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Association of Programmed Death-Ligand 1 Expression in Relation to Tumor-Infiltrating Lymphocyte Concentration and Histological Type with Outcomes of Triple-Negative Breast Cancer

Hiroko Yukawa, Akira Hirano, Yuko Ishibashi, Saki Fujita, Asaka Kodera, Yumi Anzai and Masafumi Kurosumi

Department of Breast Surgery, Tokyo Women's Medical University Adachi Medical Center, Tokyo, Japan

Background: Triple-negative breast cancer (TNBC) comprises subgroups with distinct characteristics and histological types. Tumor-infiltrating lymphocyte (TIL) concentration and programmed death-ligand 1 (PD-L1) expression are prognostic factors for TNBC. We analyzed the association of immune cell PD-L1 expression, in relation to histological type and TIL concentration, with TNBC outcomes.

Methods: Data from 86 patients with TNBC treated between 2008 and 2014 were analyzed. Those treated with immune-checkpoint inhibitors (ICIs) were excluded. PD-L1 expression in immune cells was assessed by immunohistochemistry using an SP142 clone. TIL concentration was measured with hematoxylin and eosin staining. Tumor histology was classified as basal type (G1), apocrine type (G2), metaplastic change (G3), special type (G4), and adenoid cystic carcinoma (G5).

Results: The rate of PD-L1 positivity was 2.5%, 17.3%, and 58.6% for patients with TIL concentrations classified as low (TIL-L), moderate (TIL-M), and high (TIL-H) ($p < 0.0001$). Five-year overall survival (OS) was 78.8% among patients with PD-L1-positive tumors and 81.8% among those with PD-L1-negative tumors. Among TIL-L patients, 5-year OS in PD-L1-positive and -negative tumors was 100% and 77.4%, respectively ($p = 0.9993$). Among TIL-H patients, 5-year OS for PD-L1-positive and -negative tumors was 73.0% and 83.3%, respectively ($p = 0.8241$). In multivariate analysis, tumor size and lymphatic vessel invasion were independent prognostic factors for OS.

Conclusions: The rate of PD-L1 positivity was higher in TIL-H patients. Patients classified as TIL-H and PD-L1-positive had worse TNBC outcomes.

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Key words: TILs, immune checkpoints, PD-L1, triple-negative breast cancer, histological subtypes

Introduction

Triple-negative breast cancer (TNBC) refers to tumors that do not express hormone receptors or overexpress human epidermal growth factor receptor 2 (HER2). Chemotherapy is the primary systemic therapy for TNBC[1,2]. TNBC has a high biological grade and high rates of distant metastasis and recurrence[3,4]. Combining chemotherapy with immune checkpoint inhibitors (ICIs) improves outcomes in programmed death-ligand 1 (PD-L1)-positive TNBC[5,6]. TNBC comprises multiple subgroups with distinct characteristics and histological types. These subtypes differ in prognosis[7] and sensitivity to chemotherapy[8–10]. Tumor-infiltrating lymphocytes (TILs) are crucial when treating TNBC. They have been observed in TNBC and extensively studied[11–13]. High TIL levels are associated with better outcomes for TNBC and HER2-positive breast cancer[14–16].

PD-L1 is a crucial immune checkpoint protein that prevents an immune response against tumor cells. Programmed cell death protein 1 (PD-1)/PD-L1 is a target of ICIs, and anti-PD-1/PD-L1 drugs have been developed for immune checkpoint blockade. Because of the demon-

Correspondence to Hiroko Yukawa, Department of Breast Surgery, Tokyo Women's Medical University Adachi Medical Center, 4–33–1 Kohoku, Adachi-ku, Tokyo 123–8558, Japan
E-mail: yukawa.hiroko@twmu.ac.jp
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strated effectiveness of ICIs, it is crucial to understand TIL status as an indicator of immune response in the surrounding tumor microenvironment[17,18]. TIL concentration and PD-L1 expression are prognostic factors for TNBC[19,20]; however, the association of PD-L1 expression, in relation to TIL concentration and TNBC histological type, with outcome is unclear. Therefore, we assessed PD-L1 expression and outcomes in patients with TNBC classified by histological type and TIL concentration.

Materials and Methods

The study protocol was approved by the institutional review board of Tokyo Women's Medical University (approval no. 5374R). Informed consent was obtained from all participants.

