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ORIGINAL ARTICLE

Prognostic Importance of PD-L1 Expression in Breast Carcinomas

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OBJECTIVE

Breast carcinoma (BC) is the most common cancer in women, and, in particular, some subtypes are established as suitable for immunotherapy strategies targeting immune checkpoints. Programmed cell death-1 (PD-1), as well as cytotoxic T lymphocyte-associated protein-4 (CTLA-4), has an important role in the tumor microenvironment to escape from the immune system. In this study, we examined the relationship of Programmed cell death-1 (PD-L1) expression with survival, recurrence, and other prognostic factors.

METHODS

This retrospective cohort comprised tissue microarray blocks of 391 cases examined at the same institute between 2000-2012. All cases were completely resected tumors, without neoadjuvant therapy, with more than 5 years of follow-up. Clinical follow-up and all pathologic parameters were recorded. PD-L1 immunohistochemistry (SP263) was applied, then staining details including density, percentage, and patterns were noted for tumor areas and immune cells. PD-L1 expression results were analyzed, and its relationship with prognostic parameters and survival was investigated.

RESULTS

PD-L1 expression was detected in 90 (24.8%) cases: 55 in only tumor-infiltrating lymphocytes (TILs), 35 in both tumor and TILs. Statistically, there was no significant relationship between PD-L1 expression and survival. However, high histologic grade, high scores in pleomorphism and mitosis, mild stromal reaction, dense immune cell infiltration, perineural invasion, absence of lymph node metastasis, negativity of ER and PR, HER2 expression, and high Ki-67 results had a significant relationship with PD-L1 expression.

CONCLUSION

Consistent with the literature, our results showed that PD-L1 expression in triple-negative and HER2 overexpressed types and in the presence of TILs is higher than in other breast cancers.

Keywords: Breast carcinoma; immunotherapy; PD-L1; tumor-infiltrating lymphocytes.

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INTRODUCTION

Invasive breast carcinoma (IBC) is the most common tumor in women.[1] According to Estrogen Receptor (ER), Progesterone Receptor (PR), and HER2 status, IBCs are divided into molecular subtypes such as luminal A, luminal B, HER2 expressing type, and triple-negative BC (TNBC).[2] Treatment options include surgery, radiotherapy, hormonal therapy, chemotherapy, and targeted therapy for the specific subtypes, particularly. Indeed, immune therapy is a novel option for BC patients.

Immunotherapy has been applied for the treatment of some cancers, and many studies have been conducted about it in recent years. It aims to increase the immune cells' effect while targeting checkpoints such as cytotoxic T lymphocyte-associated protein-4 (CTLA-4) and programmed death ligand-1 (PD-L1).[3] The mechanisms of checkpoint blockers prevent cancer cells from escaping immune cells and boost immune cells' activity. High PD-L1 rates provide a suitable environment for tumor proliferation. In many cancers, programmed cell death-1 (PD-1) has been found on the surface of tumor-infiltrating lymphocytes (TILs), and its ligand (PD-L1) on the surface of tumor cells. Inflammatory cytokines and immune response against tumor cells increase by using the molecules that inhibit the PD-1/PD-L1 axis.[4] PD-L1 expression was found higher in subtypes such as TNBCs and HER2 expressing IBCs. Thus, these subtypes of IBCs are novel candidates for immunotherapy.[5–7] We herein aimed to determine the prognostic importance of PD-L1 expression and its relationship with clinicopathological parameters in IBCs.

MATERIALS AND METHODS

Patients

This study was approved by the local university ethics committee (Date: 07/03/2018, Decision no: 89616).

The study included 391 cases who underwent breast-conserving surgery or mastectomy in our university hospital, were diagnosed with invasive IBC, and had at least 5 years of follow-up between 2000 and 2012. Cases that had neoadjuvant therapy, a tumor <0.4 cm, and the presence of <2 paraffin blocks were excluded.

Clinicopathologic parameters (age, gender, surgery type, histologic type, grade, grade scores, tumor size, in-situ component, stromal and inflammatory reactions grouped as mild, moderate, and dense, lymphovascular invasion (LVI), perineural invasion (PNI), lymph node (LN) status, ER, PR, HER2, and Ki-67) were recorded according to the WHO-2019 classification[1] and modified Bloom-Richardson system.[8] Follow-up information was noted from the oncology archive files.

Tissue Microarray (TMA) Construction

Invasive tumor areas without necrosis and close to peripheral TILs were identified on Hematoxylin and Eosin-stained slides. 397 samples sized 0.4 cm were taken from tumor blocks and transferred to TMA blocks.

Immunohistochemistry

The immunohistochemical staining was performed by an automatic device (Ventana Benchmark XT, Ventana Medical Systems, Tucson, Arizona) with the optiview DAB IHC detection kit and the antibody of PD-L1 sp263 clone.

34 cores on TMA slides were excluded due to missing during the staining process. Cores that were half-missed but comprised at least 200 tumor cells were included in the study.

