

Prediction of Distant Metastatic Recurrence By Tumor-Infiltrating Lymphocytes in Hormone Receptor-Positive Breast Cancer

Koji Takada

Osaka City University Graduate School of Medicine

Shinichiro Kashiwagi (✉ spqv9ke9@view.ocn.ne.jp)

Osaka City University Graduate School of Medicine <https://orcid.org/0000-0002-0460-9599>

Yuka Asano

Osaka City University Graduate School of Medicine

Wataru Goto

Osaka City University Graduate School of Medicine

Rika Kouhashi

Osaka City University Graduate School of Medicine

Akimichi Yabumoto

Osaka City University Graduate School of Medicine

Sae Ishihara

Osaka City University Graduate School of Medicine

Tamami Morisaki

Osaka City University Graduate School of Medicine

Masatsune Shibutani

Osaka City University Graduate School of Medicine

Hiroaki Tanaka

Osaka City University Graduate School of Medicine

Kosei Hirakawa

Osaka City University Graduate School of Medicine

Masaichi Ohira

Osaka City University Graduate School of Medicine

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Abstract

Background: Breast cancer subtypes are known to have different metastatic recurrence sites. Distant metastases are often observed during the post-operative course in patients with human epidermal growth factor receptor 2 (HER2)-enriched breast cancer and triple-negative breast cancer, but are relatively rare in those with hormone receptor-positive and HER2-negative (HR+/HER2-) breast cancer. Tumor-infiltrating lymphocytes (TILs) serve as an index to monitor tumor immune microenvironment and may possibly predict the prognosis and therapeutic effect in breast cancer. This study aimed to investigate the correlation between TIL density and recurrence site in HR+/HER2- breast cancer.

Methods: Four-hundred and seventy-one patients with HR+/HER2- breast cancer underwent surgery as the first treatment and received adjuvant endocrine therapy (except adjuvant chemotherapy) at the Osaka City University Hospital from April 2007 to October 2015. Needle biopsy specimens were obtained from all patients to evaluate tumor morphology and examine TILs. Morphological assessment was conducted using conventional hematoxylin and eosin staining.

Results: Breast cancer recurrence was observed in 42 patients. In patients with no TIL density, local recurrence was significantly less ($p = 0.022$), while distant metastases were significantly more ($p = 0.015$) compared to those in patients with TIL density. Therefore, for the prediction of distant metastases in HR+/HER2- breast cancer without chemotherapy, TILs could be used as predictors in univariate analysis ($p = 0.015$, odds ratio [OR] = 0.127), although not as independent factors ($p = 0.285$, OR = 0.144).

Conclusions: Our findings indicate that TILs may predict distant metastatic recurrence in stages I–II of HR+/HER2- breast cancer.

Background

Cancer, even when detected early and successfully surgically resected, the risk of its recurrence persists [1–3]. Breast cancer often has local recurrence and axillary lymph node metastasis, although distant metastases, such as to the bone, lungs, and liver, may occur. Imaging procedures, such as computed tomography (CT), ultrasonography (US), and bone scintigraphy, are necessary to detect distant metastases sub-clinically. However, some prospective studies have shown early detection of distant metastases to not affect prognosis, and routine examination is not recommended in such cases [4–6]. If distant metastasis can be detected before the onset of symptoms, both therapeutic effect and patients' quality of life can be improved. Therefore, clinicopathological factors prone to distant metastases have been studied quite extensively till date [1, 2]. Interestingly, metastatic recurrence sites vary among the different breast cancer intrinsic subtypes [1, 3]. Distant metastases are frequently found during the post-operative course in patients with human epidermal growth factor receptor 2 (HER2)-enriched breast cancer and triple-negative breast cancer (TNBC), while they are relatively rare in patients with hormone receptor-positive and HER2-negative (HR+/HER2-) breast cancer. Therefore, early detection of locoregional recurrence after surgery for HR + HER2- breast cancer has been suggested to be vital for

good prognosis [7]. However, HR+/HER2- breast cancer can also be distant from the first site of recurrence.

Invasion and metastasis of cancer involve the molecular pathological features of not only the cancer but also the surrounding interstitial region, commonly called the tumor microenvironment (TME) [8, 9]. TME consists of cancer-related fibroblasts, neovascular cells, and tumor-infiltrating lymphocytes (TILs). Recently, a correlation between TILs and lymph node metastasis was reported in gastric cancer and melanoma [10, 11], and that between TILs and axillary lymph node metastasis was reported in TNBC [12, 13]. In all these reports, lower TIL density was shown to more likely cause lymph node metastasis. However, reports on the TIL relationship with distant metastases are few compared to those on its relationship with lymph node metastases. We speculate that if TILs are involved in lymph node metastasis, which is a risk factor for distant metastasis, TILs should also be involved in distant metastasis.

We hypothesized that the site of the first recurrence may vary based on TIL density in HR+/HER2- breast cancer. Therefore, this study aimed to investigate the correlation between TIL density and recurrence site in HR+/HER2- breast cancer, specifically in patients who did not receive chemotherapy either before or after surgery.

