

Predicting therapeutic efficacy of endocrine therapy for stage IV breast cancer by tumor-infiltrating lymphocytes

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Abstract. The tumor immune environment not only modulates the effects of immunotherapy, but also the effects of other anticancer drugs and treatment outcomes. These immune responses may be evaluated by measuring tumor-infiltrating lymphocytes (TILs), which has been frequently verified clinically. In the present study, the prediction of the therapeutic effect of endocrine therapy by TILs on stage IV breast cancer was clinically analyzed. Data from 40 patients who underwent endocrine therapy as the initial drug therapy for stage IV breast cancer were used. The correlation between TILs, evaluated according to standard methods, and prognosis, including the efficacy of endocrine therapy, was investigated retrospectively. Patients with $\geq 50\%$ lymphocytic infiltration were considered to have lymphocyte-predominant breast cancer (LPBC). An analysis of outcomes revealed no difference in progression-free survival (PFS; $P=0.171$), time to treatment failure (TTF; $P=0.054$), or overall survival (OS; $P=0.641$) between the high TIL ($>10\%$) and low TIL ($\leq 10\%$) groups. Patients with LPBC ($\geq 50\%$) exhibited a significant prolongation of PFS ($P=0.005$, log-rank), TTF ($P=0.001$) and OS ($P=0.027$) compared with non-LPBC patients. On receiver operating characteristics (ROC) curve analysis, better results were obtained with LPBCs [area under the curve (AUC)=0.700] than with TILs (AUC=0.606). The present findings suggest that a high level of lymphocytic infiltration in the tumor stroma may serve as a predictor of the therapeutic efficacy of endocrine therapy in patients with stage IV estrogen receptor-positive breast cancer.

Introduction

Breast cancer with distant metastasis at first presentation (stage IV disease) is often encountered in the outpatient setting. With the recent advances in multimodal therapies for breast cancer, long-term survival may be expected, even in stage IV breast cancer with distant metastasis (1). However, one of the goals in treating metastatic disease is prolongation of survival while maintaining good quality of life (QOL). Endocrine therapy is suitable for this purpose. Adverse events are less severe with endocrine therapy compared with those with chemotherapy; therefore, due to its ability to maintain QOL, endocrine therapy is the preferred first-line treatment for non-life-threatening hormone receptor-positive advanced breast cancer (Hortobagyi's algorithm) (2). However, although endocrine therapy is useful for certain types of hormone receptor-positive breast cancer (HRBC), there are other types for which it is not very effective. In neoadjuvant endocrine therapy for early-stage breast cancer, the use of the preoperative endocrine prognostic index score and Ki67 as predictive markers has been reported (3,4). However, few studies to date have investigated the prediction of the therapeutic effect of endocrine therapy in stage IV breast cancer. Stage IV breast cancer is advanced and, if it cannot be controlled with initial drug therapy, it is life-threatening. Therefore, the ability to predict therapeutic efficacy would be of considerable value for selecting the initial drug therapy (endocrine therapy or chemotherapy).

Cancer cells harbor various gene abnormalities that allow them to proliferate spontaneously and survive, but they are also affected by the surrounding environment, which is involved in the intrinsic characteristics of cancer (5). The tumor immune environment not only modulates the effects of immunotherapy, but also the effects of other anticancer drugs and treatment outcomes (6,7). Thus, the importance of inhibiting and improving the tumor immune microenvironment is well-recognized. These immune responses may be evaluated by tumor-infiltrating lymphocytes (TILs), which has been demonstrated clinically (8-10). We also previously reported the prediction of the therapeutic efficacy of neoadjuvant chemotherapy (NAC) by TILs (11). The aim of the present study was

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to clinically verify the prediction of the therapeutic efficacy of endocrine therapy for stage IV breast cancer using TILs.