On each slide, tonsil tissue provided information about staining density, scored as 0 (no staining), 1 (low), 2 (moderate), and 3 (high). Staining density was also scored from 0 (no staining) to 3 (Fig. 1). Complete/incomplete membranous staining of invasive tumor cells and inflammatory cells, such as lymphocytes and macrophages, was evaluated for staining density and percentage individually (Fig. 1). Up to 1% and weakly staining were accepted as negative. Nuclear staining, endothelial staining, in-situ carcinoma areas, and artifacts were excluded.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) 21.0 was utilized for statistical analyses. Descriptive statistics were expressed as mean, standard deviation, median, minimum, and maximum values for quantitative parameters, while numbers and percentages were calculated for non-quantitative parameters. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used for data distribution. The Mann-Whitney U and Chi-Squared tests were exploited for comparing non-parametric data. The binary logistic regression test was used in the analysis of statistically significant data. Kaplan-Meier survival curves and Log-rank statistics (Mantel Cox) were utilized for survival analyses. Later on, the Cox proportional hazards model was performed for multivariable regression analysis. The confidence interval was accepted as 95%, and $p<0.05$ was considered significant.

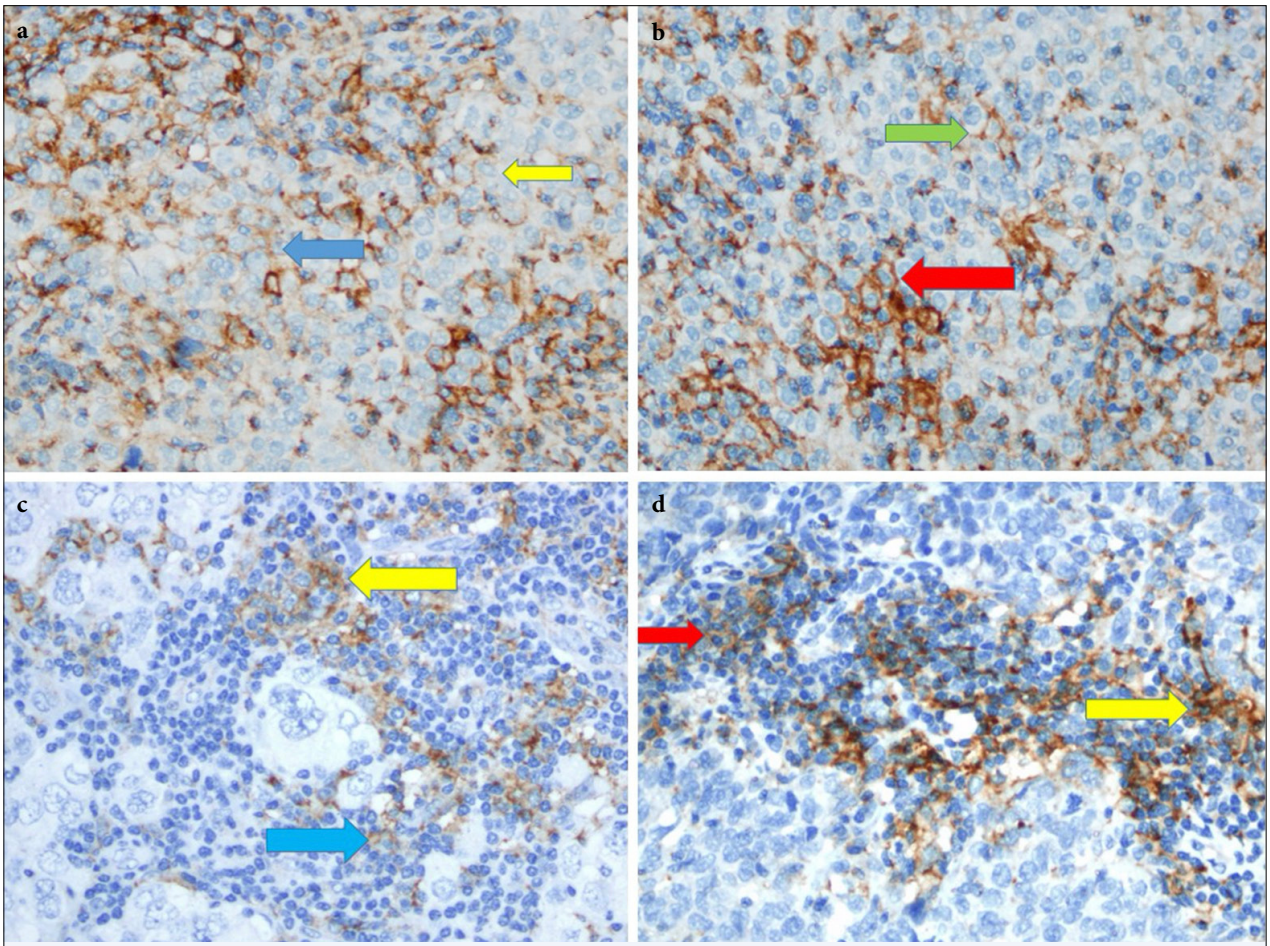

Fig. 1. Immunohistochemical staining for PD-L1 SP263: Incomplete weak (yellow arrow) and complete weak (blue arrow) staining in tumor cells. (a) Intermediate (green arrow) and strong (red arrow) staining in tumor cells. (b) Weak (blue arrow) and intermediate (yellow arrow) staining in TILs. (c) Intermediate (red arrow) and strong (yellow arrow) staining in TILs. (d) (IHC×400).

