ BC, pre- and post-NAT low TILs did not statistically affect the RFS of patients with RD. As previously reported, the clinical value of TILs differs between TNBC and HER2+ BC [20,21].

In this study, we also assessed the change between pre- and post-NAT TILs in patients with RD. While TNBC showed various change in TILs level, an increase in TILs higher than 10% was rare in HER2+ BC. Few studies have examined TILs change after NAT.

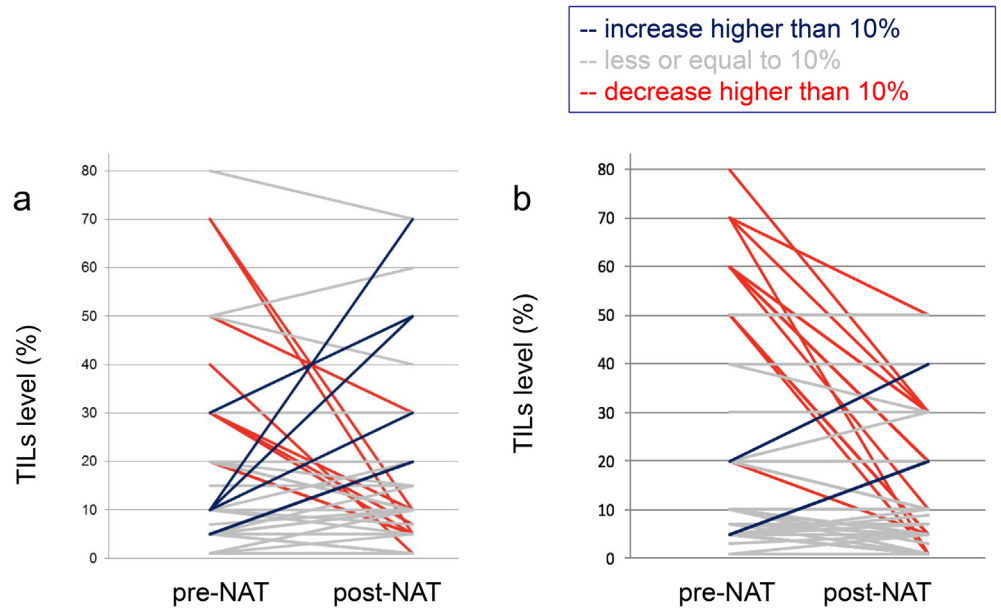

Fig. 3. Change of TILs level between pre-NAT and post-NAT samples: (a) in 55 TNBC with RD, increase in 7, no change in 39 and decrease in 9 ($p = 0.430$) and (b) in 75 HER2+ BC with RD, increase in 3, no change in 57 and decrease in 15 ($p < 0.001$). NAT, neoadjuvant therapy; TILs, tumour-infiltrating lymphocytes; TNBC, triple negative breast cancer; RD, residual disease; BC, breast cancer.

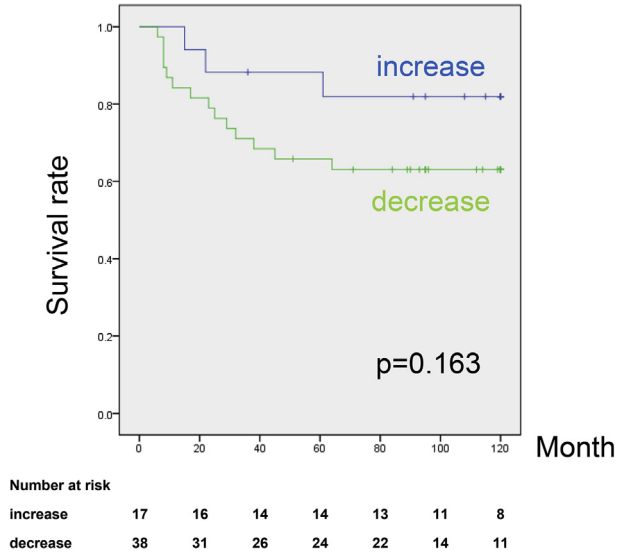

Fig. 4. The association of change of TILs level between pre- and post-NAT samples with RFS rate in 55 TNBC patients with RD (increase [n = 17], 30.9%; decrease [n = 38], 69.1%, p = 0.163). RFS, relapse-free survival; NAT, neoadjuvant therapy; TILs, tumour-infiltrating lymphocytes; TNBC, triple negative breast cancer; RD, residual disease.

Particularly, TILs change in TNBC has not been reported. Hamy et al reported that TILs level decreased in 78% of patients with HER2+ BC, and the magnitude of the decrease was positively associated with pCR [22]. According to the association between TILs change and prognosis, Pelekanou V *et al.* assessed TILs in pre-treatment and RD samples from 58 patients who were treated with NAT, including 79.3% of HR + breast cancer [23]. They reported that increase of TILs in RD after NAT was associated with longer RFS. In the present study, increased TILs did not statistically lead to longer RFS in TNBC (p = 0.163), while we could not assess the association of TILs change in HER2+ BC as the cases with increased TILs were rare.