Patients and methods

Patient background. The same methods as those used in the present study were previously used to investigate the significance of NAC for early breast cancer (11-14). Data from 40 patients who underwent endocrine therapy as the initial drug therapy for stage IV breast cancer at Osaka City University Hospital between June 2004 and December 2013 were used. The median follow-up time was 155 weeks (range, 13-553 weeks). The overall response rate (ORR), clinical benefit rate (CBR), disease control rate (DCR), overall survival (OS), time to treatment failure (TTF) and progression-free survival (PFS) were calculated to determine the efficacy of this regimen. Additionally, tumor stage and T and N factors were stratified based on the TNM Classification of Malignant Tumors, Union for International Cancer Control (UICC), 7th edition (15). Breast cancer was histologically confirmed by core needle biopsy 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 receptors (ERs), progesterone receptors (PgRs), human epidermal growth factor receptor (HER)2 and Ki67. Based on their immunohistochemical expression, the tumors were categorized into the following immunophenotypes: Luminal A (ER⁺ and/or PgR⁺, HER2⁻, Ki67-low), luminal B (ER⁺ and/or PgR⁺, HER2⁺; or ER⁺ and/or PgR⁺, HER2⁻, Ki67-high), HER2-enriched (HER2BC; ER⁻, PgR⁻, HER2⁺) and triple-negative breast cancer (TNBC; ER⁻, PgR⁻, HER2⁻) (16). In the present study, luminal A and B were considered as HRBC.

Endocrine therapy was administered on an outpatient basis in all cases. This protocol was repeated until progressive disease (PD) was detected or a severe adverse event requiring the discontinuation of the scheduled endocrine therapy was reported. The therapeutic antitumor effects were assessed according to the Response Evaluation Criteria in Solid Tumors (17). All clinical analyses in the present study were based on imaging assessment.

TTF was evaluated on a daily basis and was defined as the period from the date of treatment initiation to discontinuation for any reason, including disease progression, treatment toxicity and death. OS was evaluated on a weekly basis and was defined as the period from the date of treatment initiation to the date of death from any cause. PFS was evaluated on a weekly basis and was defined as the period from the date of treatment initiation to the date of death or the date of confirmation of PD.

Histopathological evaluation of TILs. Histopathological assessment for predictive factors was performed using core needle biopsy specimens at the time of breast cancer diagnosis. The histopathological parameters examined included nuclear grade, histological type, presence of TILs, and correlation of these parameters with intrinsic subtypes and pathological complete response. Histopathological analysis of the percentage of TILs was evaluated on a single full-face hematoxylin and eosin (HE)-stained tumor section using the

criteria described by Salgado *et al* (18). TILs were defined as the infiltrating lymphocytes within the tumor stroma and were expressed as a proportion of the field investigated; additionally, the number of TILs in the stroma surrounding the stained cancer cells was quantitatively measured in each field at a magnification, x400 (19,20). Areas of carcinoma *in situ* 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. 1). TILs were considered 'high' for scores ≥2, and 'low' for scores 1 and 0. Patients with ≥50% lymphocytic infiltration were considered to have lymphocyte-predominant breast cancer (LPBC) (21). Histopathological evaluation of TILs was jointly performed by two breast pathologists who were blinded to clinical information, including treatment allocation and outcomes.

Statistical analysis. Statistical analysis was performed using SPSS version 19.0 (SPSS Inc.). The associations between TILs, LPBC and clinicopathological variables were analyzed using Chi-squared or Fisher's exact tests, as appropriate. OS, TTF and PFS were estimated using the Kaplan-Meier method and compared using log-rank tests. Univariate and multivariate hazard ratios (HRs) were computed for the study parameters with 95% confidence intervals (CIs) using a Cox proportional hazards model, and used in a backward stepwise method for variable selection in multivariate analyses. P<0.05 was considered to indicate statistically significant differences.

Ethics approval. The present 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 following a retrospectively written research, pathological evaluation, and statistical plan (22). The research protocol conformed to the provisions of the Declaration of Helsinki as revised in 2013. All patients were informed of the investigational nature of the study and provided written informed consent. The study protocol was approved by the Ethics Committee of Osaka City University (approval no. 926).

Results

Endocrine therapy in stage IV breast cancer. The background characteristics of 40 patients who underwent endocrine therapy as the initial drug therapy for stage IV breast cancer are shown in Table I. All patients were women with a median age of 64 years (range, 44-88 years); the bone or soft tissue was the site of metastasis in 21 patients (52.5%), and visceral metastasis was present in 19 patients (47.5%). A total of 33 patients (82.5%) were strongly positive and 7 (17.5%) were weakly positive for ER expression; a total of 14 patients (35.0%) were strongly positive and 26 (65.0%) were weakly positive for PgR expression; a total of 4 patients (10.0%) were positive and 36 (90.0%) were negative for HER2 expression; as regards Ki67 expression, 7 patients (17.5%) exhibited high levels and 33 patients (82.5%) exhibited low levels. Endocrine therapy consisted of letrozole in 22 patients (55.0%), anastrozole in 11 patients (27.5%), tamoxifen ± luteinizing hormone-releasing hormone agonist in 6 patients (15.0%), and