Patients and Sample

We retrospectively analyzed data from 86 patients with metastatic TNBC (between 2008 and 2014) who did not undergo ICI therapy at our facility. This study adhered to the ethical principles of the Helsinki Declaration. TNBC was defined as the absence of estrogen receptors (ER <1%), progesterone receptors (PR <1%), and HER2 overexpression (0, 1+, 2+, and a negative result on fluorescence in situ hybridization).

Hematoxylin and Eosin Staining and Immunohistochemistry

Tumor subtype was determined by immunohistochemical (IHC) staining using core needle biopsy (CNB). The formalin fixation time ranged from 24 to 36 hours but was fixed within 48 hours.

Assessment of TIL Concentration and PDL-1 Status

TILs were collected from stromal tissues and assessed in sections stained with hematoxylin and eosin (H&E), in accordance with the guidelines of the International TILs Working Group[21]. TIL concentration was defined as low (0-10% immune cells in stromal tissue in the tumor), moderate (11-59%), and high (≥60%). PD-L1 expression was assessed in breast tissue samples obtained through CNB, using the SP142 anti-PD-L1 antibody for IHC developed by Ventana Medical Systems. PD-L1 positivity was defined as the presence of PD-L1 expressing tumor-infiltrating immune cells in 1% or more of the tumor area. Analysis was conducted by two experts on breast disease (M.K. and A.H.), who were blinded to the clinical variables.

Histological Grouping

On the basis of pathological features observed in H&E-stained sections, TNBC was categorized into five groups: Group 1 (G1) (basal-type and medullary pattern), G2 (apocrine type), G3 (metaplastic change), G4 (special type, micropapillary and invasive lobular carcinoma), and G5 (adenoid cystic cancer) (47, 22, 7, 8, and 2 cases, respectively). The evaluating pathologist adhered to international pathology guidelines.

Statistical Analysis

Continuous data are presented as median or mean ± SD. Continuous variables were analyzed nonparametrically with the Mann-Whitney U test. Categorical variables were compared with the appropriate χ^2 test. Survival analyses were conducted using the Kaplan-Meier method with the log-rank test and Cox proportional hazards models. Univariate and multivariate logistic regression analyses were used to identify factors that predicted overall survival (OS). Statistical significance was set at a p-value of < 0.05. All statistical data were analyzed using JMP Pro 16 software (SAS Institute).

Results

Table 1 shows the patient characteristics. Among the 86 patients, 26, 48, 8, and 4 had stage I, II, III, and IV disease, respectively. PD-L1-positive expression of tumor-infiltrating immune cells was detected in 21 of 86 patients, and the rate of PD-L1 positivity was 24.4% (**Table 2**). The PD-L1 positivity rates for patients with stage I, II, III, and IV disease were 19.2%, 27.1%, 25.0%, and 25.0%, respectively. The PD-L1 positivity rate was 16.7% in patients with lymphatic vessel invasion (Ly) and 25.7% in those without Ly. The PD-L1 positivity rate in relation to TIL concentration was 2.5%, 17.3%, and 58.6% for TIL-L, TIL-M, and TIL-H, respectively. The value was significantly higher in the TIL-H group than in the other groups ($p < 0.0001$).

PD-L1 Status and Outcome

The 5-year OS was 78.8% and 81.8% in patients with PD-L1-positive and -negative tumors, respectively (hazard ratio [HR]: 1.00, 95% confidence interval [CI]: 0.285-2.81; $p = 0.9933$) (**Fig. 1a**). Among TIL-L patients, 5-year OS was 100% and 77.4% in PD-L1-positive and -negative tumors, respectively (HR: < 0.0001, 95% CI: 0 to < 0.0001; $p = 0.9993$) (**Fig. 1b**). Among TIL-M patients, 5-year OS was 100% and 92.3% in PD-L1-positive and -negative tumors, respectively (HR: < 0.0001, 95% CI: 0 to <0.0001; $p = 0.9993$) (**Fig. 1c**). Among TIL-H patients, 5-year OS was 73.0% and 92.3% in PD-L1-positive and -negative tumors, respectively (HR: 0.854, 95% CI: 0.211-3.42; $p = 0.8241$) (**Fig. 1d**). Five-year distant disease-free survival was 88.9% and 91.1% in patients with PD-L1-positive and -negative tumors, respectively (HR: 1.09, 95% CI:

0.159-4.72; p = 0.9189) (**Fig. 2**). The PD-L1 positivity rates for the G1, G2, G3, G4, and G5 histological types were 36.2%, 4.6%, 14.3%, 25.0%, and 0%, respectively. This value was significantly higher for G1 than for the other types (p = 0.0242).