Methods

Patient background

A total of 709 patients with primary resectable breast cancer underwent surgery between 2007 and 2015 at the Osaka City University Hospital (**Fig. 1**). Of these, 471 patients had HR+/HER2- breast cancer and underwent surgery as the first treatment and received adjuvant endocrine therapy, except for adjuvant chemotherapy. These 471 patients were followed-up for a median of 2364 days (range, 66–4446 days) post-operatively. During postoperative follow-up, 42 patients were found to have recurrence and were enrolled in the present study. Breast cancers and their subtypes were diagnosed by core needle biopsy or vacuum-assisted biopsy. HR+/HER2- breast cancer was defined as estrogen receptor (ER)- and/or progesterone receptor (PgR)-positive and HER2-negative by immunohistological staining of the tissue. Expression of Ki67 was also examined using immunohistological staining and the cutoff value for Ki67 was set at 20% based on previous reports [14]. CT, US, and bone scintigraphy were used to evaluate the cancer stage. Cancer progression was evaluated according to the 8th edition of the TNM classification of malignant tumors (8th edition) by the Union for International Cancer Control [15]. All patients underwent either mastectomy or breast-conserving surgery, and the latter received radiation therapy in the remaining mammary gland post-surgery. For patients diagnosed with axillary lymph node metastasis by preoperative imaging, axillary lymph node dissection was also performed. In the case of breast cancer diagnosed without axillary lymph nodes metastasis, sentinel lymph node biopsy was performed using a combination of radioisotope and dye methods, as reported previously [16, 17]. Metastasis was examined pathologically by slicing sentinel lymph node at 2-mm thickness [18, 19]. Axillary lymph node dissection

was performed in cases where metastasis in the sentinel lymph node was greater than 2 mm, also called macro-metastasis. The biological characteristics (ER/PgR/Ki67/HER2) of the resected specimens were re-examined, and the results validated those of the biopsy tissue. Adjuvant chemotherapy administration was decided at the discretion of the attending physician, taking into consideration the patient's wishes and comorbidities. In the present study, disease-free survival (DFS) was defined as the time from surgery to recurrence or death; progression-free survival (PFS) was defined as the time from recurrence to relapse or death due to breast cancer after the next treatment; post-recurrence survival (PRS) was defined as the time from recurrence to death due to breast cancer.

Histopathological evaluation of TIL density

TIL density was evaluated pathologically using biopsy specimens. The pathological diagnosis and examination were jointly performed by two breast pathologists. The definition and evaluation method for TILs followed the International TILs Working Group 2014 [20]. Specifically, the density of infiltrating lymphocytes was averaged on full sections, at least five fields, of the tumor stroma. The results were divided into four groups according to previous reports (score 3: > 50%, score 2: > 10%–50%, score 1: ≤ 10%, and score 0: absent) (**Fig. 2**) [21-24].

Statistical analysis

All statistical analyses were performed using the JMP software package (SAS, Tokyo, Japan). For evaluating the correlation between two groups for each clinicopathological feature, Pearson's chi-square test was used. The odds ratio (OR) and 95% confidence interval (CI) were calculated using logistic analysis. Multivariable analysis was performed with the multivariable logistic regression model. To evaluate the hazard ratio (HR) and 95% CI related to survival outcomes, such as PFS or PRS, Cox proportional hazards models were used for univariate analysis and Cox regression models were used for multivariate analysis. Significance was defined at p-value < 0.05.

Ethics statement

Written informed consent to participate in the study was obtained from each subject in accordance with the Declaration of Helsinki. Each patient or their family was fully informed of the nature of the investigation in this study prior to obtaining their written informed consent. The study protocol was approved by the Ethics Committee of Osaka City University (approval number # 926).

Results

Clinicopathological features

Four-hundred and seventy-one patients, diagnosed with HR+/HER2- breast cancer, were not treated with adjuvant chemotherapy after resection surgery. Forty-two of them had a breast cancer recurrence. **Table 1** shows the clinicopathological features of these patients. The median age at the time of operation was 59

years (range, 37–79 years). The median tumor size was 20.5 mm (9.5–49.3 mm), and no evidence of axillary lymph node metastasis was found by pre-operative imaging, in all cases. Therefore, sentinel lymph node biopsy was performed for all. Micro-metastasis was identified in three cases (7.1%) and macro-metastasis was found in seven cases (16.7%). The latter underwent axillary lymph node dissection, and pathological examination revealed < 3 axillary lymph nodes with metastases. The expression of ER in one patient (2.4%) was negative, while that of PgR was positive. Three patients (7.1%) were negative for PgR expression and seven (16.7%) had high Ki67. Eighteen cases (42.9%) underwent breast-conserving treatment and received post-operative radiation therapy for the residual mammary gland. None of the 42 patients received post-operative radiation therapy in the axilla or supraclavicular fossa. Post-operative pathological findings showed lymphatic invasion in 21 patients (50.0%) and venous invasion in three patients (7.1%). Six patients (14.3%) were diagnosed with nuclear grade 3. All patients received adjuvant endocrine therapy; 10 patients (23.8%) were treated with tamoxifen and seven patients (16.7%) were treated with a combination of tamoxifen and a luteinizing hormone-releasing hormone agonist. The remaining 25 patients (59.5%) received anastrozole. The median DFS time was 1462 days (range, 132–3300 days). The first recurrence site was local in 18 (42.9%) patients and regional lymph node in 13 (30.9%) patients, while distant metastases were observed in 11 (26.2%) patients. Lung metastasis, detected in seven (16.6%) patients, was the most common distant metastasis. None of the patients showed simultaneous locoregional recurrence and distant metastasis. Six (14.3%) patients had TIL density higher than 10%, while in six other patients (14.3%), TIL density was not detected.