Table I. Demographic data of 40 patients receiving endocrine therapy for stage IV breast cancer.

| Parameters (n=40) | Patient no. (%) |
|---|---|
| Age (years) | 64 (range, 44-88) |
| Menopause | |
| Post-/premenopausal | 34 (85.0)/6 (15.0) |
| Degree of progression (metastasis) | |
| Bone or soft tissue/visceral | 21 (52.5)/19 (47.5) |
| Estrogen receptor | |
| Strongly positive/weakly positive | 33 (82.5)/7 (17.5) |
| Progesterone receptor | |
| Strongly positive/weakly positive | 14 (35.0)/26 (65.0) |
| HER2 | |
| Negative/positive | 36 (90.0)/4 (10.0) |
| Ki67 | |
| ≤14/>14% | 33 (82.5)/7 (17.5) |
| Treatment | |
| Letrozole/anastrozole/tamoxifen ± LHRH agonist/exemestane | 22 (55.0)/11 (27.5)/6 (15.0)/1 (2.5) |
| Clinical response | |
| CR/PR/SD ≥24 weeks/SD <24 weeks/PD | 0 (0.0)/30 (75.0)/4 (10.0)/2 (5.0)/4 (10.0) |
| Clinical response | |
| ORR/CBR/DCR | 30 (75.0)/30 (85.0)/4 (90.0) |

HER2, human epidermal growth factor receptor 2; LHRH, luteinizing hormone-releasing hormone; pCR, pathological complete response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; CBR, clinical benefit response; DCR, disease control rate.