RESULTS

Demographics of Patients and Tumor Characteristics

TMA blocks consisted of 397 core samples belonging to 391 cases. Four patients had bilateral IBCs. One case had two separate foci in both breasts four years apart. Another case had two distinct tumors, but one was depleted during the laboratory process. Finally, 363 cores from 358 cases were examined.

The mean age was 51.4 (min-max 28–87, median 50), and these results were similar in females. There were four males (1.1%), and the mean age was lower than that of females at 43.5 (min-max 33–55, median 43).

Surgery types were as follows: 169 (46.6%) modified radical mastectomy, 146 (40.2%) partial mastectomy, and 48 (13.2%) simple mastectomy. There were 185 (51%) right and 178 (49%) left breast involvements.

Most cases had a single tumor focus (294; 80.9%), and the mean size was 2.66±1.57 (min-max 0.5–14cm, median 2.5) cm. The most frequent histological type was IBC- no special type (IBC-NST), and 308 (84.8%) cases also had in-situ carcinoma. Axillary LN metastasis was in 211 (58.1%) cases. Sentinel LNs were examined in 184 cases, and 50% were positive (Table 1). ER was positive in 287 (79.1%) cases, while PR was positive in 258 (71.1%). For both ER and PR, 248 (68.3%) cases were positive, while 323 (89%) cases were positive for just one. HER2 was immunohistochemically performed in 321 cases. Some were examined by Silver In Situ Hybridization (SISH), and 69 (21.5%) ones were positive for HER2 (Table 2). Ki-67 was examined in 95 cases, and the mean Ki-67 score was 31.15% (min-max 0–92%, median 24%).

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Table 1 Distribution of the cases according to clinicopathologic parameters

Clinicopathologic parameters	n	%
Histological type		
IBC-NST	276	76.0
Invasive lobular carcinoma	14	3.9
Tubular carcinoma	1	0.3
Muscinous carcinoma	3	0.8
İnvasive apocrine carcinoma	2	0.6
İnvasive micropapillary carcinoma	1	0.3
Metaplastic carcinoma	2	0.6
Mixt carcinoma	64	17.6
Histologic grade		
I	12	3.3
II	205	56.5
III	146	40.2
Parameters of modifiye Bloom-Richardson System		
Tubulus formation		
Score 1	5	1.4
Score 2	72	19.8
Score 3	286	78.8
Pleomorphism		
Uniform cells	2	0.6
Moderate pleomorphism	161	44.4
Prominent pleomorphism	200	55.1
Mitosis		
1–9/ 10 HPF	152	41.9
10–19/10 HPF	153	42.1
>20/10 HPF	58	16
Stromal response		
Low	38	10.5
Moderate	246	67.8
Severe	79	21.8
Inflammatory response		
Low	274	75.5
Moderate	76	20.9
Severe	13	3.6
Perineural invasion		
Present	107	29.5
No	256	70.5
Lymphovascular invasion		
Present	183	50.4
No	180	49.5
Axillary lymph node metastasis		
Present	211	58.1
No	152	41.9
Sentinel lymph node metastasis*		
Present	92	50.0
No	92	50.0
Total	363	100.0

*: Sentinel lymph node examination was performed for 184 cases. IBC-NST: Invasive BC- no special type; HPF: High Power Field

Survival analyses were performed on 356 cases: 66 (18.5%) dead, 290 (81.4%) alive. The mean follow-up period was 101.8 (21–255, median 94.5) months. Recurrence analyses were performed on 358 cases. Eighty-eight (24.6%) cases had a recurrence, and the mean recurrence time was 60.9 (0–167, median 60) months.

Table 2 Hormone receptors and HER2 status

	n	%
ER		
Negative	76	20.9
Weak positive	40	11.0
Moderate positive	94	25.9
Strong positive	153	42.1
PR		
Negative	105	28.9
Weak positive	29	8.0
Moderate positive	70	19.3
Strong positive	159	43.8
HER2 (IHC)*		
0 (Negative)	116	36.1
1 (Negative)	67	20.9
2 (Equivocal)	78	24.3
3 (Positive)	60	18.7
HER2 (IHC and SISH)*		
Negative	252	78.5
Positive	69	21.5
Total	363	100.0

*HER2 status was evaluated in 321 cases. ER: Estrogen Receptor; PR: Progesterone Receptor; IHC: Immunohistochemistry; SISH: Silver In Situ Hybridization

PD-L1 Expression

90 (24.7%) of 363 tumor cores showed PD-L1 positivity: 35 in both tumor and immune cells, and 55 in only immune cells (Fig. 2). In 328 cores, tumor cells were negative for PD-L1: 316 were completely negative, while 12 showed <1% staining (Table 3).