Table 1

Clinicopathological features of 42 patients with recurrence of hormone receptor-positive / HER2 negative breast cancer not receiving chemotherapy

Parameters	Number of all patients (n = 42) (%)
Age at operation (years old)	median 59 (range, 37–79)
Tumor size (mm)	median 20.5 (range, 9.5–49.3)
Pathological lymph node metastasis pN0 / pN1mi / pN1a	32 (76.2 %) / 3 (7.1 %) / 7 (16.7 %)
Estrogen receptor Negative / Positive	1 (2.4 %) / 41 (97.6 %)
Progesterone receptor Negative / Positive	3 (7.1 %) / 39 (92.9 %)
Ki67 ≤ 20 % / >20 %	35 (83.3 %) / 7 (16.7 %)
Surgical treatment Breast conserving treatment and adjuvant radiation therapy / Mastectomy	18 (42.9 %) / 24 (57.1 %)
Lymphatic invasion ly0 / ly1	21 (50.0 %) / 21 (50.0 %)
Venous invasion v0 / v1	39 (92.9 %) / 3 (7.1 %)
Nuclear grade 1 / 2 / 3	20 (47.6 %) / 16 (38.1 %) / 6 (14.3 %)
Adjuvant endocrine therapy TAM / TAM + LH-RH agonist / ANA	10 (23.8 %) / 7 (16.7 %) / 25 (59.5 %)
Disease free survival (days)	1462 (132–3300)
Primary recurrence site Local / Regional lymph node / Lung / Bone / Liver / Bone + liver	18 (42.9 %) / 13 (30.9 %) / 7 (16.6 %) / 1 (2.4 %) / 1 (2.4 %) / 2 (4.8 %)

HER2: human epidermal growth factor receptor 2. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor-infiltrating lymphocytes.

Parameters	Number of all patients (n = 42) (%)
TILs density absent / ≤10 % / >10 %	6 (14.3 %) / 30 (71.4 %) / 6 (14.3 %)
HER2: human epidermal growth factor receptor 2. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor-infiltrating lymphocytes.	

Correlations between clinicopathological features and recurrence sites

The correlations between clinicopathological features and recurrence sites are listed in **Table 2**. According to sentinel lymph node biopsy results, two out of the seven cases with metastasis had regional lymph node recurrence and five had distant metastatic recurrence. Thus, in patients with axillary lymph node metastases, local recurrence was significantly less ($p = 0.012$), and distant metastasis was significantly more ($p = 0.003$) than that in patients without axillary lymph node metastases. In addition, in patients with longer DFS, distant metastatic recurrence was significantly higher ($p = 0.014$). Further, patients who underwent mastectomy had significantly more locoregional recurrence ($p = 0.020$). Examination of the correlations between operative procedures and clinicopathological features revealed the following significant differences in patients who underwent mastectomy: older age ($p = 0.001$), higher frequency of tumor with diameter more than 30 mm ($p = 0.006$), and lower Ki67 ($p = 0.012$) (**Additional file 1; Table S1**).

Table 2

Correlation between primary recurrence site and clinicopathological features in HR+/HER2- breast cancer not received chemotherapy

Parameters	Primary recurrence site (<i>n</i> = 42)					
	Local recurrence (<i>n</i> = 18)	Not local recurrence (<i>n</i> = 24)	<i>p</i> value	Locoregional recurrence (<i>n</i> = 31)	Distant metastasis (<i>n</i> = 11)	<i>p</i> value
Age at operation (years old)	11 (61.1 %)	14 (58.3 %)	0.856	17 (54.8 %)	8 (72.7 %)	0.299
≤ 60	7 (38.9 %)	10 (41.7 %)		14 (45.2 %)	3 (27.3 %)	
> 60						
Tumor size (mm)	9 (50.0 %)	12 (50.0 %)	1.000	15 (48.4 %)	6 (54.5 %)	0.726
≤ 20.0	9 (50.0 %)	12 (50.0 %)		16 (51.6 %)	5 (45.5 %)	
> 20.0						
Tumor size (mm)	15 (83.3 %)	19 (79.2 %)	0.734	24 (77.4 %)	10 (90.9 %)	0.328
≤ 30.0	3 (16.7 %)	5 (20.8 %)		7 (22.6 %)	1 (9.1 %)	
> 30.0						
Pathological lymph node metastasis	18 (100.0 %)	17 (70.8 %)	0.012	29 (93.5 %)	6 (54.5 %)	0.003
pN0, pN1mi	0 (0.0 %)	7 (29.2 %)		2 (6.5 %)	5 (45.5 %)	
pN1a						
Progesterone receptor	2 (11.1 %)	1 (4.2 %)	0.387	3 (9.7 %)	0 (0.0 %)	0.284
Negative	16 (88.9 %)	23 (95.8 %)		28 (90.3 %)	11 (100.0 %)	
Positive						
Ki67	16 (88.9 %)	19 (79.2 %)	0.403	26 (83.9 %)	9 (81.8 %)	0.875
≤20 %	2 (11.1 %)	5 (20.8 %)		5 (16.1 %)	2 (18.2 %)	
>20 %						
Surgical treatment	7 (38.9 %)	11 (45.8 %)	0.653	10 (32.3 %)	8 (72.7 %)	0.020
BCT and radiation therapy	11 (61.1 %)	13 (54.2 %)		21 (67.7 %)	3 (27.3 %)	
Mastectomy						

HR+/HER2- breast cancer: hormone receptor-positive and human epidermal growth factor receptor 2 negative breast cancer. BCT: breast conserving treatment. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor-infiltrating lymphocytes.

