Outcome Prediction after Neoadjuvant Chemotherapy (NAC) for Breast Cancer, using Tumor-Infiltrating Lymphocytes within Fibrotic Foci of Tumor Stroma (FF-TILs)

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Research

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Abstract

Background: Tumor-infiltrating lymphocytes (TILs), which are indicators for monitoring an immune response, are generally mononuclear immunocytes that aggregate with tumors and are thought to have a close relationship with cancer cells. On the other hand, a fibrotic focus (FF) within the stroma of a tumor is a histological formation that plays an important role in the cancer microenvironment with regard to proliferation and development. Here, we focus on TILs that exist within the FF and we have performed pathological evaluations.

Methods: Of the 320 patients were treated with neoadjuvant chemotherapy (NAC), 239 subjects who were able to evaluate FF-TILs were targeted. Lymphocytes that infiltrate the FF are FF-TILs.

Results: The disease-free survival (DFS) period after NAC for the high-FF-TIL group was found to be significantly longer than that for the low-FF-TIL group for all cases ($p < 0.001$) and for all subtypes of triple-negative breast cancer (TNBC) ($p = 0.001$), human epidermal growth factor receptor 2-enriched breast cancer (HER2BC) ($p = 0.010$), and hormone receptor-positive breast cancer (HRBC) ($p = 0.003$). In multivariable analysis as well, high-FF-TIL group classification was an independent factor for recurrence after NAC for all cases ($p < 0.001$, hazard ratio (HR) = 0.198) and all subtypes of TNBC ($p = 0.006$, HR = 0.172), HER2BC ($p = 0.025$, HR = 0.135), and HRBC ($p = 0.007$, HR = 0.228).

Conclusions: It is suggested that FF-TILs are a useful factor for predicting recurrence of breast cancer after NAC.

Article headings: NAC for breast cancer by evaluation of FF-TILs

Background

Cancer cells have various mutations that allow them to proliferate spontaneously and survive. However, the surrounding environment also influences cancer cells in their entirety and their intrinsic characteristics [1]. Therefore, monitoring the host immune response to tumors in the cancer microenvironment helps predict treatment response and outcome [1, 2]. Tumor-infiltrating lymphocytes (TILs), which are indicators for monitoring an immune response, are generally mononuclear immunocytes that aggregate with tumors and are thought to have a close relationship with cancer cells. Recently, the usefulness of methods that histopathologically evaluate the TILs function *in situ* has been elucidated [3–5]. Moreover, it has been shown that morphological evaluation of TILs is useful for outcome prediction in the treatment of breast cancer, as well as for predicting the therapeutic effects of drugs; therefore, TILs are considered novel biomarkers [6–9].

Methods for *in situ* evaluation of TILs and their cutoff values have not assimilated previously, but rather have depended on the previous reports [7, 10, 11]. Thus, in 2014, a recommendation concerning evaluation methods for TILs was created by the International Working Group [3]. TILs can be classified on the basis of the region in which they exist as either stromal TILs (within the stroma of the tumor) or intratumoral TILs (existing within tumor foci and therefore in contact with cancer cells). According to this recommendation, it is desirable to evaluate TILs according to the quantity of immune cells within the cancer stroma; therefore, this recommendation employs evaluation with stromal TILs. However, it has been suggested that the heterogeneity of TILs deters clear cutoff values from being defined.

Recently, it has been reported that the biological state of the cancer stroma is closely related to cancer proliferation and development [12–14]. Therefore, the evaluation of histomorphological images of tumor stroma has come to be considered one of the major keys to understanding the cancer microenvironment. A fibrotic focus (FF) within the stroma of a tumor is a histological formation that plays an important role in the cancer microenvironment with regard to proliferation and development [15, 16]. Infiltrating tumor cells surround the FF, which can be recognized as a converged focus of the tissue component that exists within the tumor. In breast cancer, it has been reported that tumors with wide-ranging FF have a high likelihood of malignancy and poor prognosis [15, 17, 18]. That is, it is thought that morphological evaluation of FF is an important indicator in the cancer microenvironment for understanding the transformation to malignancy [19, 20].