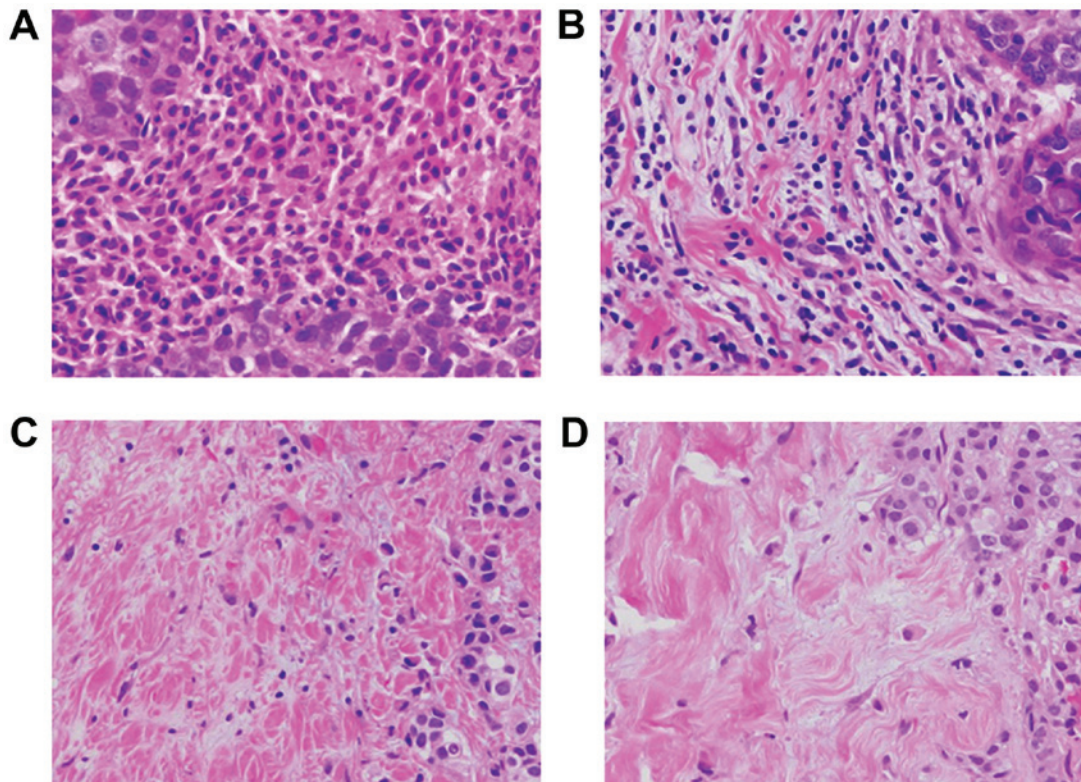


Figure 1. Histopathological TIL evaluation. TILs were measured by examining the occupation ratio of immune cells present in the tumor stroma of hematoxylin and eosin-stained specimens at a magnification, x400. Proportional scores of 3, 2, 1 and 0 were assigned if the area of stroma containing lymphoplasmacytic infiltration around invasive tumor cell nests comprised (A) >50%, (B) 10-50%, (C) ≤10% and (D) 0%, respectively, of the total area.

Table II. Correlation of clinicopathological characteristics with TILs and LPBC in 40 stage IV breast cancer patients.

| Parameters | TILs, n (%) | | P-value | LPBC, n (%) | | P-value |
|--------------------------------|-------------|------------|---------|-------------|-----------------|---------|
| | High (n=13) | Low (n=27) | | LPBC (n=9) | Non-LPBC (n=31) | |
| Age at surgery, years | | | | | | |
| ≤64 | 7 (53.8) | 14 (51.9) | 0.906 | 7 (77.8) | 14 (45.2) | 0.088 |
| >64 | 6 (46.2) | 13 (48.1) | | 2 (22.2) | 17 (54.8) | |
| Menopausal status | | | | | | |
| Premenopausal | 2 (15.4) | 4 (14.8) | 0.649 | 2 (22.2) | 4 (12.9) | 0.410 |
| Postmenopausal | 11 (84.6) | 23 (85.2) | | 7 (77.8) | 27 (87.1) | |
| Estrogen receptor | | | | | | |
| Strongly positive | 9 (69.2) | 24 (88.9) | 0.139 | 7 (77.8) | 26 (83.9) | 0.504 |
| Weakly positive | 4 (30.8) | 3 (11.1) | | 2 (22.2) | 5 (16.1) | |
| Progesterone receptor | | | | | | |
| Strongly positive | 4 (30.8) | 10 (37.0) | 0.491 | 4 (44.4) | 10 (32.3) | 0.383 |
| Weakly positive | 9 (69.2) | 17 (63.0) | | 5 (55.6) | 21 (67.7) | |
| HER2 | | | | | | |
| Negative | 12 (92.3) | 24 (88.9) | 0.608 | 8 (88.9) | 28 (90.3) | 0.656 |
| Positive | 1 (7.7) | 3 (11.1) | | 1 (11.1) | 3 (9.7) | |
| Ki67, % | | | | | | |
| ≤14 | 10 (76.9) | 23 (85.2) | 0.408 | 7 (77.8) | 26 (83.9) | 0.504 |
| >14 | 3 (23.1) | 4 (14.8) | | 2 (22.2) | 5 (16.1) | |
| Degree of progression | | | | | | |
| Bone or soft tissue metastasis | 7 (53.8) | 14 (51.9) | 0.906 | 4 (44.4) | 17 (54.8) | 0.431 |
| Visceral metastases | 6 (46.2) | 13 (48.1) | | 5 (55.6) | 14 (45.2) | |
| Objective response rate | | | | | | |
| ORR | 9 (69.2) | 21 (77.8) | 0.414 | 7 (77.8) | 23 (74.2) | 0.601 |
| Non-ORR | 4 (30.8) | 6 (22.2) | | 2 (22.2) | 8 (25.8) | |

HER2, human epidermal growth factor receptor 2; ORR, objective response rate; LPBC, lymphocyte-predominant breast cancer; TILs, tumor-infiltrating lymphocytes.