Parameters	Primary recurrence site (<i>n</i> = 42)					
	Local recurrence (<i>n</i> = 18)	Not local recurrence (<i>n</i> = 24)	<i>p</i> value	Locoregional recurrence (<i>n</i> = 31)	Distant metastasis (<i>n</i> = 11)	<i>p</i> value
Lymphatic invasion	10 (55.6 %)	11 (45.8 %)	0.533	16 (51.6 %)	5 (45.5 %)	0.726
ly0		13 (54.2 %)		15 (48.4 %)	6 (54.5 %)	
ly1	8 (44.4 %)					
Venous invasion	17 (94.4 %)	22 (91.7 %)	0.729	29 (93.5 %)	10 (90.9 %)	0.770
v0		2 (8.3 %)		2 (6.5 %)	1 (9.1 %)	
v1	1 (5.6 %)					
Nuclear grade	15 (83.3 %)	21 (87.5 %)	0.703	26 (83.9 %)	10 (90.9 %)	0.567
1, 2		3 (12.5 %)		5 (16.1 %)	1 (9.1 %)	
3	3 (16.7 %)					
Adjuvant endocrine therapy	8 (44.4 %)	9 (37.5 %)	0.650	13 (41.9 %)	4 (36.4 %)	0.746
TAM (+ LH-RH agonist)	10 (55.6 %)	15 (62.5 %)		18 (58.1 %)	7 (63.6 %)	
ANA						
Disease free survival (days)	12 (66.7 %)	9 (37.5 %)	0.061	19 (61.3 %)	2 (18.2 %)	0.014
≤1462		15 (62.5 %)		12 (38.7 %)	9 (81.8 %)	
>1462	6 (33.3 %)					
TILs density	15 (83.3 %)	21 (87.5 %)	0.703	27 (87.1 %)	9 (81.8 %)	0.667
≤10		3 (12.5 %)		4 (12.9 %)	2 (18.2 %)	
>10	3 (16.7 %)					
TILs density	0 (0.0 %)	6 (25.0 %)	0.022	2 (6.5 %)	4 (36.4 %)	0.015
Absent	18 (100.0 %)	18 (75.0 %)		29 (93.5 %)	7 (63.6 %)	
Not absent						

HR+/HER2- breast cancer: hormone receptor-positive and human epidermal growth factor receptor 2 negative breast cancer. BCT: breast conserving treatment. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor-infiltrating lymphocytes.

Focusing on TIL density, no significant difference was observed when the cutoff value of TIL density was set at 10%. However, of the six patients with no TIL density, two had regional lymph node recurrence and

four had distant metastatic recurrence. Therefore, in patients with no TIL density, local recurrence was significantly less ($p = 0.022$) and distant metastasis was significantly more ($p = 0.015$) than that in patients with TIL density.

Correlations between clinicopathological features and TILs

We examined the correlation between clinicopathological features and TILs (**Table 3**); TIL density tended to be absent in patients with tumor size > 20 mm ($p = 0.078$) and 10% or more in patients with tumor size < 20 mm ($p = 0.078$). Macro-metastasis was found by sentinel lymph node biopsy in 4/6 patients with no TIL density. Significantly more lymph node metastasis was found in patients with no TIL than that in patients with TIL ($p < 0.001$). Significantly higher frequency of venous invasion was found in patients with TIL $\geq 10\%$ than that in patients with TIL $< 10\%$ ($p = 0.007$).

Table 3

Correlation between TILs and clinicopathological features in HR+/HER2- breast cancer not received chemotherapy

Parameters	Tumor- infiltrating lymphocytes (n = 42)					
	Absent (n = 6)	Not absent (n = 36)	<i>p</i> value	≤ 10 (n = 36)	> 10 (n = 6)	<i>p</i> value
Age at operation (years old)	2 (33.3 %)	23 (63.9 %)	0.158	22 (61.1 %)	3 (50.0 %)	0.608
≤ 60	4 (66.7 %)	13 (36.1 %)		14 (38.9 %)	3 (50.0 %)	
> 60						
Tumor size (mm)	1 (16.7 %)	20 (55.6 %)	0.078	16 (44.4 %)	5 (83.3 %)	0.078
≤ 20.0	5 (83.3 %)	16 (44.4 %)		20 (55.6 %)	1 (16.7 %)	
> 20.0						
Tumor size (mm)	4 (66.7 %)	30 (83.3 %)	0.336	28 (77.8 %)	6 (100.0 %)	0.199
≤ 30.0	2 (33.3 %)	6 (16.7 %)		8 (22.2 %)	0 (0.0 %)	
> 30.0						
Pathological lymph node metastasis	2 (33.3 %)	33 (91.7 %)	< 0.001	29 (80.6 %)	6 (100.0 %)	0.237
pN0, pN1mi	4 (66.7 %)	3 (8.3 %)		7 (19.4 %)	0 (0.0 %)	
pN1a						
Progesterone receptor	1 (16.7 %)	2 (5.6 %)	0.328	2 (5.6 %)	1 (16.7 %)	0.328
Negative	5 (83.3 %)	34 (94.4 %)		34 (94.4 %)	5 (83.3 %)	
Positive						
Ki67	6 (100.0 %)	29 (80.6 %)	0.237	29 (80.6 %)	6 (100.0 %)	0.237
≤20 %	0 (0.0 %)	7 (19.4 %)		7 (19.4 %)	0 (0.0 %)	
>20 %						
Surgical treatment	2 (33.3 %)	16 (44.4 %)	0.611	15 (41.7 %)	3 (50.0 %)	0.703
BCT and radiation therapy	4 (66.7 %)	20 (55.6 %)		21 (58.3 %)	3 (50.0 %)	
Mastectomy						

TILs: tumor- infiltrating lymphocytes. HR+/HER2- breast cancer: hormone receptor-positive and human epidermal growth factor receptor 2 negative breast cancer. BCT: breast conserving treatment. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor- infiltrating lymphocytes.

