Original paper

Prognosis-related novel immunostaining pattern for programmed cell death ligand 1 and prognostic value of tumour-infiltrating lymphocytes in triple-negative breast cancer

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This study aims to determine the prognostic significance of programmed cell death ligand 1 (PD-L1) expression and tumour-infiltrating lymphocytes (TILs) in triple-negative breast cancer (TNBC).

PD-L1 expression and TIL percentage were determined in TNBCs that did not receive neoadjuvant therapy. The relationship between PD-L1 expression and the percentage of TILs with survival was investigated.

The presence of intratumoural PD-L1-positive tumour-infiltrating immune cells (TIICs) in tumours with ≥ 1% PD-L1 expression was identified as a new PD-L1 evaluation parameter. The presence of intratumoural PD-L1-positive TIICs as a new parameter in PD-L1-positive cases increased overall survival. The percentage of TILs increased in both overall and distant metastasis-free survival ($p = 0.040$ and $p = 0.006$, respectively). As a result, it was found that the risk of death was increased 5.18-fold ($p = 0.013$) in patients without intratumoural PD-L1-positive TIICs. This risk of death was calculated to be 5.40-fold higher in patients with TIL percentage ≤ 10% than in those with > 40% ($p = 0.024$), and the risk of distant metastasis was calculated to be 11.95 times higher.

In our study, we discovered that the percentage of TILs made a statistically significant difference in TNBC survival. The presence of intratumoural PD-L1-positive TIICs in PD-L1-positive cases significantly increased survival.

Key words: immune checkpoint blockade, programmed cell death 1, programmed cell death ligand 1, triple-negative breast cancer, tumour-infiltrating lymphocyte.

Introduction

Over the last 2 decades, the use of screening programs in breast carcinoma and the availability of hormone receptors or human epidermal growth factor receptor 2 (HER2)-targeted treatment modality have significantly improved breast carcinoma survival [1]. However, triple-negative breast carcinoma (TNBC), which is defined by the absence of oestrogen receptor (ER), progesterone receptor (PR), and *HER2* amplification, accounts for 20% of all breast carcinoma cases [2]. TNBC is associated with a poor prognosis, and new treatment options are required in these tumours. Targeted therapy is hampered by the absence of a primary genomic driver mutation and high-tumour heterogeneity [3].

Programmed cell death 1 (PD-1) is a member of the co-stimulatory CD28/CTLA-4 family that inhibits the immune response by transmitting an inhibitory signal to T-lymphocytes [4]. Treatments targeting PD-1 and programmed cell death ligand1 (PD-L1)

attempt to restore T-lymphocytes' ability to destroy tumour cells [4].

Obtaining good results in targeted therapy with detectable HER2 expression by immunohistochemistry method have been promising for identifying cases that will benefit from anti-PD-1/PD-L1 therapy using PD-L1 immunohistochemistry. PD-L1 expression in TNBCs is primarily observed in tumour-infiltrating immune cells (TIICs) rather than tumour cells, and it inhibits the anti-tumour immune response [2]. However, conflicting publications on prognosis report that PD-L1 positivity is a positive or negative factor in TNBC [1].

Avoiding host immunity is critical for tumour growth and progression [6]. The immune system can stimulate both cancer cell rejection and tumour growth regulators [7]. Although breast carcinomas have not traditionally been classified as immunogenic, tumour-infiltrating lymphocytes (TILs) have been associated with a good prognosis in cohort studies [8]. In breast carcinoma, TILs are higher in TNBCs and HER2+ tumours than in ER+/HER2– tumours [9]. The high prevalence of TILs in TNBCs and HER2+ tumours indicates that these tumour groups are more immunogenic [9]. The use of TILs as a biomarker in breast carcinoma allows for the identification of a patient population with a better prognosis, for whom adjuvant therapy can be avoided, and the targeting of cases that may benefit from immune checkpoint blockade therapy. Studies have suggested that the percentage of TILs detected in haematoxylin-eosin (H&E) stained preparations and the ratio of PD-L1 by immunohistochemistry could be used to select the patient population that would respond to immune checkpoint blockade agents [9].

Material and methods

Patient sample selection

Between 2011 and 2017, cases diagnosed with immunohistochemically ER-, PR-, and CerbB2-negative, breast carcinoma at the Department of Pathology, Ege University School of Medicine, were included in the study. Specific tumour types with a better prognosis in the TNBC phenotype, cases that received neoadjuvant therapy, and cases with tumours smaller than 1 cm or lacking sufficient tumour tissue in paraffin blocks to prevent tumour tissue loss were excluded from the study. The study included 104 cases that met these criteria.

PD-L1 immunohistochemistry

3-μm slices from formalin-fixed, paraffin-embedded blocks containing the entire tumour area were deparaffinized, dehydrated with xylene, and rehydrated in a graded array alcohol solution. Immunostaining was performed using an automated immunostained (Ventana BenchMark XT) according to the instructions on the package insert for the PD-L1 VENTANA SP142 assay.