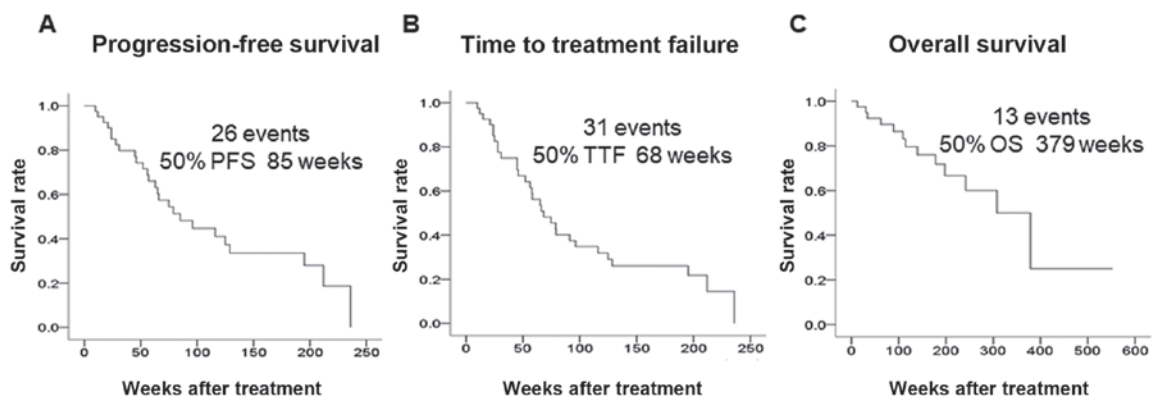


Figure 2. Outcomes of endocrine therapy for stage IV breast cancer. Kaplan-Meier curves of the indicated clinical effects of endocrine therapy are shown. (A) The median PFS was 85 weeks, (B) the median TTF was 68 weeks, and (C) the median OS was 379 weeks. PFS, progression-free survival; TTF, time to treatment failure; OS, overall survival.

exemestane in 1 patient (2.5%). As regards tumor response, the ORR was 75.0%, the CBR was 85.0%, and the DCR was 90.0%. The median PFS was 85 weeks, the median TTF was 68 weeks, and the median OS was 379 weeks (Fig. 2).

Prediction of therapeutic effect using TILs. Among the 40 patients, TIL levels were high (>10%) in 13 (32.5%) and low (≤10%) in 27 (67.5%) patients. A total of 9 patients (22.5%) had LPBC (≥50% lymphocyte infiltration), and

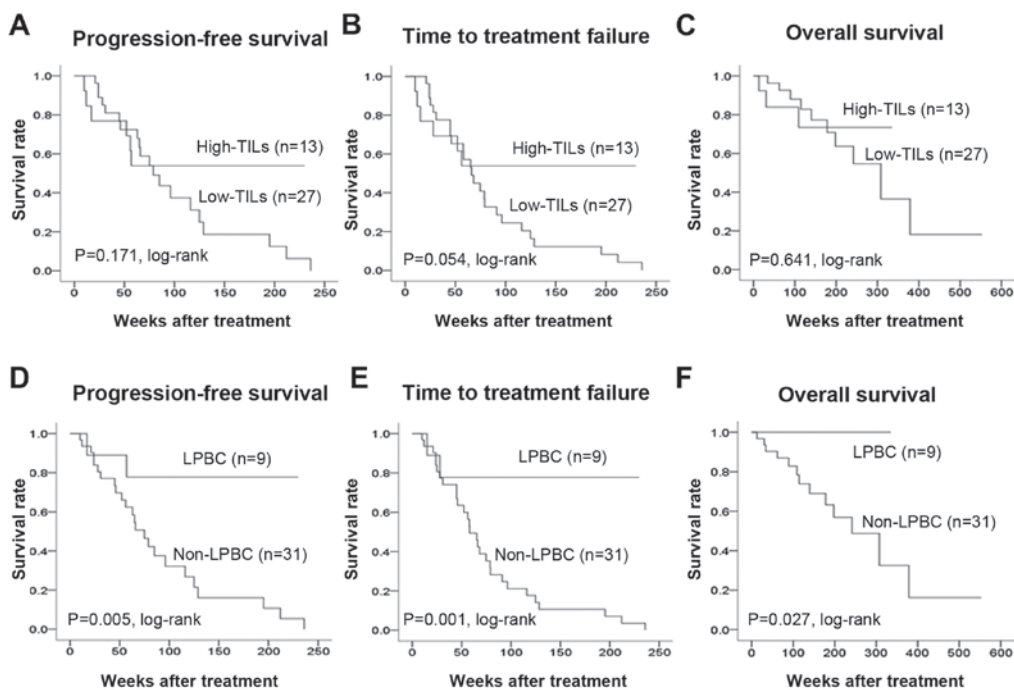


Figure 3. PFS, TTF and OS in stage IV breast cancer according to TIL levels. An analysis of outcomes of the high and low TIL groups revealed no difference in (A) PFS ($P=0.171$, log-rank), (B) TTF ($P=0.054$, log-rank), or (C) OS ($P=0.641$, log-rank) between the two groups. LPBC patients exhibited a significant prolongation of the (D) PFS ($P=0.005$, log-rank), (E) TTF ($P=0.001$, log-rank) and (F) OS ($P=0.027$, log-rank) compared to non-LPBC patients. PFS, progression-free survival; TTF, time to treatment failure; OS, overall survival; TILs, tumor-infiltrating lymphocytes; LPBC, lymphocyte-predominant breast cancer.