Parameters	Tumor- infiltrating lymphocytes (n = 42)					
	Absent (n = 6)	Not absent (n = 36)	p value	≤ 10 (n = 36)	> 10 (n = 6)	p value
Lymphatic invasion	3 (50.0 %)	18 (50.0 %)	1.000	18 (50.0 %)	3 (50.0 %)	1.000
ly0	3 (50.0 %)	18 (50.0 %)		18 (50.0 %)	3 (50.0 %)	
ly1	3 (50.0 %)	18 (50.0 %)		18 (50.0 %)	3 (50.0 %)	
Venous invasion	6 (100.0 %)	33 (91.7 %)	0.463	35 (97.2 %)	4 (66.7 %)	0.007
v0	6 (100.0 %)	33 (91.7 %)		35 (97.2 %)	4 (66.7 %)	
v1	0 (0.0 %)	3 (8.3 %)		1 (2.8 %)	2 (33.3 %)	
Nuclear grade	5 (83.3 %)	31 (86.1 %)	0.857	31 (86.1 %)	5 (83.3 %)	0.857
1, 2	5 (83.3 %)	31 (86.1 %)		31 (86.1 %)	5 (83.3 %)	
3	1 (16.7 %)	5 (13.9 %)		5 (13.9 %)	1 (16.7 %)	
Adjuvant endocrine therapy	1 (16.7 %)	16 (44.4 %)	0.199	15 (41.7 %)	2 (33.3 %)	0.700
TAM (+ LH-RH agonist)	1 (16.7 %)	16 (44.4 %)		15 (41.7 %)	2 (33.3 %)	
ANA	5 (83.3 %)	20 (55.6 %)		21 (58.3 %)	4 (66.7 %)	
Disease free survival (days)	1 (16.7 %)	20 (55.6 %)	0.078	18 (50.0 %)	3 (50.0 %)	1.000
≤1462	1 (16.7 %)	20 (55.6 %)		18 (50.0 %)	3 (50.0 %)	
>1462	5 (83.3 %)	16 (44.4 %)		18 (50.0 %)	3 (50.0 %)	
Primary recurrence site	0 (0.0 %)	18 (50.0 %)	0.022	15 (41.7 %)	3 (50.0 %)	0.703
Local recurrence	0 (0.0 %)	18 (50.0 %)		15 (41.7 %)	3 (50.0 %)	
Not local recurrence	6 (100.0 %)	18 (50.0 %)		21 (58.3 %)	3 (50.0 %)	
Primary recurrence site	2 (33.3 %)	29 (80.6 %)	0.015	27 (75.0 %)	4 (66.7 %)	0.667
Locoregional recurrence	2 (33.3 %)	29 (80.6 %)		27 (75.0 %)	4 (66.7 %)	
Distant metastasis	4 (66.7 %)	7 (19.4 %)		9 (25.0 %)	2 (33.3 %)	

TILs: tumor- infiltrating lymphocytes. HR+/HER2- breast cancer: hormone receptor-positive and human epidermal growth factor receptor 2 negative breast cancer. BCT: breast conserving treatment. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor- infiltrating lymphocytes.

Prediction of distant metastases in patients with HR+/HER2- breast cancer without chemotherapy

Examining distant metastasis predictors based on the results, axillary lymph node metastasis (p = 0.003, OR = 16.723) and operative method (p = 0.026, OR = 0.044) were found to be independent factors (**Table**

4). TILs (absent vs. present) were predictors of distant metastases as per univariate analysis ($p = 0.015$, $OR = 0.127$), though not independent factors ($p = 0.285$, $OR = 0.144$).

HR+/HER2- breast cancer: hormone receptor-positive and human epidermal growth factor receptor 2 negative breast cancer. CI, confidence intervals. BCT: breast conserving treatment. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor-infiltrating lymphocytes. **Prognosis after recurrence** Using univariate analysis with PFS after recurrence, no clear predictive factor could be identified, since treatment after recurrence was variable (**Additional file 2; Table S2**). On the other hand, as per multivariate analysis with PRS, lymph node metastasis during surgery was found to be a poor prognostic factor ($p = 0.042$, $HR = 17.339$) (**Table 5**).

Table 4. Univariate and multivariate analysis with distant metastasis for HR+/HER2- breast cancer not received chemotherapy

Parameters	Univarite analysis			Multivarite analysis		
	Odd ratio	95 % CI	<i>p</i> value	Odd ratio	95 % CI	<i>p</i> value
Age at operation (years old) ≤ 60 vs > 60	0.455	0.101-2.048	0.299			
Tumor size (mm) ≤ 20.0 vs > 20.0	0.781	0.197-3.106	0.726			
Tumor size (mm) ≤ 30.0 vs > 30.0	0.343	0.037-3.161	0.328			
Pathological lymph node metastasis pN0, pN1mi vs pN1a	12.083	1.880-77.665	0.003	16.723	1.197-520.348	0.050
Progesterone receptor Negative vs Positive	-	-	0.284			
Ki67 ≤20 % vs >20 %	1.156	0.190-7.037	0.875			
Surgical treatment BCT and radiation therapy vs Mastectomy	0.179	0.039-0.821	0.020	0.044	0.001-0.420	0.026
Lymphatic invasion ly0 vs ly1	1.280	0.322-5.088	0.726			
Venous invasion v0 vs v1	1.450	0.118-17.766	0.770			
Nuclear grade 1, 2 vs 3	0.520	0.054-5.021	0.567			
Adjuvant endocrine therapy TAM (+ LH-RH agonist) vs ANA	0.867	0.217-3.461	0.839			
Disease free survival (days) ≤1462 vs >1462	7.125	1.309-38.771	0.014	6.693	0.954-81.406	0.080
TILs density ≤10 vs >10	1.500	0.234-9.611	0.667			

TILs	0.127	0.018- 0.797	0.015	0.144	0.003- 5.060	0.285
Absent vs Not absent						

HR+/HER2- breast cancer: hormone receptor-positive and human epidermal growth factor receptor 2 negative breast cancer. CI, confidence intervals. BCT: breast conserving treatment. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor-infiltrating lymphocytes.