Here, we focus on TILs that exist within the FF and we have performed pathological evaluations. TILs within FF are called FF-TILs, and we hypothesized that the evaluation of TILs that exist within these regions would be a more precise indicator than previous methods of evaluating TILs. Among patients undergoing neoadjuvant (pre-surgical) chemotherapy (NAC) for breast cancer, we evaluated the prediction of treatment effects using FF-TILs.

Methods

Patient background

A total of 320 patients with resectable, early-stage breast cancer diagnosed as stage IIA (T1, N1, M0 or T2, N0, M0), IIB (T2, N1, M0 or T3, N0, M0), or IIIA (T1-2, N2, M0 or T3, N1-2, M0) were treated with NAC between 2005 and 2015. The present study included 239 participants, following the exclusion of 24 patients in whom the evaluation of TILs from the biopsy samples was difficult, and another 57 patients in whom the evaluation of FF-TILs was difficult (Fig. 1). Tumor stage and T and N factors were stratified on the basis of the TNM Classification of Malignant Tumors, Union for International Cancer Control (UICC) Seventh Edition [21]. Breast cancer was confirmed histologically by core needle biopsy (CNB) or vacuum-assisted biopsy (VAB), and staged by systemic imaging studies using computed tomography (CT), ultrasonography (US), and bone scintigraphy. Breast cancer was classified into subtypes according to the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor (HER) 2, and Ki67. Based on their immunohistochemical expression profiles, the tumors were categorized into the following immunophenotypes: luminal A (ER + and/or PgR+, HER2-, Ki67-low); luminal B (ER + and/or PgR+, HER2+) (ER + and/or PgR+, HER2-, Ki67-high); HER2-enriched (HER2BC) (ER-, PgR-, and HER2+); and triple-negative breast cancer (TNBC) (negative for ER, PgR and HER2). In this study, luminal A and luminal B were considered to be hormone receptor-positive breast cancer (HRBC) [22].

All patients received a standardized protocol of NAC consisting of four courses of FEC100 (500 mg/m^2 fluorouracil, 100 mg/m^2 epirubicin, and 500 mg/m^2 cyclophosphamide) every 3 weeks, followed by 12 courses of 80 mg/m^2 paclitaxel administered weekly. The patients with HER2-positive breast cancer, were additionally administered weekly (2 mg/kg) or tri-weekly (6 mg/kg) trastuzumab during paclitaxel treatment [23–25]. All patients underwent chemotherapy as outpatients. Therapeutic anti-tumor effects were assessed in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) criteria [26]. Patients underwent mastectomy or breast-conserving surgery following NAC [27]. The pathological effect of chemotherapy was assessed for resected primary tumors after NAC. Pathological complete response (pCR) was defined as the complete

disappearance of the invasive components of the lesion with or without intraductal components, including that in the lymph nodes according to the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 protocol [28]. All patients who underwent breast-conserving surgery were administered postoperative radiotherapy to the remnant breast. The standard postoperative adjuvant therapy for the subtype concerned was administered.

Overall survival (OS) time was the period from the initiation of NAC to the time of death from any cause. Disease-free survival (DFS) was defined as freedom from all local, locoregional, and distant recurrences. All patients received follow-up with physical examination every 3 months, US every 6 months, and CT and bone scintigraphy annually. The median follow-up period was 3.7 years (range, 0.2–6.0 years) for the assessment of OS and 3.4 years (range, 0.1–6.0 years) for DFS.

Ethics

This study was conducted at Osaka City University Graduate School of Medicine, Osaka, Japan, according to the Reporting Recommendations for Tumour Marker prognostic Studies (REMARK) guidelines and a retrospectively written research, pathological evaluation, and statistical plan [29]. Written informed consent was obtained from all patients. This research conformed to the provisions of the Declaration of Helsinki in 2013. The study protocol was approved by the Ethics Committee of Osaka City University (#926).