Most tumors had heterogeneous staining patterns in terms of complete and incomplete membranous staining (Fig. 1). Staining rates of complete patterns varied between 1–60%, and it was detected in 40% (n=14) of cases. Staining rates of incomplete patterns were heterogeneous between 40–99%, and this pattern was observed in 60% (n=21) of cases. In 32 cases, staining was entirely (100%) incomplete without including any complete membranous positivity. All cores were also stained heterogeneously in terms of staining density (Fig. 3).

Relationship of PD-L1 Expression with Clinicopathological Parameters and Survival

The correlation between PD-L1 expression and clinicopathological parameters was summarized in Table 4. PD-L1 positivity was significantly higher in grade III tumors (p<0.001). The cases with high scores for pleomorphism and mitosis showed higher PD-L1 positivity, but tubule formation was not correlated with PD-L1 expression (p<0.001, p<0.001, p=0.076). PD-L1 was significantly higher in cases with a mild stromal response, dense immune response, no PNI, and negative LNs (p<0.001, p<0.001, p=0.045, p=0.041). No relationship was found with patient age, gender, tumor size, histological type, and LVI (p=0.984, p=0.089, p=0.122, p=0.082, p=0.806).

The year of diagnosis and PD-L1 expression weren't correlated (p=0.127); however, when years were divided into four groups, the PD-L1 positivity ratio was lower in older years (2000–2002: 5%, 2004–2006: 12.7%, 2007–2009: 31.7%, 2010–2012: 50.7%).

PD-L1 positivity was higher in ER-negative, PR-negative, and HER2-positive cases (p<0.001, p<0.001, p<0.001). Ki-67 was higher in PD-L1 positive cases (p=0.001). When we classified the cases as "Luminal

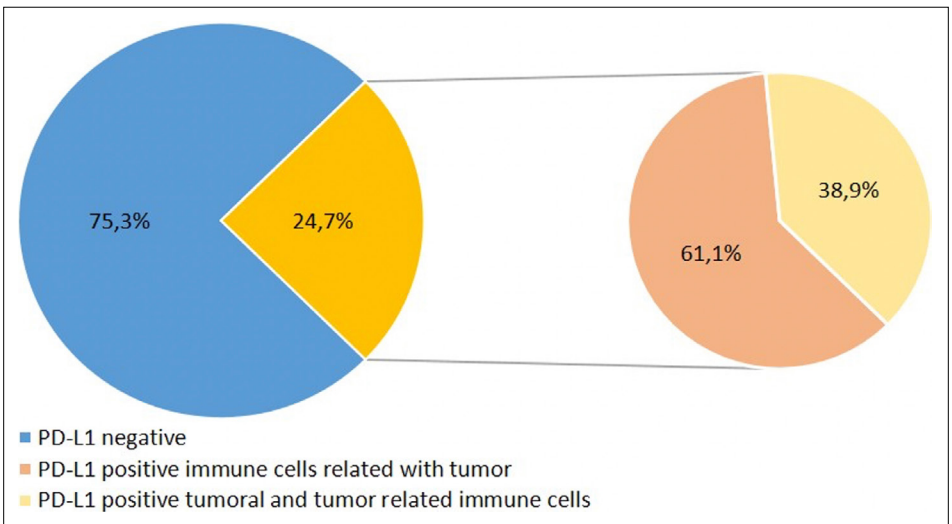

Fig. 2. Distribution of PD-L1 expression among cells.

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Table 3 PD-L1 expression in tumor and tumor related immune cells

	PD-L1 negative		PD-L1 positive	
	n	%	n	%
Tumor cells	328	90.4	35	9.6
Immune cells	273	75.2	90	24.8

	Negative		Weak (1)		Intermediate (2)		Strong (3)	
	n	%	n	%	n	%	n	%
Tumor cells	316	87	45*	12.4	11	3	4	1.2
Immune cells	273	75.2	88	24.2	12	6.1	9	2.5

*: 12-cases stained <1%.

A (ER+, PR+, HER2-), Luminal B (ER/PR+, HER2-), HER2 expressing type (ER-, PR-, HER2+) and TNBC (ER-, PR-, HER2-)", TNBC and HER2 expressing types showed higher PD-L1 positivity (p<0.001).

Among logistic regression analysis, PD-L1 positivity was correlated with stromal and immune response and loss of HER2 expression (Table 5).

However, PD-L1 expression had no relationship with overall survival (OS) or disease-free survival (DFS) (p=0.157, p=0.160) (Fig. 4, 5).