PD-L1 immunohistochemical evaluation

The immunohistochemical evaluation of the VENTANA PD-L1 SP142 clone in TNBCs was performed in accordance with the interpretation guideline. As a result, TIICs are found in the intratumoural and contiguous peritumoural stroma, alongside lymphocytes, macrophages, dendritic cells, and granulocytes. The proportion of tumour area occupied by PD-L1 staining TIIC of any intensity was used to calculate TIICs. PD-L1 positivity was evaluated according to the following criteria in accordance with the guideline:

PD-L1 negative

Absence of any discernible PD-L1 staining or the presence of discernible PD-L1 staining of any intensity in TIICs covering $< 1\%$ of tumour area occupied by tumour cells, associated intratumoural and contiguous peritumoural stroma.

PD-L1 positive

Presence of discernible PD-L1 staining of any intensity in TIICs covering $\geq 1\%$ of tumour area occupied by tumour cells, associated intratumoural and contiguous peritumoural stroma (Fig. 1A, B).

The score did not include staining of carcinoma cells or neutrophils in necrotic areas.

Intratumoural PD-L1-positive TIICs

The presence of intratumoural PD-L1-positive TIICs in tumours with $\geq 1\%$ PD-L1 expression was identified as a new PD-L1 assessment parameter. These are TIICs that have direct cell-cell contact with carcinoma cells showing PD-L1 expression (Fig. 1C, D).

Evaluation of tumour-infiltrating lymphocytes

Tumour-infiltrating lymphocytes were scored in H&E-stained preparations by 2 pathologists, as recommended by the international TIL working group convened to evaluate TILs in breast cancer [10]. According to these recommendations, TILs were scored in the stroma between the areas of carcinoma, and all mononuclear cells (lymphocytes and plasma cells) were included. TILs in the stroma were calculated as a percentage of the stromal area alone. The total assessed surface area did not include the carcinoma cells and large areas of central necrosis and fibrosis. The assessment of stromal TILs did not include peritumoural follicular aggregates and tertiary lymphoid structures with germinal centres.