Histopathological Evaluation Of Tils

Histopathological assessment of predictive factors was made for CNB or VAB specimens at the time of the breast cancer diagnosis. Histopathological parameters examined included nuclear grade, histological type, presence of TILs, and correlation of these parameters with intrinsic subtypes and pCR. Histopathological analysis of the percentage of TILs was evaluated on a single full-face hematoxylin and eosin (HE)-stained tumor section according to criteria described by Salgado et al [3]. TILs were defined as the infiltrating lymphocytes within tumor stroma and were expressed by the proportion to the field investigated, and the number of TILs in stroma surrounding the stained cancer cells was quantitatively measured in each field under 400-times magnification [11, 30]. The area of *in situ* carcinoma and crush artifacts were not included. Proportional scores were defined as 3, 2, 1, and 0 if the area of stroma with lymphoplasmacytic infiltration around invasive tumor cell nests was > 50%, > 10–50%, ≤ 10%, and absent, respectively **(**Fig. 2A-D**)**. TILs were considered high when scores were ≥ 2 and low when scores were 1 and 0.

Histopathological evaluation of FF-TILs

An FF is a scar-like lesion consisting of an area of mainly collagen and fibroblasts, often located near the center of a carcinoma. And, an FF is a converged focus of the tissue component of the stroma of a tumor, and it is surrounded by infiltrating tumor cells. An FF is defined as “FF often consisted of fibrous bands radially expanding to the surrounding area, and FF was located within the tumor, was surrounded by a more cellular zone of infiltrating ductal carcinoma cells, and occupied various percentage of the tumor area” [15, 16]. The fibroblasts and collagen fiber that form the FF show a storiform pattern, and propagate intertwined with each other. Lymphocytes that infiltrate the FF are FFTILs. We refer to the high TIL group (TIL score ≥ 2) within an FF as the high-FF-TIL group, and the low TIL group (TIL score: 1 or 0) within a FF as the low-FF-TIL group. We refer

to the prediction of treatment effect using previous TIL evaluation methods as the Training Set (TS), and that using FF-TILs as the Validation Set (VS). Histopathologic evaluation of TILs and FF was jointly performed by two breast pathologists (MO, YK) who were blinded to clinical information, including treatment allocation and outcomes.

Sectional analysis

Statistical analysis was performed using the SPSS version 19.0 statistical software package (IBM Corp., Armonk, NY). The associations between FF-TILs, and clinicopathological variables were examined using the chi-square test (or Fisher's exact test when necessary). Multivariable analysis of pCR was carried out using a binary logistic regression model. The Kaplan-Meier method was used to estimate DFS and OS, and the results were compared between groups with log-rank tests. The Cox proportional hazards model was used to compute univariable and multivariable hazard ratios (HR) for the study parameters with 95% confidence intervals (CIs), and used in a backward stepwise method for variable selection in multivariable analyses. A p value < 0.05 was considered significant. Cutoff values for different biomarkers included in this study were chosen before statistical analysis.

Results

FF-TILs and clinicopathological investigation

Among the 239 patients who underwent NAC, 123 (51.5%) were in the high-FF-TIL group, and 116 (48.5%) were in the low-FF-TIL group. In the high-FF-TIL group, the Ki67 value was significantly high (p = 0.049), the proportion of HRBC was significantly low (p = 0.013), and the pCR rate was significantly high (p = 0.004) (Table 1). With regard to subtypes, there were 83 cases of TNBC (34.7%), 46 cases of HER2BC (19.2%), and 110 cases of HRBC (46.1%). In an investigation that considered the clinicopathological background, regarding HRBC, in the high-FF-TIL group the Ki67 values were significantly high (p = 0.001) (Table 2). However, for all the subtypes, no correlation with the pCR rate was found (TNBC: p = 0.154, HER2BC: p = 0.489, HRBC: p = 0.083).

Table 1
Correlation between clinicopathological features and FF-TILs in 239 breast cancers.