Table 5. Univariate and multivariate analysis with post-recurrence survival after recurrence

Parameters	Univarite analysis			Multivarite analysis		
	Hazard ratio	95 % CI	<i>p</i> value	Hazard ratio	95 % CI	<i>p</i> value
Age at operation (years old) ≤ 60 vs > 60	0.871	0.040-9.101	0.910			
Tumor size (mm) ≤ 20.0 vs > 20.0	1.798	0.172-38.728	0.623			
Tumor size (mm) ≤ 30.0 vs > 30.0	-	-	0.265			
Pathological lymph node metastasis pN0, pN1mi vs pN1a	21.520	1.878-488.577	0.016	17.339	1.112-530.855	0.042
Progesterone receptor Negative vs Positive	-	-	0.545			
Ki67 ≤20 % vs >20 %	1.806	0.084-18.864	0.642			
Surgical treatment BCT and radiation therapy vs Mastectomy	0.422	0.020-4.410	0.467			
Lymphatic invasion ly0 vs ly1	1.906	0.182-41.094	0.589			
Venous invasion v0 vs v1	3.760	0.174-39.725	0.327			
Nuclear grade 1, 2 vs 3	-	-	0.294			
Adjuvant endocrine therapy TAM (+ LH-RH agonist) vs ANA	0.422	0.020-4.410	0.467			
Disease free survival (days) ≤1462 vs >1462	1.229	0.056-13.152	0.870			
Primary recurrence site Locoregional recurrence vs Distant metastasis	9.331	0.877-202.892	0.063	6.057	0.414-178.741	0.187

TILs density ≤10 vs >10	3.405	0.158- 35.582	0.360
TILs Absent vs Not absent	0.211	0.018- 4.800	0.269

CI, confidence intervals. BCT: breast conserving treatment. TAM: tamoxifen. LH-RH: luteinizing hormone-releasing hormone. ANA: anastrozole. TILs: tumor-infiltrating lymphocytes.

Discussion

There are various reports on the prediction of distant metastasis; however, the recurrence site in these reports differs depending on the subtype, and tumor size and lymph node metastasis are common risk factors [2, 25–27]. In addition, young age [2], histopathological grade, and lymphovascular invasion have been reported as risk factors [2, 26], although there are reports suggesting otherwise, as well. In this study, a combination of “BCT and radiation therapy” as post-operative procedures was found to be predictive factor for distant metastasis. The predictive factor status may be attributed to the fact that “BCT and radiation therapy” group was significantly younger, and had larger tumor diameter and higher Ki67 compared to the “mastectomy” group. They are the risk factors listed above. Although each parameter alone was not a predictor, they were found to turn into a predictor when combined.

For the prediction of distant metastasis, some research groups have examined the molecular pathological features of cancer cells; for example, co-expression of $\alpha 6\beta 4$ integrin and neuroepithelioma transforming gene 1 (Net1) [28], expression of YKL-39 (a kind of chitinase-like protein) [29], and overexpression of phosphorylated PLC-gamma-1 (one of the phosphoinositides) were reported in this regard [30]. These expression levels have been shown to promote invasion and metastasis of cancer cells both *in vivo* and *in vitro*; hence, these studies retrospectively examined the use of clinical samples as a predictors of distant metastases. There have also been reports that examined the molecular characteristics of cancer cells with respect to the circulating tumor cells (CTCs) that are deeply involved in distant metastasis [31, 32]. One such report suggested that CTCs may be indicators of the therapeutic effect of chemotherapy or endocrine therapy [33], while other studies reported them to be predictors of distant metastatic recurrence of breast cancer based on clinical data [34, 35]. A few publications have even reported the prediction of distant metastasis using genetic assays [36]. Mutations in p53, a gene involved in deoxyribonucleic acid (DNA) repair, are involved in recurrence; Narod reported p53 accumulation to be a strong predictor of recurrence [37]. Filipits et al. predicted distant metastasis using RNA-based multigene score [38]. A genomic evaluation study showed a higher mutation burden to accompany recurrence than primary tumor [39]. These results together suggest gene mutations to potentially cause local or host immune tolerance at the distant metastatic site [1].

Formation of distant metastases in cancer generally follows the concept of “seed and soil.” While we have discussed the “seed” until now [40], TME corresponds to the “soil” as per this concept [8, 9]. TILs are also included in the cells that constitute the TME. In breast cancer, TILs have been reported to vary by subtype. In particular, HER2-enriched breast cancer and TNBC have been reported to show significantly higher TIL density than HR+/HER2- breast cancer, and TILs have been proven to predict the therapeutic effect of chemotherapy [13, 41–43]. In contrast, there are very few reports examining the correlation between TILs and clinicopathological factors or therapeutic effects in HR+/HER2- breast cancer.

In this study, we excluded patients who had undergone neoadjuvant/adjuvant chemotherapy based on three reasons. First, chemotherapy affects the immune microenvironment, including TILs, in preoperative chemotherapy study, and related changes may affect prognosis [44]. TILs are also predictors of the therapeutic effect of adjuvant chemotherapy; therefore, adjuvant chemotherapy may also affect the tumor immune microenvironment. Secondly, axillary lymph node metastasis before adjuvant chemotherapy was diagnosed based on image only, and therefore, the diagnosis was not accurate. Lastly, different chemotherapy regimens are known to have different effects, and neoadjuvant chemotherapy causes more local recurrence than adjuvant therapy [45].

There are some reports on the relationship between recurrence sites and TILs. Park et al. reported patients with TILs > 10% in early-stage TNBC to show significantly more locoregional recurrence than those with lower TILs [46]. In cervical squamous cell carcinoma, low TILs are also likely to cause distant metastasis [47]. Moreover, distant metastasis is reportedly predicted from pathological features, including lymphocytes of lymph nodes in breast cancer with lymph node metastasis, although not from the density of lymphocytes around the tumor [48]. Bidwell et al. have shown innate immune escape to promote bone metastasis, based on clinical data and experiments in mice [49]. Some studies using breast cancer cell lines *in vivo* also reported that immunosuppression in the tumor immune environment increased the risk of lung metastasis [50, 51]. In this study, TIL density was suggested to possibly be a distant metastatic predictor, although not an independent factor. We previously reported that TILs may also be involved in lymph node metastasis in HR+/HER2- breast cancer [52]. In this study, TILs were considered to be strongly correlated with lymph node metastasis, and were involved in distant metastasis prediction as well as prognosis after recurrence.