DISCUSSION

In BCs, the response to immunotherapy seems better than chemotherapy in some tumors expressing PD-L1; however, there is no standard approach yet regarding the suitable antibody clone and cut-off value.[9] To understand which cases are convenient for this therapy, IHC is the most widely used method to determine PD-L1 expression in tumoral and immune cells such as macrophages and lymphocytes. However, many variables affect PD-L1 expression. The PD-1/PD-L1 signaling pathway is part of normal immunity and is induced by T lymphocyte activation, which is more pronounced in long-term inflammation. Therefore, the PD-L1 evaluation area should not have an old biopsy site and a chronic inflammatory process. Besides, IHC results may vary depending on the fixation solution and time.[10] For accurate results, the cold ischemia time (time to fixation) should be <30 minutes and should not exceed 1 hour. It is known that the most

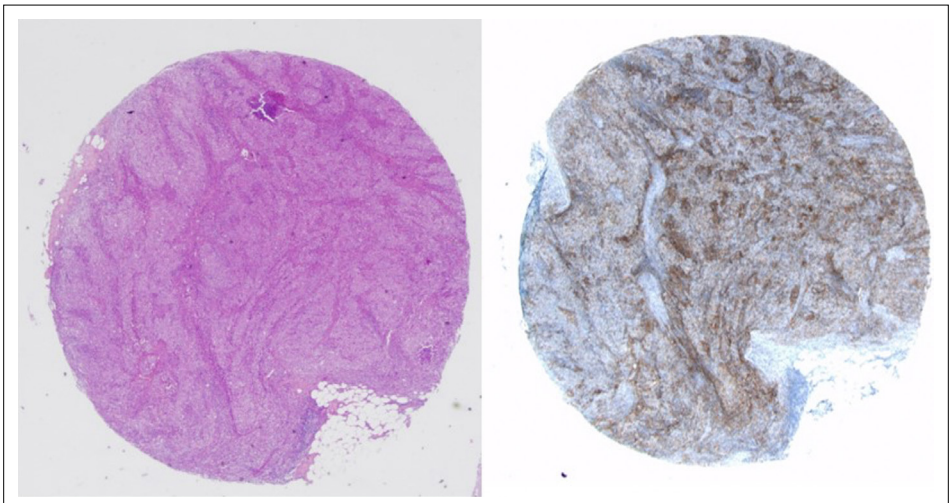

Fig. 3. The heterogeneous staining pattern in tumor cells for PD-L1 SP263 (H&E and IHC ×20).

Table 4 Relationship of PD-L1 expression with clinicopathological parameters

Clinicopathological parameters	PD-L1 negative		PD-L1 positive		p
	n	%	n	%	
Histologic grade					
I	12	100	-	-	**<0.001**
II	174	84.9	31	15.1	
III	87	59.6	59	40.4	
Tubulus formation (score)					
1	5	100	-	-	0.076
2	60	83.3	12	16.7	
3	208	72.7	78	27.3	
Pleomorphism (score)					
1	2	100	-	-	**<0.001**
2	138	85.7	23	14.3	
3	133	66.5	67	33.5	
Mitotic rate (score)					
1	130	85.5	22	14.5	**<0.001**
2	120	78.4	33	21.6	
3	23	39.7	35	60.3	
Stromal response					
Mild	20	52.6	18	47.4	**<0.001**
Intermediate	183	74.4	63	25.6	
Dense	70	88.6	9	11.4	
Immune response					
Mild	234	85.4	40	14.6	**<0.001**
Intermediate	36	47.4	40	52.6	
Dense	3	23.1	10	76.9	
Perineural invasion					
Absent	185	72.3	71	27.7	**0.045**
Present	88	82.2	19	17.8	
Lymphatic invasion					
Absent	136	76.0	44	24.5	0.806
Present	137	74.9	46	25.1	
Lymph node metastasis					
Absent	106	69.7	46	30.3	**0.041**
Present	167	79.1	44	20.9	
ER					
Negative	33	43.4	43	56.6	**<0.001**
Positive	240	83.6	47	16.4	
PR					
Negative	59	56.2	46	43.8	**<0.001**
Positive	214	82.9	44	17.1	
HER2*					
Negative	205	81.3	47	18.7	**<0.001**
Positive	34	49.3	35	50.7	
Molecular subtypes					
Luminal A	160	89.4	19	10.6	**<0.001**
Luminal B	245	83.6	48	16.4	
Triple Negative	15	39.5	23	60.5	
HER2	9	37.5	15	62.5	

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Table 4 Cont.