Parameters	FF-TILs in all breast cancers (n = 239)		p value
	High (n = 123)	Low (n = 116)	
Age at operation	60 (48.8%)	56 (48.3%)	0.938
≤55	63 (51.2%)	60 (51.7%)	
>55			
Menopause	52 (42.3%)	46 (39.7%)	0.680
Negative	71 (57.7%)	70 (60.3%)	
Positive			
Tumor size	23 (18.7%)	15 (12.9%)	0.223
≤2 cm	100 (81.3%)	101 (87.1%)	
>2 cm			
Lymph node status	36 (29.3%)	30 (25.9%)	0.556
Negative	87 (70.7%)	86 (74.1%)	
Positive			
Nuclear grade	97 (78.9%)	92 (79.3%)	0.932
1, 2	26 (21.1%)	24 (20.7%)	
3			
Ki67	47 (38.2%)	59 (50.9%)	0.049
≤14 %	76 (61.8%)	57 (49.1%)	
>14 %			
Intrinsic subtype	47 (38.2%)	36 (31.0%)	0.244
TNBC	76 (61.8%)	80 (69.0%)	
non-TNBC			
Intrinsic subtype	29 (23.6%)	17 (14.7%)	0.080
HER2BC	94 (76.4%)	99 (85.3%)	
non- HER2BC			

FF, fibrotic focus. TILs, tumor-infiltrating lymphocytes. TNBC, triple-negative breast cancer. HER2BC, human epidermal growth factor receptor 2-enriched breast cancer. HRBC, hormone receptor-positive breast cancer. pCR, pathological complete response.

Parameters	FF-TILs in all breast cancers (n = 239)		p value
	High (n = 123)	Low (n = 116)	
Intrinsic subtype	47 (38.2%)	63 (54.3%)	0.013
HRBC	76 (61.8%)	53 (45.7%)	
non-HRBC			
Pathological response	51 (41.5%)	28 (24.1%)	0.004
pCR	72 (58.5%)	88 (75.9%)	
non-pCR			

FF, fibrotic focus. TILs, tumor-infiltrating lymphocytes. TNBC, triple-negative breast cancer. HER2BC, human epidermal growth factor receptor 2-enriched breast cancer. HRBC, hormone receptor-positive breast cancer. pCR, pathological complete response.

Table 2
Correlations between FF-TILs and clinicopathological parameters in 83 triple-negative, 46 HER2 enriched, and 110 luminal type breast cancers.

Parameters	TNBC (n = 83)		p value	HER2BC (n = 46)		p value	HRBC (n = 110)		p value
	High (n = 47)	Low (n = 36)		High (n = 29)	Low (n = 17)		High (n = 47)	Low (n = 63)	
Age at operation	25 (53.2%)	19 (52.8%)	0.970	14 (48.3%)	7 (41.1%)	0.641	24 (51.1%)	34 (54.0%)	0.763
≤55	22 (46.8%)	17 (47.2%)		15 (51.7%)	10 (58.9%)		23 (48.9%)	29 (46.0%)	
>55									
Menopause	19 (40.4%)	14 (38.9%)	0.887	11 (37.9%)	7 (41.1%)	0.828	22 (46.8%)	25 (39.7%)	0.455
Negative	28 (59.6%)	22 (61.1%)		18 (62.1%)	10 (58.9%)		25 (53.2%)	38 (60.3%)	
Positive									
Tumor size	7 (14.9%)	5(13.9%)	0.897	7 (24.1%)	2 (11.8%)	0.268	9 (19.1%)	8 (12.7%)	0.355
≤2 cm	40 (85.1%)	31 (86.1%)		22 (75.9%)	15 (88.2%)		38 (80.9%)	55 (87.3%)	
>2 cm									
Lymph node status	14 (29.8%)	9 (25.0%)	0.629	10 (34.4%)	5 (29.4%)	0.723	12 (25.5%)	16 (25.4%)	0.987
Negative	33 (70.2%)	27 (75.0%)		19 (65.6%)	12 (70.6%)		35 (74.5%)	47 (74.6%)	
Positive									
Nuclear grade	36 (76.6%)	25 (69.4%)	0.464	25 (86.2%)	12 (70.6%)	0.182	36 (76.6%)	55 (87.3%)	0.142
1, 2	11 (23.4%)	11 (30.6%)		4 (13.8%)	5 (29.4%)		11 (23.4%)	8 (12.7%)	
3									
Ki67	17 (36.2%)	8 (22.2%)	0.170	13 (44.8%)	8 (47.1%)	0.883	17 (36.2%)	43 (68.3%)	0.001
≤14 %	30 (63.8%)	28 (77.8%)		16 (55.2%)	9 (52.8%)		30 (63.8%)	20 (31.7%)	
>14 %									
Pathological response	23 (48.9%)	12 (33.3%)	0.154	15 (51.7%)	7 (41.2%)	0.489	13 (27.7%)	9 (14.3%)	0.083
pCR	24 (51.1%)	24 (66.7%)		14 (48.3%)	10 (58.8%)		34 (72.3%)	54 (85.7%)	
non-pCR									