We also showed that TNBC patients with low pre-NAT and post-NAT TILs had high risk of recurrence. For such cases, new treatment strategies with additional drugs, such as adjuvant capecitabine may be useful (CREATE-X trial) [24]. The evaluation of TIL before and after NAT may help selecting those patients in need of further treatments, who may benefit by the addition of novel immunostimulatory treatments within clinical trials.

We acknowledge that this study has some limitations. First of all, its retrospective design and the relatively small number of patients included. Therefore, some results including no statistical difference of prognosis according to TILs level might be affected. Second, over 98 months of observation, only 44 patients had recurrence. Third, 30% of HER2+ BC patients did not receive trastuzumab, which may have significantly affected the results and would anyway describe a different population as compared to the actual one [25,26]. Fourth, HER2+ BC patients contained various degrees of Allred score in ER status, thus possibly affecting the response to NAT. However, TILs is a predictive and prognostic factor, which may be integrated with other clinicopathological variables to further improve treatment, and therefore outcome.

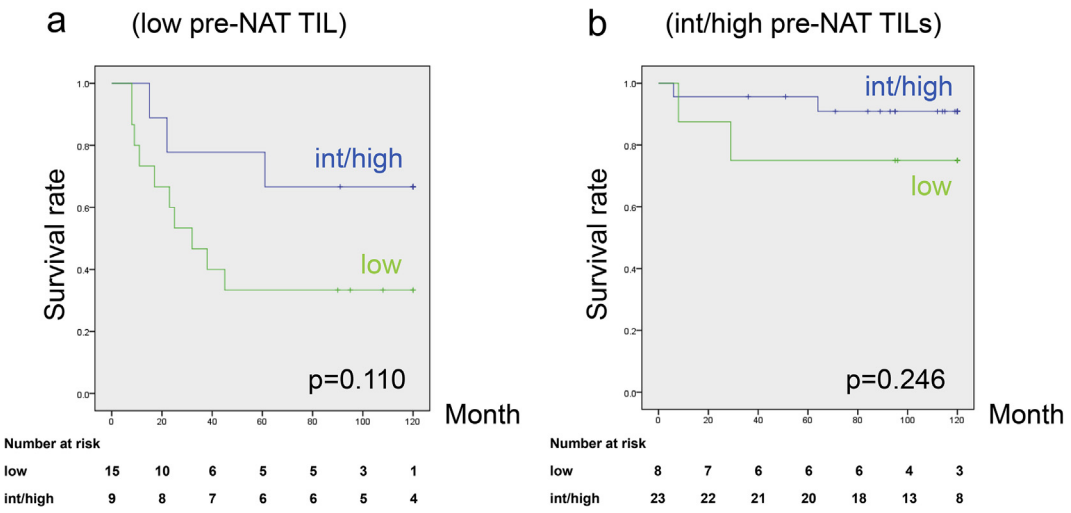

Fig. 5. The prognostic value of post-NAT TILs according to pre-NAT TIL level in 55 TNBC with RD: (a) among 24 patients with low pre-NAT TILs, low post-NAT TILs had a trend towards shorter RFS period as compared to int/high post-NAT TILs (p = 0.110); (b) among 31 patients with int/high pre-NAT TILs, low post-NAT TILs were not associated with shorter RFS period (p = 0.246). NAT, neoadjuvant therapy; TILs, tumour-infiltrating lymphocytes; TNBC, triple negative breast cancer; RD, residual disease; RFS, relapse-free survival.

Table 3
Association of TILs with relapse-free survival in patients with TNBC and RD.

Factor	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p value	HR (95% CI)	p value
Pre-NAT TILs low vs int/high	5.333 (1.731–16.427)	0.004	3.844 (1.190–12.421)	0.024
Post-NAT TILs low vs int/high	4.271 (1.498–12.173)	0.007	2.836 (0.951–8.457)	0.061
Change of TILs decrease/equal vs increase	2.361 (0.678–8.218)	0.177	–	–
Age ≦50 vs >50	1.681 (0.640–4.417)	0.292	–	–
Nodal status pN + vs pN0	1.548 (0.544–4.408)	0.413	–	–

TILs, tumour-infiltrating lymphocytes; NAT, neoadjuvant therapy; RD, residual disease; TNBC, triple negative breast cancer; HR, hazard ratio; CI, confidence interval.

5. Conclusions

We have demonstrated that TILs before and after NAT is a predictive and prognostic factor in TNBC, and predictive in HER2+ BC. These results suggest that TILs level should be taken into account as a stratification factor when additional adjuvant treatments are considered in patients with RD.

Conflict of interest statement

There is no conflict of interest to declare for this study.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee.

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