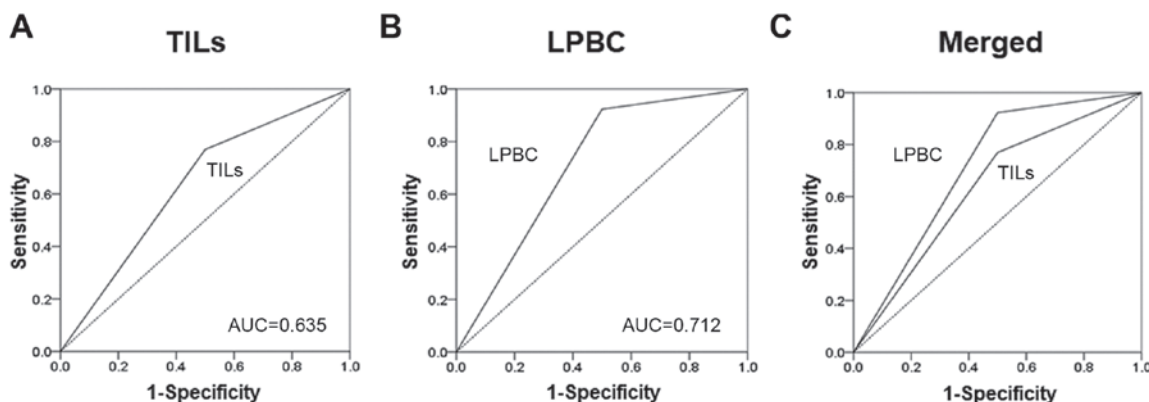


Figure 4. On receiver operating characteristics (ROC) curve analysis, better results were obtained with LPBC ($AUC=0.700$) than with TILs ($AUC=0.606$). (A) TILs, (B) LPBCs and (C) merged. AUC, area under the ROC curve; TILs, tumor-infiltrating lymphocytes; LPBC, lymphocyte-predominant breast cancer.

31 patients (77.5%) had non-LPBC. Investigation of the clinicopathological characteristics of the patients revealed no significant differences between the high TIL and low TIL groups (Table II). There were also no significant differences between LPBC and non-LPBC patients. An analysis of outcomes revealed no difference in PFS ($P=0.171$, log-rank), TTF ($P=0.054$, log-rank), or OS ($P=0.641$, log-rank) between the high and low TIL groups (Fig. 3). LPBC patients had significantly prolonged PFS ($P=0.005$, log-rank), TTF ($P=0.001$, log-rank) and OS ($P=0.027$, log-rank) compared with non-LPBC patients. Univariate analysis of PFS found that responding to treatment ($HR=0.179$, $P<0.001$) and LPBC ($HR=0.158$, $P=0.013$) were factors associated with a favorable prognosis (Table III). Multivariate analysis confirmed that responding to treatment ($HR=0.048$,

$P<0.001$) and LPBC ($HR=0.058$, $P=0.001$) were independent factors associated with a favorable prognosis.

On receiver operating characteristics (ROC) curve analyses, better results were obtained with LPBCs [area under the curve (AUC)=0.700] compared with TILs ($AUC=0.606$) (Fig. 4).