FF, fibrotic focus. TILs, tumor-infiltrating lymphocytes. TNBC, triple-negative breast cancer. HER2BC, human epidermal growth factor receptor 2-enriched breast cancer. HRBC, hormone receptor-positive breast cancer. pCR, pathological complete response.

Prognostic Analysis Using Ff-til

The DFS period after NAC for the high-FF-TIL group was found to be significantly longer than that for the low-FF-TIL group for all cases ($p < 0.001$, log-rank) and for all subtypes of TNBC ($p = 0.001$, log-rank), HER2BC ($p = 0.010$, log-rank), and HRBC ($p = 0.003$, log-rank) (Fig. 3A-D). In comparison of OS between the high-FF-TIL group and the low-FF-TIL group, for all cases ($p = 0.160$, log-rank) and for all subtypes a significant lengthening was not found (TNBC: $p = 0.414$, HER2BC: $p = 0.888$, HRBC: $p = 0.147$, log-rank) (**Supplemental Fig. 1A-D**). In univariable analysis, for the high-FF-TIL cases, all cases ($p < 0.001$, HR = 0.182), and all subtypes of TNBC ($p = 0.004$, HR =- 0.154), HER2BC ($p = 0.022$, HR = 0.136), and HRBC ($p = 0.007$, HR = 0.224) contributed to a significant lengthening of the DFS period (Table 3) (Fig. 4A-D). In multivariable analysis as well, high-FF-TIL group classification was an independent factor for recurrence after NAC for all cases ($p < 0.001$, HR = 0.198) and all subtypes of TNBC ($p = 0.006$, HR = 0.172), HER2BC ($p = 0.025$, HR = 0.135), and HRBC ($p = 0.007$, HR = 0.228).

Table 3
Univariable and multivariable analysis with respect to disease-free survival in breast cancer subtypes.

		Univariable analysis			Multivariable analysis		
Parameter		Hazard ratio	95 % CI	p value	Hazard ratio	95 % CI	p value
		all breast cancers (n = 239)					
Tumor size (cm)	≤ 2 vs > 2	1.594	0.631–4.030	0.324			
Lymph node status	Negative vs Positive	2.2494	1.058–5.879	0.037	2.286	0.969–5.393	0.059
Nuclear grade	1–2 vs 3	1.126	0.573–2.214	0.730			
Ki67 (%)	≤ 14 vs > 14	0.717	0.403–1.277	0.259			
Pathological response	pCR vs non-pCR	0.561	0.291–1.083	0.085	0.783	0.401–1.529	0.474
FF-TILs	High vs Low	0.182	0.088–0.379	< 0.001	0.198	0.094–0.418	< 0.001
		TNBC (n = 83)					
Tumor size (cm)	≤ 2 vs > 2	0.701	0.200-2.464	0.580			
Lymph node status	Negative vs Positive	1.059	0.340–3.293	0.921			
Nuclear grade	1–2 vs 3	0.934	0.253–3.453	0.919			
Ki67 (%)	≤ 14 vs > 14	0.961	0.334–2.765	0.941			
Pathological response	pCR vs non-pCR	0.380	0.122–1.182	0.095	0.492	0.157–1.545	0.225
FF-TILs	High vs Low	0.154	0.044–0.543	0.004	0.172	0.048–0.609	0.006
		HER2BC (n = 46)					
Tumor size (cm)	≤ 2 vs > 2	0.176	0.220-14.649	0.584			
Lymph node status	Negative vs Positive	3.201	0.393–26.099	0.277			
Nuclear grade	1–2 vs 3	0.452	0.055–3.688	0.458			