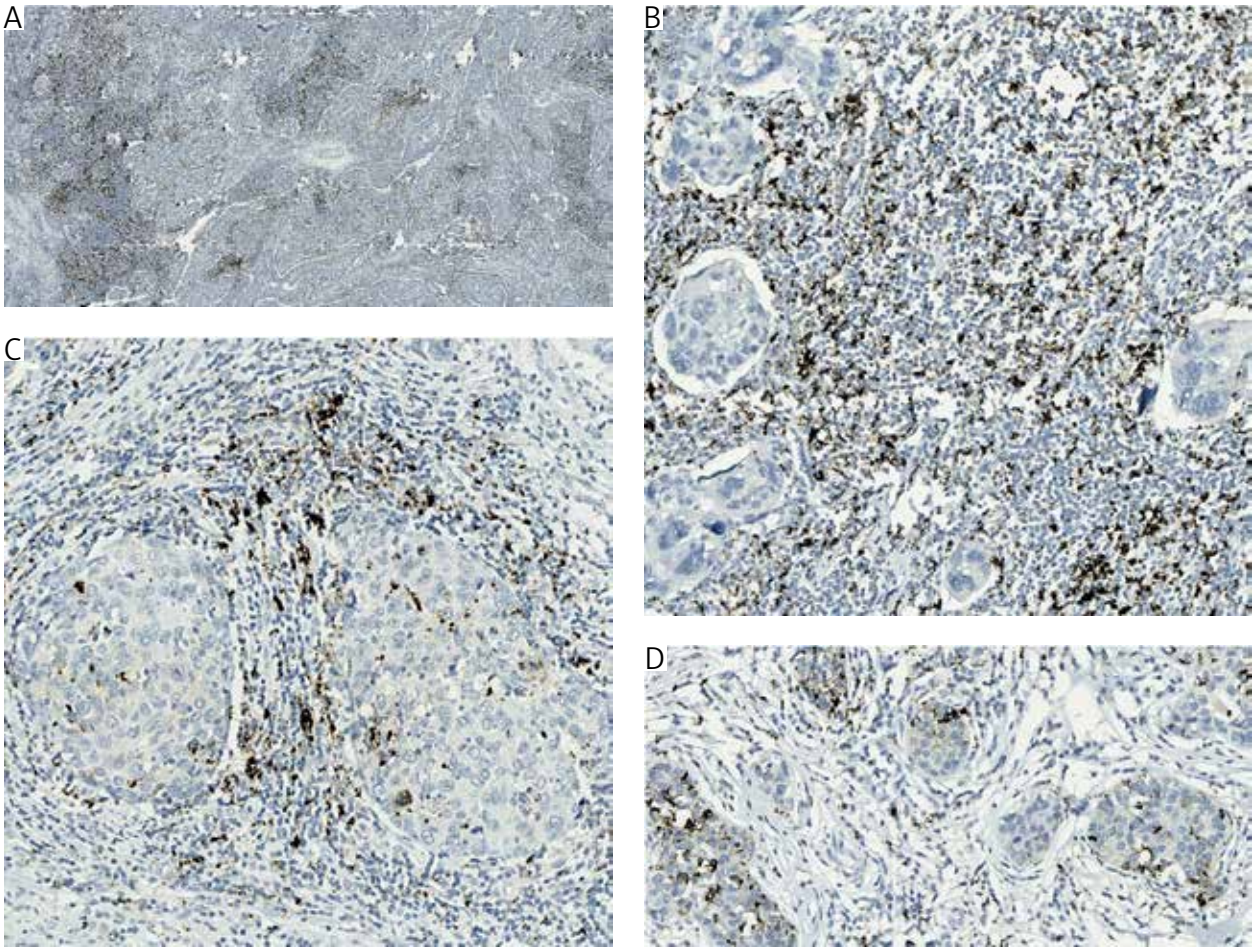

Fig. 1. Programmed cell death ligand 1 (PD-L1) immunohistochemical staining in triple-negative breast carcinoma. A, B) Positive (≥ 1%) PD-L1 staining in tumour-infiltrating immune cells (TIICs) (25× and 100×); C, D) Presence of intratumoural PD-L1 TIICs in PD-L1-positive (≥ 1%) cases (100× and 400×)

Categorization of parametric data

A total of 104 cases were divided into 2 groups based on whether the PD-L1 immunohistochemical evaluation was positive (≥ 1%) or negative (< 1%). Furthermore, 51 PD-L1-positive cases were classified as having intratumoural PD-L1-positive TIICs or not. TILs were divided into 3 groups 10: 0–10%, 11–40%, and > 40–100% (Fig. 2).

Biostatistical analysis

For numerical variables with a normal distribution, Student's T test was used; for other variables, the Mann-Whitney *U* test and Kruskal-Wallis H test were used. Chi-square (χ^2) was used to compare categorical variables, and Fisher's exact test was used when necessary. The Kaplan-Meier method was used to evaluate survival curves, survival rates, and average survival estimates, and the log-rank statistic was used to compare groups. Univariate and multivariate hazard ratios (HRs) were estimated using Cox regression analysis. Statistical analysis was performed using IBM SPSS statistic 25.0 software. $P < 0.05$ was considered significant for all statistical tests.

Ethics statement

The study was approved by the Ethics Committee of Faculty of Medicine, Ege University, Izmir, Turkey (Approval No. 20-11.1T/46) and conducted according to the principles of the Helsinki Declaration.

Results

Our study included 104 cases with immunohistochemically negative hormone receptor and HER2 expression. At the time of diagnosis, the mean age of the cases was 54.25 (range 28–82) years.

PD-L1 positivity

In our study, PD-L1-positive (≥ 1%) and PD-L1-negative (< 1%) cases, immunohistochemically, were 51 (49%) and 53 (51%), respectively. The presence of intratumoural PD-L1-positive TIICs evaluated in 51 tumours with PD-L1 expression ≥ 1% was found in 54.9% ($n = 28$) of the cases. There were 9 cases (8.7%) with PD-L1 staining in tumour cells at any rate.