Discussion

The immune system has recently been found to affect the therapeutic efficacy, and it plays an important role in patient prognosis, even in the field of breast cancer (5,23,24). TILs, an indicator for monitoring antitumor immune responses, are reported to be chemosensitive in breast cancers such as TNBC and HER2BC, which display a high degree of immune

Table III. Univariate and multivariate analysis with respect to progression-free survival in 40 stage IV breast cancer patients.

| Parameters | Univariate analysis | | | Multivariate analysis | | |
|-----------------------|---------------------|-------------|---------|-----------------------|-------------|---------|
| | Hazard ratio | 95% CI | P-value | Hazard ratio | 95% CI | P-value |
| Age, years | | | | | | |
| ≤64 | 1.138 | 0.516-2.511 | 0.749 | | | |
| >64 | | | | | | |
| Menopausal status | | | | | | |
| Premenopausal | 2.209 | 0.515-9.478 | 0.286 | | | |
| Postmenopausal | | | | | | |
| Degree of progression | | | | | | |
| Bone or soft tissue | 0.605 | 0.271-1.355 | 0.222 | | | |
| Visceral metastases | | | | | | |
| TILs | | | | | | |
| High | 0.527 | 0.208-1.339 | 0.178 | | | |
| Low | | | | | | |
| Ki67, % | | | | | | |
| ≤14 | 1.049 | 0.391-2.815 | 0.925 | 1.578 | 0.540-4.610 | 0.405 |
| >14 | | | | | | |
| Response rate | | | | | | |
| ORR | 0.179 | 0.072-0.443 | <0.001 | 0.048 | 0.011-0.199 | <0.001 |
| Non-ORR | | | | | | |
| LPBC | | | | | | |
| Yes | 0.158 | 0.037-0.680 | 0.013 | 0.058 | 0.010-0.333 | 0.001 |
| No | | | | | | |

CI, confidence interval; TILs, tumor-infiltrating lymphocytes; ORR, objective response rate; LPBC, lymphocyte-predominant breast cancer.

activity (10,25-27). However, although it is known that there are few TILs in ER-positive breast cancer, the association of these TILs with endocrine therapy has yet to be determined (20).

Cancer cells have various gene abnormalities that allow them to proliferate spontaneously and survive, but they are also affected by the surrounding environment (cancer microenvironment), which is involved in the intrinsic characteristics of cancer (5). On the other hand, the ER, which is crucial for the development and progression of breast cancer, is activated not by estrogen alone, but by a complex signaling cascade, including a pathway that is mediated by growth factor-stimulated phosphorylation, and its regulation depends on the microenvironment surrounding the cancer cells (28). Specifically, even in endocrine therapy, which exerts an antitumor effect by blocking estrogen activity and inhibiting estrogen production, the regulation and improvement of the cancer microenvironment are crucial to therapy. In the present study, it was possible to predict the therapeutic effect of endocrine therapy in LPBC, which displays a high degree of lymphocyte infiltration. In ER-positive breast cancer, due to the small number of TILs, a high degree of lymphocytic infiltration is considered predictive of the therapeutic effect.

In addition, immunogenic cell death (ICD) is induced by both chemotherapy and radiotherapy (29-31). Cancer cells that cause ICD release nucleoproteins, such as high mobility group box 1 protein (HMGB1). HMGB1 acts on toll-like receptor

4, which is expressed on dendritic cells (DC) to induce DC maturation, and promotes antigen presentation and T-cell activation (32). In this manner, anticancer treatments markedly affect the immune response of the body, and it is believed that an immune response is activated by a similar mechanism in endocrine therapy as well. The present findings suggest that LPBC patients, who display a high level of immunoactivity, respond to treatment with a greater level of sensitivity, as the tumor immune microenvironment is modulated by endocrine therapy.

In the present study, confirmation of LPBC by evaluating TILs in biopsy samples in stage IV breast cancer was found to contribute to the selection of the appropriate initial drug therapy. However, the fact that this was a small-scale retrospective study is a major limitation. It will be necessary to perform future analyses of subsets of TILs and to verify the immunologically relevant genes involved in endocrine therapy. In the future, it is expected that the suitability of LPBC as a predictive factor of therapeutic efficacy will be investigated in other studies, including the PROACT study, in which neoadjuvant endocrine therapy is currently being investigated, as well as in the ACOSOG Z1031 study (4,33).

The findings of the present study suggest that a high level of lymphocytic infiltration in the tumor stroma may serve as a predictor of the therapeutic efficacy of endocrine therapy for patients with stage IV ER-positive breast cancer.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YA participated in the design of the study and drafted the manuscript. SK participated in the design of the study and manuscript editing. WG, KoT, MS, RA and TT helped with data collection and manuscript preparation. KaT and ST helped with study data collection and participated in its design. KH and MO conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This research conformed to the provisions of the Declaration of Helsinki, 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 (approval no. 926).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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