CI, confidence interval. FF, fibrotic focus. TILs, tumor-infiltrating lymphocytes. TNBC, triple-negative breast cancer. HER2BC, human epidermal growth factor receptor 2-enriched breast cancer. HRBC, hormone receptor-positive breast cancer. pCR, pathological complete response.

		Univariable analysis			Multivariable analysis		
Ki67 (%)	≤ 14 vs > 14	0.377	0.088–1.619	0.190	0.391	0.089–1.721	0.214
Pathological response	pCR vs non-pCR	0.572	0.136–2.403	0.445			
FF-TILs	High vs Low	0.136	0.025–0.748	0.022	0.135	0.024–0.775	0.025
		HRBC (n = 110)					
Tumor size (cm)	≤ 2 vs > 2	4.418	0.595–32.821	0.146			
Lymph node status	Negative vs Positive	7.711	1.039–57.236	0.046	7.592	1.022–56.411	0.048
Nuclear grade	1–2 vs 3	1.234	0.456–3.341	0.679			
Ki67 (%)	≤ 14 vs > 14	0.703	0.304–1.624	0.410			
Pathological response	pCR vs non-pCR	0.816	0.301–2.210	0.689			
FF-TILs	High vs Low	0.224	0.076–0.663	0.007	0.228	0.077–0.672	0.007

CI, confidence interval. FF, fibrotic focus. TILs, tumor-infiltrating lymphocytes. TNBC, triple-negative breast cancer. HER2BC, human epidermal growth factor receptor 2-enriched breast cancer. HRBC, hormone receptor-positive breast cancer. pCR, pathological complete response.

When receiver operating characteristic (ROC) analysis was performed, for all breast cancer cases, VS (rea under the curve [AUC]: 0.701) was shown to have better results than TS (AUC: 0.582) (Fig. 5A). Similar results were also found with investigation based on subtype: TNBC (AUC: TS = 0.616, VS = 0.735), HER2BC (AUC: TS = 0.618, VS = 0.730), and HRBC (AUC: TS 0.530, VS = 0.660) (Fig. 5B-D).

Discussion

Recently, in many large-scale prospective clinical trials, TILs have been proven to be useful as a prognostic factor [4] [6, 8, 9, 31]. These reports have suggested that TILs can be useful as clinical biomarkers, and today attempts to unify their evaluation methods are underway [3, 4]. According to the International Working Group, in the evaluation of TILs, the percentage of immunocytes within a tumor stroma should be measured at the border of the cancer infiltration region [3]. However, there is not yet a standardized opinion regarding determination of the region within the tumor stroma. Therefore, we also paid attention to FF in regions that were within the tumor stroma and were surrounded by infiltrating tumor cells. Wide-ranging FF contribute to the tumor's acquisition of a malignant condition due to the hypoxic environment within the tumor, and are related to prognosis and drug resistance [32–34]. In the present study, when we investigated TILs that exist within FF as FF-TILs in patients undergoing NAC, the high-FF-TIL group had a longer DFS period after NAC than did the low-FF-TIL group.

When studied by subtype, TILs have been considered useful as prediction markers for treatment effect in subtypes with high immunological action, such as TNBC and HER2BC [4, 8, 9, 31]. However, in HRBC, which has the highest frequency, there are few reports that show a clinical relationship with TILs. In the present study, FF-TILs have been proven useful as prognostic factors following NAC not only for TNBC and HER2BC, but also for HRBC. Furthermore, for non-HRBC, such as TNBC and HER2BC, it is thought that wide-ranging FF exist [34], and in the present study as well, for non-HRBC, we observed that FF-TILs were significantly high.