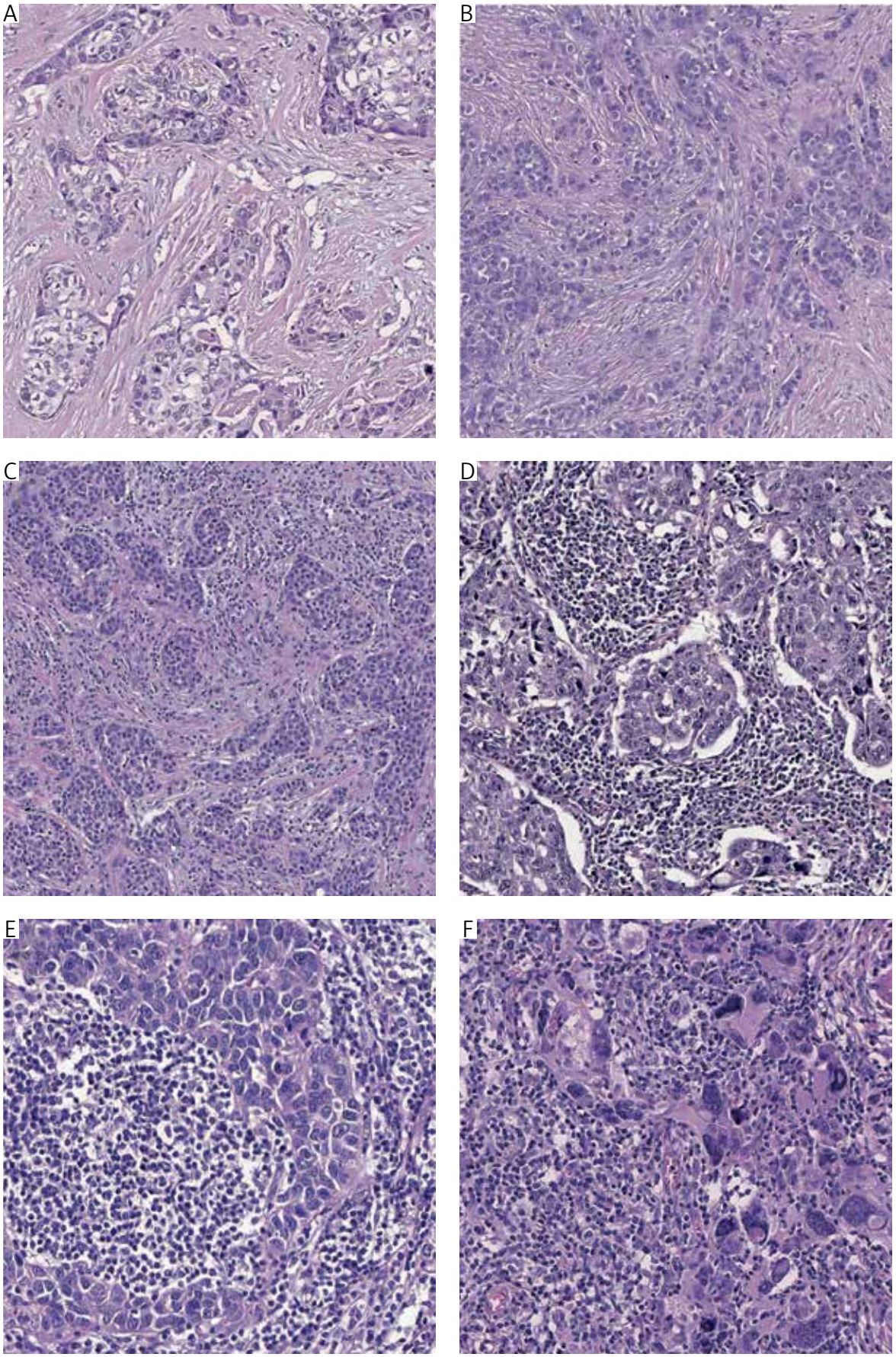

Fig. 2 Percentage of tumour-infiltrating lymphocytes (TILs). A, B) TILs ≤ 10% (100×); C) TILs 11–40% (100× and 200×); D–F) TILs > 40% (400×)

Tumour-infiltrating lymphocytes percentage

There were 56 (52.8%), 23 (22.1%), and 23 (22.1%) cases when they were divided into 3 groups according to TIL percentage: ≤ 10%, 11–40%, and > 40%, respectively.

Correlation between clinicopathological factors and PD-L1 expression or tumour-infiltrating lymphocytes

We found no statistically significant difference between PD-L1 positivity in TIICs and the mean age at diagnosis, tumour multicentricity, tumour size, tumour grade, presence of lymphovascular invasion, and axillary lymph node metastasis. In the presence of intratumoural PD-L1-positive TIICs in tumours with ≥ 1% PD-L1 expression and the percentage of TILs in the tumour, a similar situation was observed (Table I).

Relationship between PD-L1 and tumour-infiltrating lymphocytes

PD-L1 positivity increased statistically significantly as the percentage of TILs increased. While PD-L1 positivity was seen in 28.1% of cases with a TIL percentage of ≤ 10%, it increased to 91.7% in cases with a TIL percentage of > 40%, a statistically significant difference ($p = 0.001$). The presence of intratumoural PD-L1-positive TIICs increased as the percentage of TILs increased, but this difference was not statistically significant ($p = 0.123$) (Table II).

Survival outcome

In our study, we obtained follow-up data on 102 of 104 cases. The mean follow-up period was 61.5 months (range 3–145 months). At the end of the follow-up period, 72.55% ($n = 74$) of the cases remained alive, whereas 27.45% ($n = 28$) died.