The greatest limitation of this study was that very few cases of recurrence were examined. Another limitation was that the patients received different types of adjuvant endocrine therapy. Moreover, younger and lymphovascular invasion, which were previously reported as risk factors, were not found to be predictors in this study. TILs may also be involved in venous invasion. Furthermore, considering that the TIL subtypes have different functions, detailed studies are necessary to identify the organs that are prone to metastasis. Nevertheless, TILs can be easily evaluated using needle biopsy specimens for the diagnosis of breast cancer and are highly useful. Filipits et al. predicted distant metastases using a combination of the above-mentioned predictors [38] and TILs may be considered to be an additional predictor. Currently, postoperative follow-up involves patient interview, palpation, and mammography. Whole-body imaging for asymptomatic breast cancer patients post resection surgery is not recommended

[4–6]. However, late diagnosis of distant metastasis, that is, after emergence of detectable symptoms, leads to impairment of the patient's quality of life. Moreover, the risk of reduced treatment options, owing to the deterioration in the general condition as a result of recurrence, prevails. Therefore, it is important to identify and diagnose breast cancer that is prone to distant metastasis.

Conclusions

In stages I–II of HR+/HER2- breast cancer, locoregional recurrence is common and distant metastasis is relatively rare. However, prediction of distant metastasis in breast cancer recurrence is important for better prognosis and patient's quality of life. This study suggested TILs to possibly be one of predictors of distant metastatic recurrence in stages I–II of HR+/HER2- breast cancer in patients who do not undergo chemotherapy.

Abbreviations

BC: breast cancer, CI: confidence interval, CT: computed tomography, CTCs: circulating tumor cells, DFS: disease free survival, DNA: deoxyribonucleic acid, ER: estrogen receptor, HER2: human epidermal growth factor receptor 2, HR+/HER2- breast cancer: hormone receptor-positive and HER2-negative breast cancer, HRs: hazard ratios, Net1: neuroepithelioma transforming gene 1, OR: odd ratio, PFS: progression-free survival, PgR: progesterone receptor, PRS: post-recurrence survival, TNBC: triple-negative breast cancer, TILs: tumor infiltrating lymphocytes, TME: tumor microenvironment, US: ultrasonography

Declarations

Ethics approval and consent to participate

A written informed consent to participate in the study was obtained from each subject in accordance with the declaration of Helsinki principles. Each patient or the patient's family was fully 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 (approve number #926).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable 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. KT collected the data and wrote the manuscript. SK, YA, WG, RK, AY, SI, and TM performed the operation and designed the study. KT and SK summarized the data and revised the manuscript. MS, HT, KH, and MO provided a 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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Author's information

¹Department of Breast and Endocrine Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan.

²Department of Gastrointestinal Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan.

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Figures

Fig. 1 Takada K. et al.