In the current study, a reason that outcome prediction was possible even for HRBC is the heterogeneity of TILs in the cancer microenvironment. ER, which is important for the occurrence and development of HRBC, is activated not just by estrogen but also by the signal cascade of a pathway via phosphorylation due to various growth factors, and the control of ER depends on the cancer microenvironment [35]. However, there are reports that, in a hypoxic environment, expression of hormone receptors is weakened [36, 37]. That is, it is thought that, in an FF region, hormone receptors are weakened owing to the hypoxic environment; therefore, biomarkers for ER-negative cancers are useful. It is possible to dynamically understand the changes in the cancer microenvironment more precisely with FF-TILs than with the previous evaluation range of TILs; therefore, we believe it may be a better evaluation method.

In the present study, patients in the low-FF-TIL group experienced a high recurrence rate, and perhaps, depending on the subtype, adjuvant therapy should have been added. That is, in the selection of post-surgical adjuvant therapy for patients undergoing NAC, it is suggested that determination using FF-TILs can contribute to the choice of a proper treatment strategy.

However, the retrospective nature of this study is a limitation, and regarding adjuvant therapy after NAC, since there are differences among cases, and since it is difficult to evaluate FF with CNB specimens, it is necessary to obtain more tissue through the use of methods such as VAB during diagnoses. In the previous report, we classified FF long diameter 8 mm as positive / negative as cutoff. However, with this method, evaluable FFs are limited. In this study, we used FF as the convergent focal point of the fiber component surrounded by infiltrating tumor cells without using the cutoff value. That is, it was possible to evaluate FF even if the area was small. Therefore, the evaluation rate of FF is higher than the previous report. Even a minute specimen at CNB or VAB, in the method we used. Evaluation of FF becomes possible.

Conclusions

It is suggested that FF-TILs are a useful factor for predicting recurrence of breast cancer after NAC, and they may be a more precise indicator than previous evaluation methods with TILs.

Abbreviations

NAC: neoadjuvant chemotherapy, TILs: tumor-infiltrating lymphocytes, FF: fibrotic foci, UICC: Union for International Cancer Control, CNB: core needle biopsy, VAB: **vacuum-assisted biopsy,** CT: computed tomography, US: ultrasonography, ER: estrogen receptor, PgR: progesterone receptor, HER: human epidermal growth factor receptor, HER2BC: HER2-enriched, TNBC: triple-negative breast cancer, HRBC: hormone receptor-positive breast cancer, RECIST: Response Evaluation Criteria in Solid Tumors, pCR: pathological complete response, OS: overall survival, DFS: disease-free survival, REMARK: Reporting Recommendations for Tumor Marker prognostic

Studies, HE: hematoxylin and eosin, TS: Training Set, VS: Validation Set, HR: hazard ratio, CI: confidence interval, ROC: receiver operating characteristic, AUC: area under the curve.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all subjects. This research conformed to the provisions of the Declaration of Helsinki in 2013. All patients were informed of the investigational nature of this study and provided their written, informed consent. The study protocol was approved by the Ethics Committee of Osaka City University (#926).

Consent for publication

Not applicable.

Availability of data and materials

The data and materials used and analyzed in the current study would be available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors were involved in the preparation of this manuscript. YA collected the data, and wrote the manuscript. SK, RK, AY, KT, SI, WG and TM performed the operation and designed the study. YA, SK, MS and HT summarized the data and revised the manuscript. KH and MO substantial contribution to the study design, performed the operation, and revised the manuscript. All authors read and approved the final manuscript.