Survival analyses (Kaplan-Meier)

The mean overall survival (OS) of our cases was calculated to be 107.02 ±6.15 months (95% CI: 94.97–119.06), with distant metastasis observed in 25.49% ($n = 26$) during follow-up. The mean distant metastasis-free survival (DMFS) was calculated to be 91.25 ±4.72 months (95% CI: 81.98–100.51). During the follow-up period, 6.86% ($n = 7$) of the cases experienced local recurrence. The mean local recurrence-free survival time was 111.43 ±3.08 months (95% CI: 105.39–117.46). Recurrence or distant metastasis was observed in 30.39% ($n = 31$) of the cases during the follow-up period. DFS was calculated as a mean of 86.85 ±4.81 months (95% CI: 77.43–96.28). All our cases had a 5-year OS rate of 80%, and the patients with the lowest 5-year OS were those with a TIL percent-

Table I. Correlation between clinicopathological factors and programmed cell death ligand 1 expression or tils percentage

Clinicopathological features	PD-L1 (%)			Intratumoural PD-L1 positive TIICs[a]			TILs (%)			
	Negative (< 1)	Positive (≥ 1)	*P*- value	Absent	Present	*P*-value	≤ 10	11–40	> 40	*P*- value
Age (years)	55.08 ±11.24	53.39 ±13.63	0.493	56 ±10.59	51.25 ±15.56	0.219	55.28 ±10.68	56.39 ±14.21	49.75 ±13.91	0.159
Tumour multicentricity										
Negative	47	43	0.514	20	23	0.638	50	21	19	0.441
Positive	6	8		3	5		7	2	5	
Tumour size [cm]										
≤ 2	25	20	0.413	9	11	0.991	23	10	12	0.726
> 2	28	31		14	17		34	13	12	
Histological grade										
II	16	10	0.213	6	4	0.291	17	3	6	0.292
III	37	41		17	24		40	20	18	
Lymphovascular invasion										
Negative	25	27	0.556	11	16	0.507	30	9	13	0.494
Positive	28	24		12	12		27	14	11	
Lymph-node metastasis										
Negative	32	33	0.649	12	21	0.09	36	12	17	0.413
Positive	21	18		11	7		21	11	7	

PD-L1 – programmed death ligand 1, TIIC – tumour-infiltrating immune cell, TIL – tumour-infiltrating lymphocyte
[a] Among PD-L1 positive 51 cases

Table II. Correlation between programmed cell death ligand 1 expression and tumour-infiltrating lymphocytes percentage

Parameters	TILs (%)			
	≤ 10	11–40	> 40	*p*-value
PD-L1 positive TIICs				
Positive < 1	41	10	2	0.001
Negative ≥ 1	16	13	22	
Intratumoural PD-L1 positive TIICs[a]				
Absent	10	6	7	0.171
Present	6	7	15	

PD-L1 – programmed death ligand 1, TIIC – tumour-infiltrating immune cell, TIL – tumour-infiltrating lymphocyte
[a] Among PD-L1 positive 51 cases

age ≤ 10% (70%). This was followed by cases with no intratumoural PD-L1-positive TIICs in tumours with ≥ 1% PD-L1 expression (72%). The patients with the highest 5-year OS were those with a TIL > 40% (100%). All our cases had a 5-year DMFS of 74%. Cases with TILs ≤ 10% and those without intratumoural PD-L1-positive TIICs (in tumours with ≥ 1% PD-L1) had the lowest 5-year DMFS (63% and 65%, respectively; data not shown).

There was no statistically significant difference between PD-L1-positive and -negative cases in terms of OS, DFS, and DMFS ($p = 0.632$, 0.453, and 0.333, respectively). Patients with intratumoural PD-L1-positive TIICs had a statistically significantly longer OS than those without ($p = 0.006$), but there was no statistically significant difference in terms of mean DMFS ($p = 0.113$) in PD-L1-positive cases. OS, DFS, and DMFS were statistically significantly longer in cases with TIL > 40% than in cases with < 10% ($p = 0.040$, 0.019, and 0.006, respectively). While the mean DFS was 76.08 months in cases with TIL < 10%, it was 110.41 months, nearly double, in cases with TIL > 40%. In addition, mean DMFS increased approximately 2-fold (77.45 and 115.18 months, respectively). The mean OS in cases with PD-L1 tumour cell staining was 82.85 (±16.01) months, and 107.59 (±6.41) months in cases without tumour cell staining; DMFS was calculated as 81.12 (±16.23) and 91.61 (±4.91), respectively. OS and DMFS differences were not statistically significant ($p = 0.561$ and 0.586, respectively) (Fig. 3).

COX regression analysis results

There was no statistically significant difference between PD-L1-positive and -negative cases in terms of death risk or the development of distant metastasis. However, among the PD-L1-positive cases, the risk of death was statistically significantly increased 5.18-fold (95% CI: 1.42–18.90, $p = 0.013$) in those without intratumoural PD-L1-positive TIICs. Although the risk of developing metastasis was 2.61-fold higher, the difference was not statistically significant (95% CI: 0.76–8.91, $p = 0.127$). Patients with TIL < 10% had a 5.40-fold higher risk of death and an 11.95-fold higher risk of developing distant metastases than patients with TIL > 40%, both of which were statistically significant (95% CI: 1.24–23.46, $p = 0.024$ and 95% CI: 1.59–90.13, $p = 0.016$, respectively). There was no statistical difference between cases with and without PD-L1 tumour cell staining in terms of death risk and risk of distant metastasis (HR: 1.42; 95% CI: 0.43–4.73, $p = 0.565$ and HR: 1.40; 95% CI: 0.41–4.78, $p = 0.588$, respectively) (Table III).