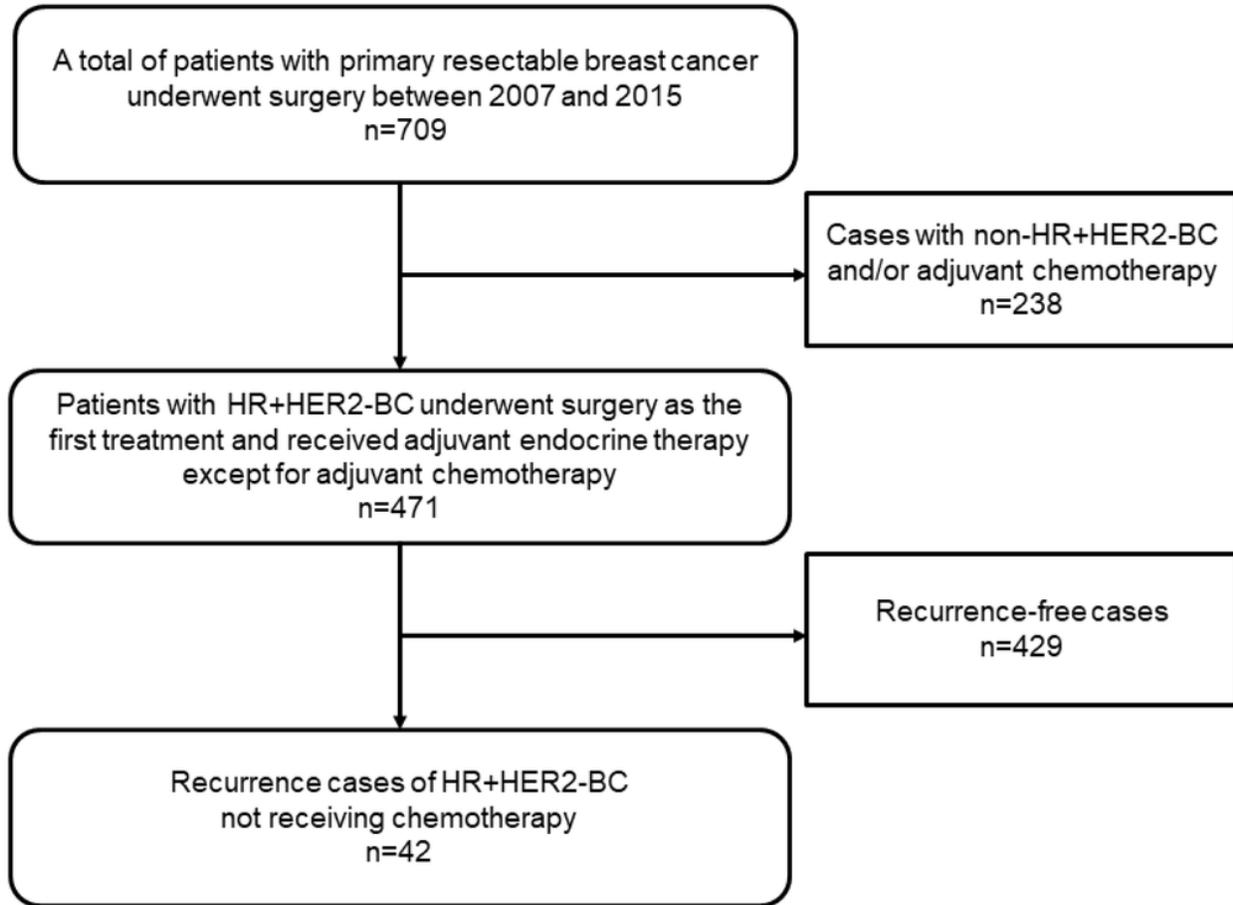


Figure 1

Consort diagram. A total of 709 patients with primary resectable breast cancer underwent surgery between 2007 and 2015 at the Osaka City University Hospital. Two-hundred and thirty-eight patients were excluded from this study because they had non-HR+/HER2- breast cancer and/or received adjuvant chemotherapy. Four-hundred and seventy-one patients with HR+/HER2- breast cancer underwent surgery as the first treatment and received adjuvant endocrine therapy except for adjuvant chemotherapy. Since four-hundred and twenty-nine patients did not have recurrence during postoperative follow-up, forty-two patients with recurrence were targeted in this study.

Fig. 2 Takada K. et al.

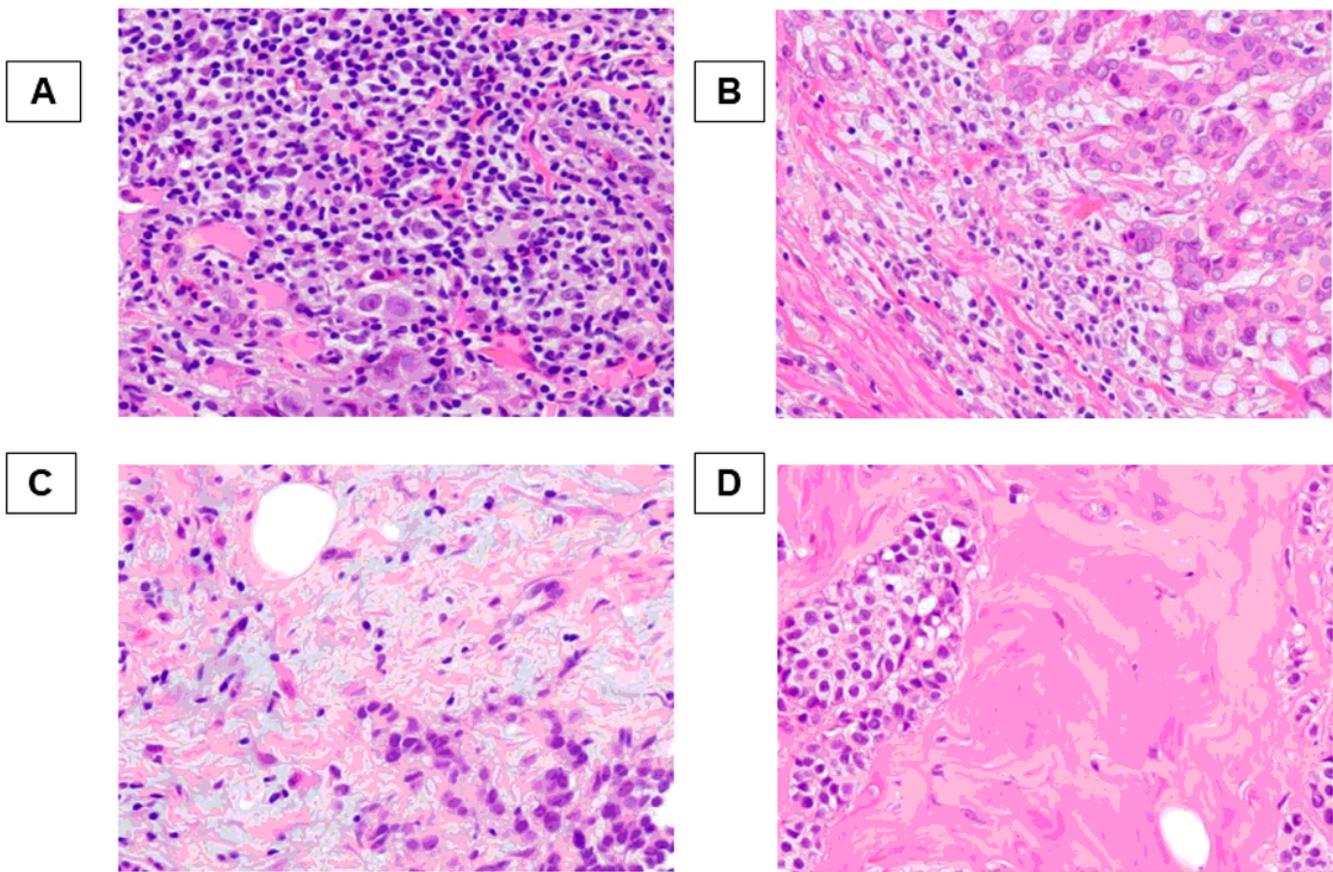


Figure 2

Histopathologic evaluation of the tumor-infiltrating lymphocytes (TILs) density was performed on hematoxylin and eosin-stained tumor section. The density of them was averaged on full sections, at least five fields, of the tumor stroma. The results were divided into four groups (>50% (A); >10–50% (B); ≤10% (C); and absent (D), respectively).

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