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Figures

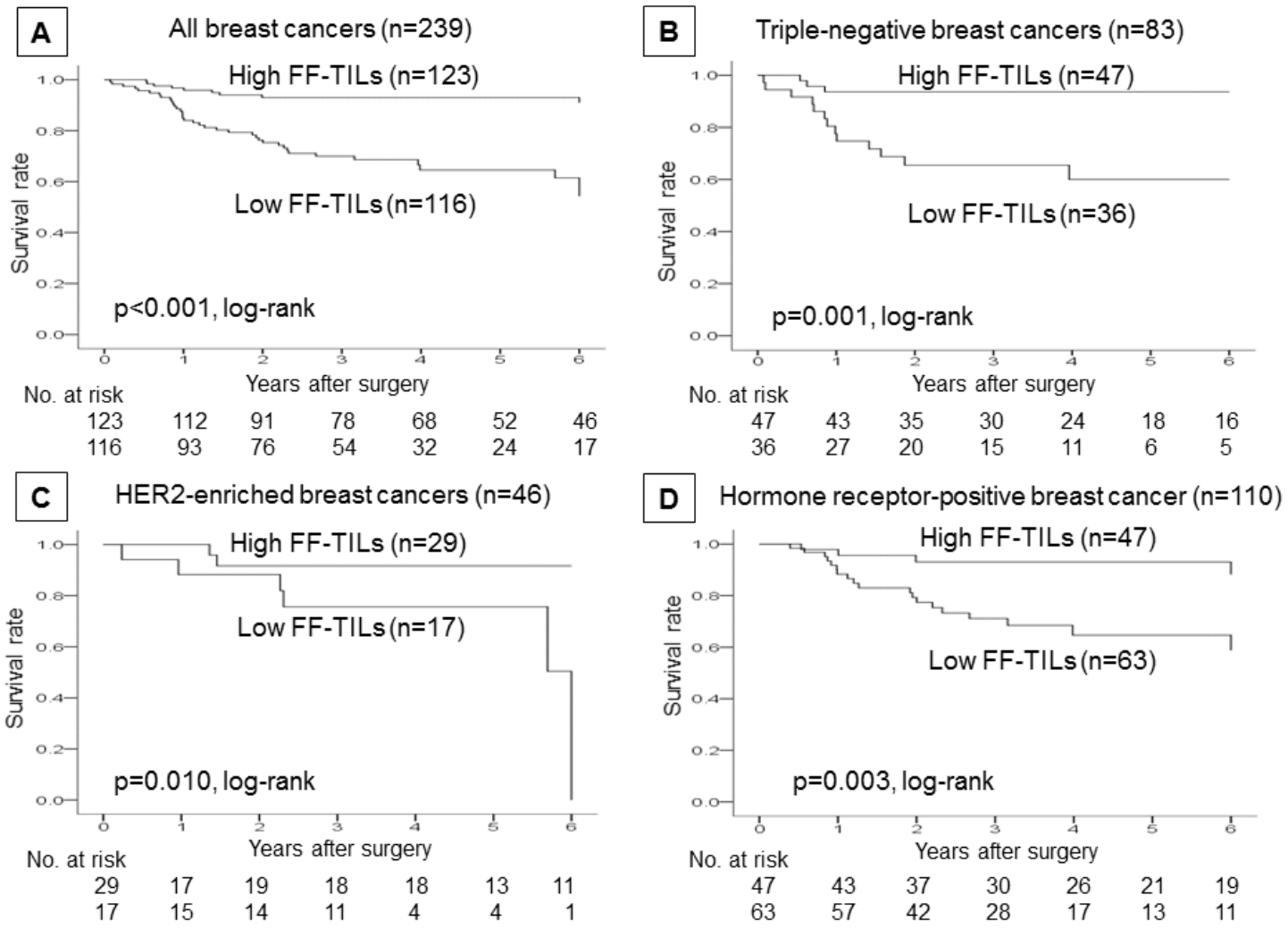

Figure 3

The disease-free survival (DFS) period after NAC. The DFS period after NAC for the high-FF-TIL group was found to be significantly longer than that for the low-FF-TIL group for all cases ($p < 0.001$, log-rank) (A) and for all subtypes of TNBC ($p = 0.001$, log-rank) (B), HER2BC ($p = 0.010$, log-rank) (C), and HRBC ($p = 0.003$, log-rank) (D).