Discussion

Triple-negative breast cancers are malignancies with a poor prognosis among breast carcinomas, with no targeted therapy options, such as anti-hormone in hormone-positive tumours and anti-HER2 in HER2+ tumours. New treatment regimens that can be targeted in TNBCs have emerged in recent years.

The PD-1/PD-L1 pathway is a key T-lymphocyte checkpoint mechanism that transmits an inhibitory signal to T-lymphocytes, thereby inhibiting the immune response [11]. One of the most promising targets in cancer immunotherapy is the inhibition of PD-1 and PD-L1 [12]. TNBCs are the most immunogenic group of breast carcinomas, and activating anti-tumour immunity through immune checkpoint blocking has emerged as a new target in these tumours [13]. The use of atezolizumab, an anti-PD-L1 agent in TNBCs, was approved by the U.S. Food and Drug Administration, and subsequently, immunohistochemistry-based detection of PD-L1 was suggested as a predictive biomarker for selecting cases that would benefit from this therapy [14]. However, clinical studies have revealed that only 10–20% of TNBC cases benefit from this treatment. Therefore, it is important to understand the relationship between PD-L1 and TNBC [13].

In studies evaluating PD-L1 expression in breast carcinomas, positive cases ranged 6–56.6% [12, 15, 16]. In studies limited to TNBCs, this rate ranged 19–78% [13, 16–20]. The reason for such a wide distribution of positive case rates may be due to the different subjective PD-L1 used in the studies. In our study, PD-L1-positive (≥ 1%) and PD-L1-negative (< 1%) cases, immunohistochemically, were 51 (49%) and 53 (51%), respectively.

High TIL has been reported to be associated with PD-L1 expression in breast carcinomas [12]. There is a similar correlation in TNBCs [1]. In a recent study, PD-L1 was found to be positive in almost all cases with a TIL percentage above 20% [14]. In our study,