Among patients with G1 tumors, 5-year OS was 74.8% and 79.6% in those with PD-L1-positive and -negative tumors, respectively (HR: 1.03, 95% CI: 0.310-3.43; p = 0.9588) (**Fig. 3**). For other histological types, there was no correlation between PD-L1 expression and outcome.

In the multivariate OS analysis, tumor size and Ly were independent prognostic factors but PD-L1 positivity was not a significant prognostic factor (HR: 0.663, 95% CI: 0.169-2.60; p = 0.5559) (**Table 3**).

Table 1 Characteristics of patients (n=86)

		Case (%)
Stage	I	26 (30.2)
	II	48 (55.8)
	III	8 (9.3)
	IV	4 (4.7)
Ly	0	74 (86)
	1-3	12 (14.0)
Lymph node status	positive	56 (65.1)
	negative	30 (34.9)
TIL	L (low)	40 (46.5)
	M (moderate)	17 (19.8)
	H (high)	29 (33.7)
Histological type	G1 (basal type, medullary pattern)	47 (54.7)
	G2 (apocrine type)	22 (25.6)
	G3 (metaplastic change)	7 (8.1)
	G4 (special type, invasive micropapillary ca., invasive lobular ca.)	8 (9.3)
	G5 (adenoid cystic ca.)	2 (2.3)

Ly: lymphatic invasion, TIL: tumor-infiltrating lymphocytes, ca.: carcinoma

Discussion

In this study, the rate of PD-L1 positivity was relatively low, 24.4%, but previously reported rates vary. Variation in antibody differences ranged from 6-66% in studies limited to SP142[5,22–24]. Differences in formalin fixation time and pathologist diagnoses likely contribute to the wide range in reported positivity rates. Because biopsy specimens are more consistent than surgical specimens, we used biopsy specimens to assess PD-L1 expression in the present study. Current therapy for treating TNBC involves neoadjuvant chemotherapy with ICI, and assessment of PD-L1 from biopsy specimens is the standard method.

The rate of PD-L1 positivity was consistent across all stages. Our expectation that the positivity rate would increase as disease stage advanced was not correct, but the small size of the specimens may have resulted in selection bias. The hypothesis that cancer progression induces PD-L1 expression and distant metastasis is not supported by the present findings.

The rate of PD-L1 positivity was higher in TIL-H patients than in TIL-L and TIL-M patients, perhaps because the cancer is recognized as non-self, and antigen-presenting cells activate lymphoid T and B cells. Antigen-primed lymphocytes then migrate to the surrounding cancer environment and increase TIL concentration. To prevent an immune response, PD-L1 expression is induced by certain mechanisms, which might explain why PD-L1 positivity was high in the TIL-H group.

The G1 (basal type, medullary pattern) histological group had a significantly higher rate of PD-L1 positivity. Because of high cellular atypia and cancer antigenicity,

Table 2 PD-L1 positivity (n=86)

		PD-L1 positivity rate	
Overall	PD-L1 positive	24.2% (21/86)	
Stage	I	19.2% (5/26)	
	II	27.1% (13/48)	
	III	5.0% (2/8)	
	IV	25.0% (1/4)	
Ly	0	25.7% (19/74)	
	1-3	16.7% (2/12)	
Lymph node	positive	26.8% (15/56)	
	negative	20.0% (6/30)	
TILs	L	2.5% (1/40)	
	M	17.3% (3/17)	p<0.0001
	H	58.6% (17/29)	