Clinicopathological parameters	PD-L1 negative		PD-L1 positive		p
	n	%	n	%	
Year of the diagnosis					
2000	2	50.0	2	50.0	0.127
2001	4	80.0	1	20.0	
2002	2	66.7	1	33.3	
2003	4	66.7	2	33.3	
2004	9	90.0	1	10.0	
2005	18	90.0	2	10.0	
2006	8	50.0	8	50.0	
2007	22	81.5	5	18.5	
2008	32	86.5	5	13.5	
2009	38	74.5	13	25.5	
2010	46	67.6	22	32.4	
2011	41	70.7	17	29.3	
2012	47	81.0	11	19.0	
Survival**					
Dead	62	93.9	4	6.1	0.157
Alive	261	90.0	29	10.0	
Recurrence					
Present	71	80.7	17	19.3	0.160
Absent	199	73.7	71	26.3	
Total	273	75.2	90	24.8	

*: 321 cases were known for HER2 status; **: Survival analysis was performed on 356 cases.

ideal fixative is 10% buffered formalin, and the fixation time is recommended as 6–48 hours for core needle biopsies and 24–48 hours for excision materials. Polioudaki et al.[11] showed that long or short fixation with Triton X can disrupt the membranous staining of PD-L1, and staining may occur in the ER-golgi zone. Kai et al.[10] showed that there was no significant change in PD-L1 staining after fixation for up to 1 week, but PD-L1 became negative in 1 case after fixation longer than three months. To minimize differences in fixation, we excluded the consultation cases. Moreover, the age of the paraffin block is another important factor. In a study about gastric adenocarcinomas, PD-L1 positivity was lower in materials older than 42 days.[12] In our study, the positivity rate decreased in the old years, but it wasn't significant statistically.

On the other hand, the evaluation of PD-L1 by IHC is still in process and has many varieties regarding the originated organ, cell types, clone of the antibody, and therapy agent. In gastric carcinomas, cytoplasmic staining was also considered positive.[13] The International Association for the Study of Lung Cancer (IASLC) considers positive different cut-off values among agents and any intensity of complete or partial membranous staining in tumor cells. The cut-off value was considered as ≥25% staining in tumor cells for the SP263 clone in the cases under Durvalumab treatment, while recommended reporting ranges such as <1%, 1–5%, 5–10%, and >10% for the cases under Nivolumab treatment.[14] In BCs, there is no cut-off value determined according to different drugs or antibody clones.[15] Since there was no standardized cut-off value, we noted all the staining and accepted the cut-off value as "1%", which is the lowest value that determines whether the patient with lung carcinoma will receive treatment. Thus, we considered 12 cases with a staining rate <1% as negative. Besides, in our three cases, nuclear positivity in tumor cells was excluded. PD-L1 can also be expressed in endothelium, fibroblasts, and nerve cells; even if its reason is unknown, it can be used as an internal positive control.[16] In our series, there was endothelial staining in one case.

We excluded cytological material or small biopsies and included cases with complete removal of tumor tissue. Even if TMA samples were evaluated in our study, the staining was heterogeneous in all our posi-

Table 5. Logistic regression analysis results of PD-L1 expression and clinicopathological parameters

	B	SE	Wald	Sig.	OR	CI 95%	
						Lower	Upper
Perineural invasion							
Absent							
Present	–0.203	0.381	0.284	0.594	0.816	0.387	1.721
Regional lymph node metastasis							
Absent							
Present	–0.293	0.377	0.602	0.438	0.746	0.356	1.563
ER							
Negative							
Positive	–0.753	0.513	2.158	0.142	0.471	0.172	1.286
PR							
Negative							
Positive	–0.424	0.44	0.927	0.336	0.654	0.276	1.551
HER2							
Negative							
Positive	1.446	0.366	15.601	<0.001*	4.246	2.072	8.701
Stromal response							
Mild (ref)							
Intermediate	–0.977	0.49	3.978	0.046*	0.376	0.144	0.983
Dense	–2.074	0.631	10.787	0.001*	0.126	0.036	0.433
Immune response							
Mild (ref)							
Intermediate	1.598	0.372	18.4	<0.001*	4.942	2.381	10.255
Dense	3.184	0.966	10.859	0.001*	24.132	3.633	160.306
Histologic grade							
1 (ref)							
2	–20.698	11401.192	0	0.999	0	0	.
3	0.164	0.403	0.166	0.684	0.849	0.385	1.869
Constant	0.063	0.702	0.008	0.928	0.939		

R^2=0.31(Cox&Snell) 0,46(Nagelkere) χ^2 =118,846, p<0,001; *: p<0,05. B: Beta; SE: Standard error; Sig.: Significance; OR: Odds ratio; CI: Convidence interval

tive cases for both tumor cells and immune cells. Dill et al.[17] similarly found heterogeneity and accepted that a >50% cut-off value as diffuse positivity. However, some studies reported that PD-L1 expression in tumor cells and TILs were parallel. Zhou et al.[18] found that PD-L1 positivity rates were similar in tumor cells and TILs in IBCs.