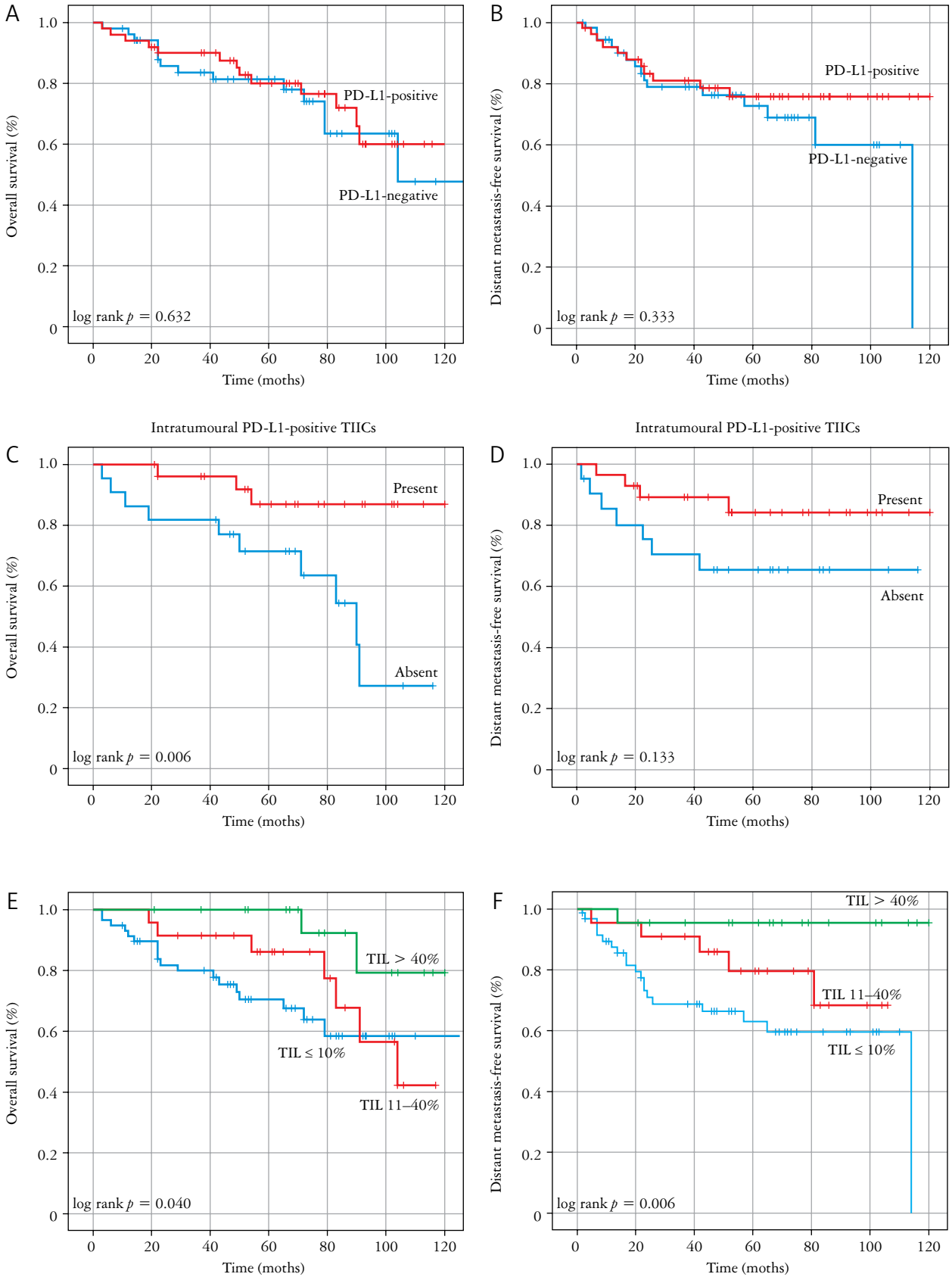

Fig. 3. Associations of programmed cell death ligand 1 (PD-L1) expression (positive vs. negative), presence of intratumoural PD-L1-positive tumour-infiltrating immune cells (TIICs) among PD-L1-positive cases and tumour-infiltrating lymphocyte (TIL) percentage with overall survival (OS) and distant metastasis-free survival (DMFS). There were no associations between PD-L1 positivity and OS or DMFS. However, there was a significant association between the presence of intratumoural PD-L1-positive TIICs and OS in PD-L1-positive cases ($p = 0.006$). Moreover, the TIL percentage (tumour-infiltrating lymphocytes ≤ 10% vs. > 40% cases) was significantly associated with OS ($p = 0.040$) and DMFS ($p = 0.006$)

Table III. Univariate Cox regression analysis for survival in cases programmed cell death ligand 1 expression and tumour-infiltrating lymphocytes percentage

Parameters	Overall survival					Distant disease-free survival				
	Death		HR	95% CI	*p*-value	Distant metastasis		HR	95% CI	*p*-value
	n	%				n	%			
PD-L1 positive TIICs (%)										
Positive (≥ 1)	13	26	1.00			11	22.4	1.00		
Negative (< 1)	15	28.8	1.20	0.56–2.56	0.634	15	29.4	1.47	0.67–3.20	0.338
Intratumoural PD-L1 positive TIICs[a]										
Absent	3	10.7	1.00			4	14.3	1.00		
Present	10	45.5	5.18	1.42–18.90	0.013	7	33.3	2.61	0.76–8.91	0.127
TILs percentage (%)										
> 40	2	9.1	1.00			1	4.5	1.00		
11–40	7	30.4	3.62	0.75–17.41	0.109	5	22.7	6.05	0.69–52.78	0.104
≤ 10	19	33.3	5.40	1.24–23.46	0.024	20	35.7	11.95	1.59–90.13	0.016

HR – hazard ratio, PD-L1 – programmed death ligand 1, TIIC – tumour-infiltrating immune cell, TIL – tumour-infiltrating lymphocyte
[a] Among PD-L1 positive 51 cases

PD-L1 positivity increased significantly with the increase in TIL percentage ($p = 0.001$). In tumours with a TIL percentage of ≤ 10 and > 40%, PD-L1 positivity was 28.1% and 91.7%, respectively. The correlation between PD-L1 and TIL percentage could be explained by the fact that tumours with a high mutation load are rich in TILs, and TILs have signals that activate PD-L1 [13].

Considering the relationship between PD-L1 expression and survival in breast carcinomas, there are many conflicting results. Publications report that PD-L1 improves OS [15].

In one study, it was emphasized that increased PD-L1 mRNA level in the tumour increases survival independent of known prognostic features (tumour size, lymph node involvement, hormone receptors, and HER2 status) [21]. The presence of a strong anti-tumour immune response leading to PD-L1 expression can explain the association between PD-L1 expression and prolonged survival in breast carcinomas followed in these studies [15]. On the other hand, PD-L1 expression in breast carcinomas was associated with reduced OS [4]. This was supported by different studies reporting that OS is statistically significantly longer in PD-L1 negative patients [22]. PD-L1 expression was also found to be associated with decreased survival in a meta-analysis of breast carcinomas, and removing studies that only included TNBCs did not change this conclusion [23]