Ly: lymphatic invasion, TILs: tumor-infiltrating lymphocytes

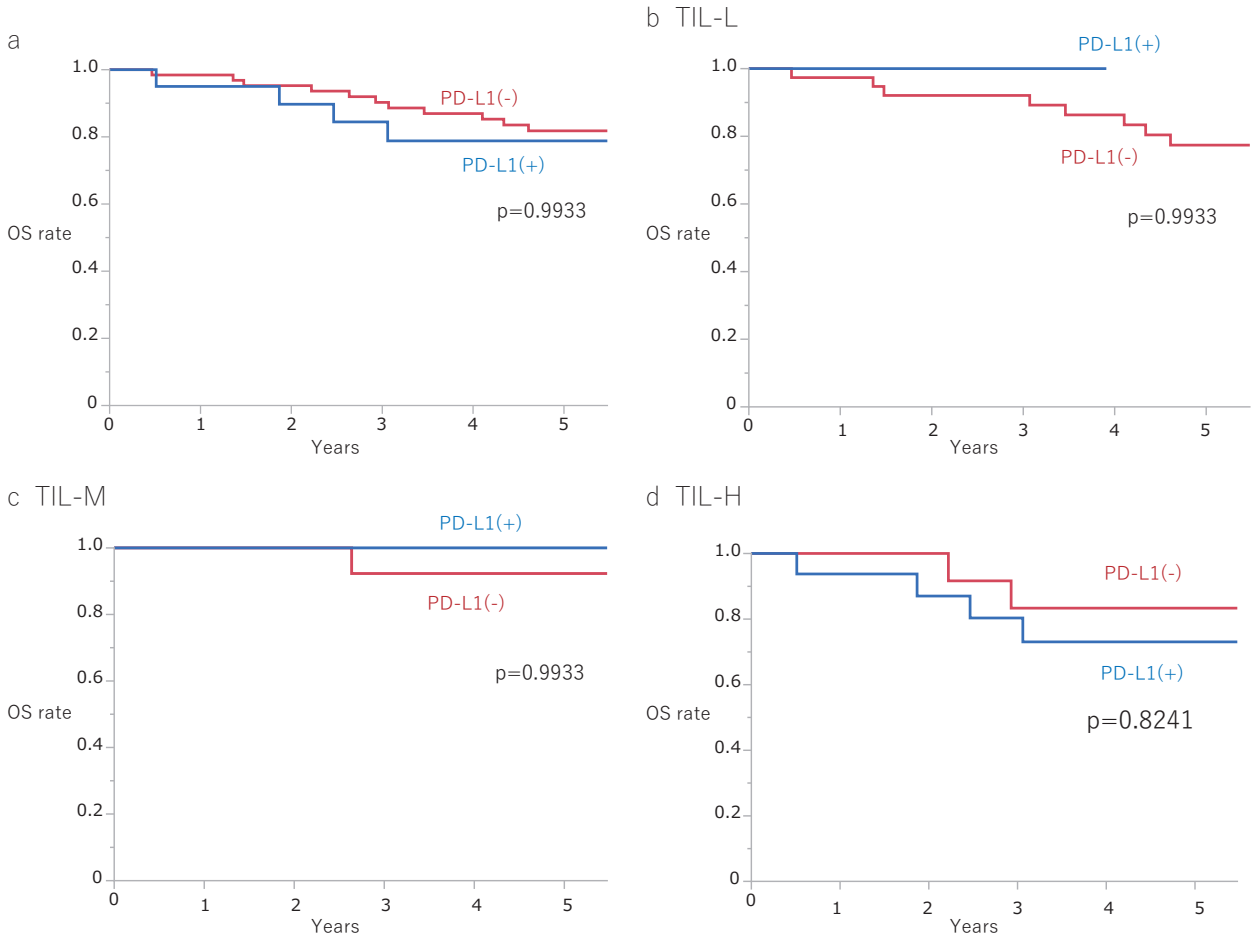

Fig. 1 The 5-years OS in each status

a) Kaplan-Meier curves for 5-year overall survival (OS) in relation to programmed death-ligand 1 (PD-L1) status.
b) 5-year OS for patients with a low concentration of tumor-infiltrating lymphocytes (TIL-L).
c) 5-year OS for patients with a moderate concentration of tumor-infiltrating lymphocytes (TIL-M).
d) 5-year OS for patients with a high concentration of tumor-infiltrating lymphocytes (TIL-H).