Regarding the relation of PD-L1 expression and histomorphologic features, some studies showed that PD-L1 was expressed in IBC with medullary features and in tumors with apocrine and metaplastic features.[19] We couldn't find a correlation between specific histological types. Nevertheless, in our study, PD-L1 expression increased as the histological grade increased. PD-L1 positivity in grade III tumors was significantly higher, as in the literature.[6,15,17–23] It also increased as pleomorphism and mitosis increased. Similarly, Zawlik et al.[22] found that PD-L1 expression increased in high-grade IBCs. In addition, PD-L1 positivity increased in cases whose Ki67 proliferation index was above the median value, similar to some studies.[15,18,21,24]

Higher PD-L1 expression in HER2 expressing IBC and TNBCs, which are known as worse prognostic subtypes, was shown in many studies. Parallelly in our study, PD-L1 expression was significantly correlated with ER/PR negativity and HER2 positivity and was higher in the same subtypes of IBCs. In one study, PD-L1 mRNA level was lower in ER-α positive IBCs. In this study, performed in cell cultures of TNBCs, PD-L1 mRNA level decreased in the presence of ectopic ER-α expression.[25] Similar results were obtained in several studies showing the correlation of ER and PR status with PD-L1.[6,17–19,21,26]

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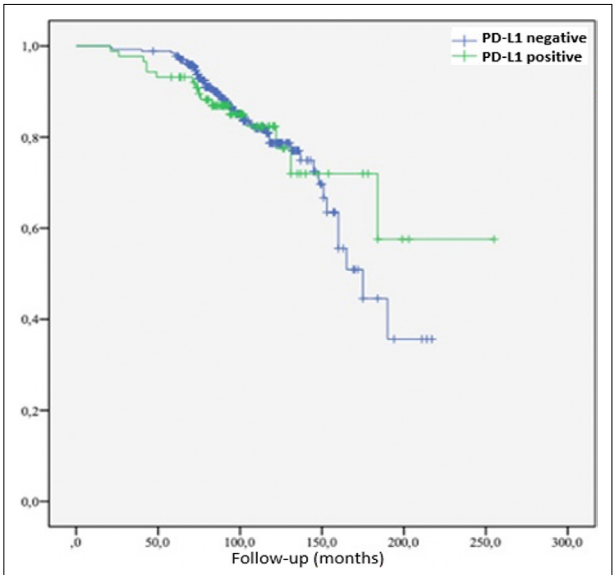

Fig. 4. Overall survival in terms of PD-L1 expression.

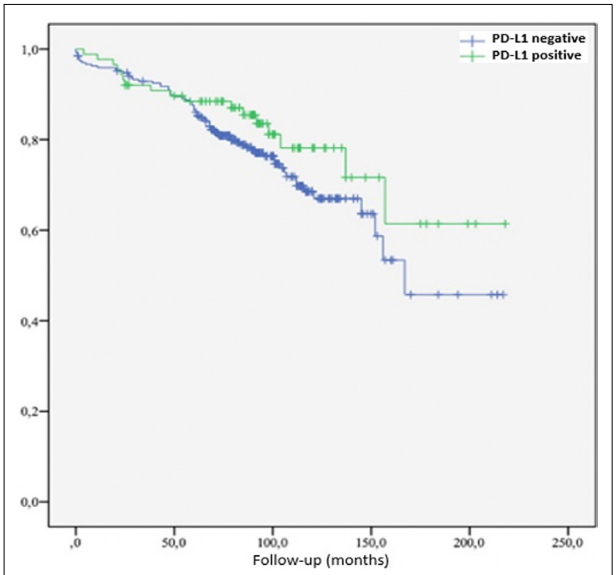

Fig. 5. Disease-free survival in terms of PD-L1 expression.

PD-L1 expression in TILs was also reported as significant.[27,28] The "TIL Working Group" proposed some criteria to determine TIL rate, and according to these criteria, it was demonstrated that a high TIL rate, tertiary lymphoid structures, and PD-L1 expression were seen in more aggressive tumors.[27,29] In our study, cases with the presence of dense TILs had higher PD-L1 expression. An increased level of PD-L1 expression in TILs was also shown to be predictive for neoadjuvant therapy and prognostic for adjuvant therapy.[28] Noske et al.[26] found that PD-1 and PD-L1 (sp263) expression was associated with TILs density in 1318 BCs, and the TILs rate was higher in TNBCs and HER2 expressing types. In another study including 180 cases with neoadjuvant therapy, a correlation was found between PD-1/PD-L1 expression, high TILs rate, and complete regression. Additionally, PD-L1 expression was found mostly in TNBCs.[30] In vimentin-expressing invasive IBCs, it was found that stromal TILs increased and PD-L1 expression decreased.[31]

The presence and density of TILs had prognostic significance in HER2 expressing and TNBCs.[32] In particular, TNBCs were reported as the most immunogenic BCs and had a significant relationship between TILs and prognosis. They are also the most potent candidates for immunotherapy because of higher PD-L1 expression.[5,7] It was shown that when TILs were grouped as low (<10%), moderate (10–50%), and intense (>50%), the prognosis was better with adjuvant treatment in TNBCs with intense TILs, but this wasn't detected in HER2-expressing cases. In this group, the presence of TILs after neoadjuvant therapy was associated with complete remission and a positive prognostic finding, but it wasn't statistically significant.[33] Mori et al.[34] found a high TILs rate in 46.7% of TNBCs, however, couldn't demonstrate its prognostic significance. In studies on molecular subtypes, a high TILs rate was also found in the HER2-expressing type and this rate was associated with PD-L1 expression.[9,21,35] A higher TILs rate was associated with a good prognosis in TNBCs, but the prognostic significance of TILs couldn't be demonstrated in HER2-expressing tumors.[35] In the KEYNOTE 028 study performed on ER+ BCs, PD-L1 positivity was 19% in ER+HER2- cases, PD-L1 expression decreased as ER positivity increased, and PD-L1 expression was higher in luminal B.[36]