Given the relationship between PD-L1 expression and OS in TNBCs, some publications have reported that PD-L1 expression correlates with increased OS [24]. However, most studies investigating the association of PD-L1 with OS in TNBCs found no significant association [1, 25–27]. Similarly, no statistically significant difference was found between PD-L1 expression and OS in our study. When the effect of PD-L1 on DFS in TNBCs was investigated, no significant association was found [1, 27]. In contrast, some studies have found that PD-L1 expression in TIICs increases DFS, and PD-L1 staining in tumour cells decreases survival [24]. In our study, the mean DMFS was calculated as 91 ±4.72 months, and no statistically significant relationship between PD-L1 expression and DMFS was found. The presence of an immune active microenvironment or immune cells making the tumour more sensitive to chemotherapy was emphasized in studies in which PD-L1 was associated with positive prognostic factors in TNBCs [24]. These conflicting results regarding survival in the literature may be due to different PD-L1 assessment methods, cut-offs, and antibody clones [1].

Interestingly, in our study, there was a significant correlation between the presence of intratumoural PD-L1-positive TIICs and a longer OS in PD-L1 ≥ 1% tumours ($p = 0.006$). As a result, the risk of death in cases without intratumoural PD-L1-positive TIICs is 5.18-fold higher than those with existing intratumoural PD-L1-positive TIICs ($p = 0.013$). Although previous studies have reported that different PD-L1 staining patterns influence prognosis, we found no data on the importance of finding intratumoural PD-L1-positive TIICs. The higher percentage of TILs in these tumours may explain the better prognosis of intratumoural PD-L1-positive TIICs. At the same time, these tumours are more likely to

be mutated and have a high neo-antigen burden. As a result, because the rate of intratumoural TIICs is higher in these tumours, the probability of PD-L1 staining may increase. More research is needed to fully understand this finding.

Considering the relationship between TIL density and prognosis in TNBCs, several studies have shown that TILs are associated with long-term OS [1, 13, 28–30]. A high TIL percentage has a positive effect not only on the OS but also on DFS and DMFS [28]. TILs have taken their place as a favourable prognostic factor reducing the risk of relapse and death in TNBCs [12]. Each 10% increase in TILs has been shown to reduce the risk of death [28, 30]. There is a positive correlation between TILs and prognosis in TNBC, and immunity in TNBCs is important for the clinical course [29]. In our study, there was a statistically significant increase in OS in cases with a TIL percentage $> 40\%$ compared with cases with a TIL percentage $\leq 10\%$ ($p = 0.040$). In our sample, the 5-year estimated survival was 100% in cases with a TIL percentage $> 40\%$, and 70% in cases with a TIL percentage $\leq 10\%$. In terms of death risk, cases with a TIL percentage $\leq 10\%$ had a 5.40-fold higher risk than cases with a TIL percentage $> 40\%$ ($p = 0.024$). It has been shown that increased TIL density in TNBCs also increases DMFS [1, 6, 27]. In TNBC, each 10% increase in TILs reduces the risk of distant metastasis by 13% ($p = 0.02$) [6]. When the relationship between TILs and DMFS was compared in our study, a statistically significant increase in DMFS was observed as the percentage of TILs increased ($p = 0.019$). The 5-year DMFS for TIL $\leq 10\%$ and $> 40\%$ cases was 63% and 96%, respectively. As a result, the risk of developing distant metastasis increased 11.95-fold in TIL $\leq 10\%$ compared with $> 40\%$ cases. These findings in our study support the literature finding that increased TIL density in TNBCs is associated with a favourable prognosis.

Conclusions

In our TNBC study, we compared PD-L1 expression and the percentage of TILs in 104 cases with clinicopathological and survival data. The limitations of our study are that it is retrospective and the number of cases is limited. Because this is a single-centre study, the findings must be confirmed by multicentre studies with larger cohorts.

Many studies have highlighted the positive corelation between TIL and survival. Although our study is consistent with the literature, it is noteworthy that tumours with TIL $\leq 10\%$ have approximately 5-fold higher risk of death and a 12-fold higher risk of distant metastasis than those with $> 40\%$. We believe that dividing the percentage of TILs investigated in the literature with different threshold values into 3 groups will improve reproducibility and agreement between observers. Pathologists will be able to more accurately correct the extreme groups with a clear prognostic difference if they divide them into 3 groups and leave a grey zone between them.

A significantly higher rate of PD-L1 positivity in tumours with high TIL indicates a synergistic effect between the immune system and PD-L1 and is consistent with previous findings. The statistically significant increase in survival of patients with intratumoural PD-L1-positive TIICs may be a novel parameter for PD-L1 evaluation and reporting in TNBCs. Furthermore, we believe that the percentage of TILs in each breast carcinoma pathology should be reported because it provides valuable prognostic information.

Acknowledgments

This study was supported by grants from Ege University Scientific Research Fund (BAP) (Approval No. 22498).

The authors declare no conflict of interest.

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