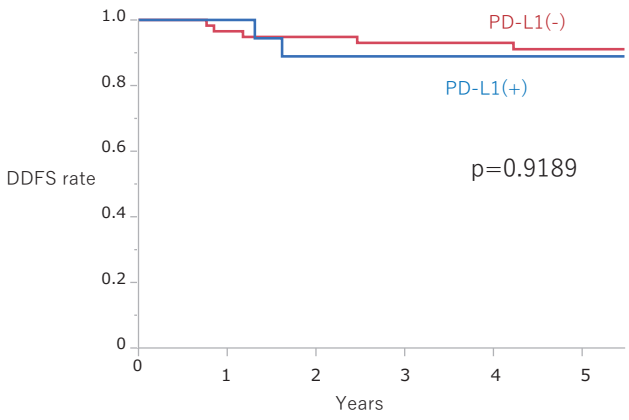

Fig. 2 The 5-years DDFS

Kaplan-Meier curves of 5-year distant disease-free survival (DDFS) in relation to programmed death-ligand 1 (PD-L1) status.

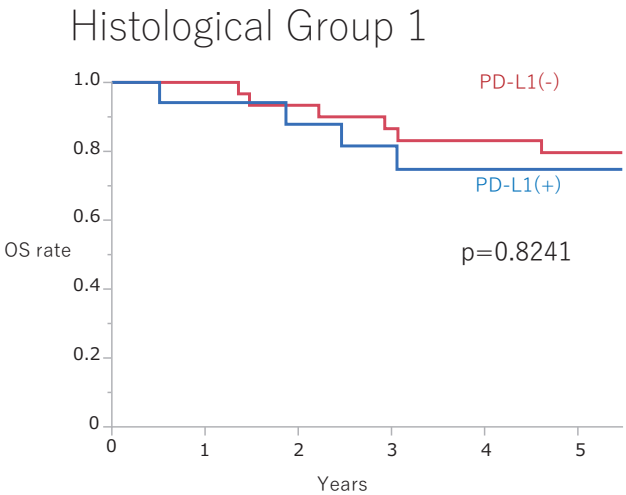

Fig. 3 The 5-years OS

Kaplan-Meier curves of 5-year overall survival (OS) among patients with histological Group 1 tumors (basal type, medullary pattern) in relation to programmed death-ligand 1 (PD-L1) status.

Table 3 Univariate and multivariate analysis of overall survival

		Univariate analysis (95%CI)	P-value	Multivariate analysis (95%CI)	P-value
PD-L1	+ vs −	1.00 (0.33-3.06)	0.9933	0.66 (0.169-2.60)	0.5559
Histological type	G1 vs other	1.64 (0.61-4.38)	0.3209	0.45 (0.15-1.41)	0.1720
s-TIL	H vs L/M	1.65 (0.65-4.19)	0.2894	0.91 (0.29-2.83)	0.8690
Ly	+ vs −	2.80 (1.00-7.86)	0.0508	3.97 (1.12-14.1)	0.0330
T	T1 vs other	0.21 (0.05-0.90)	0.0355	5.98 (1.18-30.2)	0.0306
NG	3 vs other	2.78 (0.92-8.45)	0.0713	0.30 (0.08-1.03)	0.0562
Pathological node status	+ vs −	2.21 (0.88-5.57)	0.0926	1.06 (0.35-3.21)	0.9212

this type more readily triggers an immune response, resulting in a higher PD-L1 positivity rate. Medullary pattern TNBC exhibits greater induction of TILs[25]. Therefore, the high rate of PD-L1 positivity is consistent with this finding. However, no correlation between PD-L1 expression and OS was observed without ICI for any histological type. G1 histology may thus be a favorable indicator for ICI treatment.

A tumor size >T2 and Ly positivity were dependent factors in multivariate analysis of OS. However, PD-L1 expression was not a dependent prognostic factor. Although this study showed no correlation between PD-L1 expression and OS, PD-L1 positivity of tumor cells was a negative prognostic factor in a meta-analysis[26]. In contrast, the same meta-analysis also reported that PD-L1 positivity of immune cells, including TILs, is associated with favorable outcomes[26].

Because this study focused on a population that was not treated with ICIs, it is crucial for future studies to examine the beneficial effect of ICIs in patients with G1, for whom PD-L1 positivity of immune cells is high. In contrast, ICI may be excessive in patients with G2 and G5 and low PD-L1 positivity rates.

A limitation of this study is the small sample used for analysis of outcomes. Larger patient cohorts should be enrolled in future studies.

In conclusion, the rate of PD-L1 positivity was higher in TIL-H patients. PD-L1-positive TIL-H patients with TNBC had worse outcomes. This subset of TNBC cases may be a therapeutic target for ICIs.

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Conflict of Interest: The authors declare no conflict of interest in relation to this study.

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