Hou et al.[6] found that PD-L1 expression was associated with negative LNs in HER2-expressing tumors. Bae et al.[21] obtained a similar result in 465 BCs. Our results were consistent with them. On the contrary, Soliman et al.[37] found higher PD-L1 expression in cases with LN metastasis. Also, while Karnik et al.[15] found a correlation between PD-L1 expression and lymphatic invasion, we couldn't find it.

Our study included three cases who underwent synchronous bilateral breast surgery. In two cases, PD-L1 was negative in both tumors and TILs in both breasts. In the other case, there was 1% weak staining in both tumor cells and TILs in the larger tumor (4.5 cm, grade III) and 10% staining in TIL in the other BC

focus (0.8 cm, grade II). This may be due to the limitation of sampling or differences in PD-L1 expression according to tumor size and grade. Indeed, considering all our cases, there also was no significant relationship between tumor diameter and PD-L1 expression, as in the literature.[15,18,21]

In one of our cases, both breasts were operated on for IBC-NST (HER2+, ER-, PR-) with an interval of two years: On one side, there was no PD-L1 expression in tumor cells, whereas 10% weak positivity in TILs (4 cm, grade II). In the other breast, there was 2% weak staining in tumor cells and 20% in TILs (1.7 cm, grade III). This result may be due to treatment. Although PD-L1 levels were found to be high in patients treated with Trastuzumab, a direct relationship between HER2 and PD-L1 was not reported in the Cancer Genome Atlas.[38]

In BCs, the prognostic importance of PD-L1 is controversial. In some studies, as PD-L1 expression increased, survival decreased.[39,40] PD-L1 expression was also found to be associated with poor prognostic parameters such as large tumor size, higher grade, ER/PR negativity, and HER2 positivity.[41,42] In a study including 5454 BCs, Sabatier et al.[19] found that PD-L1 had no prognostic significance; however, survival increased as PD-L1 expression increased in basal-type cancers.

The density of TILs is also effective in survival. TILs are grouped as low, medium, and intense, according to density.[29] In a study grouping the proportion of stromal lymphocytes according to a 30% cut-off value, a low lymphocyte ratio was associated with shorter recurrence time but had no effect on OS. Considering the PD-L1 staining rates, the cases with more PD-L1 positive lymphocytes had a worse prognosis.[43] We couldn't obtain significant results in terms of TILs density in survival analyses.

Schmid et al.[44] evaluated the immune cells infiltrating the tumor for PD-L1 SP142 in metastatic TNBCs. In the PD-L1 positive group, 1-year DFS was 29.1% in cases receiving chemotherapy+atezolizumab, and 16.4% in those receiving chemotherapy+placebo. OS was 25 and 15.5 months, respectively. In pilot applications and phase I-II studies on BCs, immunotherapy was applied as a single agent blocking the PD-1/PD-L1 axis or in combination with chemotherapy as an adjuvant or neoadjuvant therapy.[44–46] In these studies, responses ranged from complete remission to stabilization of the disease for a while. These studies included limited cases and had many differences such as patient characteristics, antibody clones, evaluation criteria, and cut-off values.

In a meta-analysis, there was no correlation between patient age and PD-L1 expression similar to our study.[20] In addition, we detected that PD-L1 expression was similar in both genders. This result may be since there were only 4 males in our series. However, in a study, PD-1 and PD-L1 expressions were compared in 165 male and 246 female BCs, and no important difference was found, but less PD-1 expression was detected in male BCs.[23]

We couldn't find a statistically significant relationship between PD-L1 expression and survival such as Li et al.[47] Even if we couldn't find a significant correlation between OS/DFS, PD-L1-expressed cases had better survival. Moreover, Beckers et al.[48] showed OS increased as PD-L1 expression increased, particularly in the presence of TILs. In another study including TNBCs, PD-1 positive immune cells had a greater effect on DFS/OS.[49] In another study including TNBCs, DFS/OS were longer in the presence of PD-L1.[24] On the contrary, in a study including 21 cases with BCs during pregnancy, PD-L1 expression was associated with poor prognosis, and even PD-L1 expression in tumor cells was an independent factor in terms of DFS/OS.[50]

Limitations of the Study

As a method, TMA studies facilitate the evaluation of many cases at the same time and produce quite important results. On the other hand, it should not be forgotten that evaluation is limited to a small area. Particularly in the case of having heterogeneous results in the same case, evaluation of TMA may lead to false positive or negative results. Even if the tumor samples were obtained 0.4 cm in size in our study, the limited sampling may not be representative of the entire tumor, and positive cells may not be detected in the sampled part due to the heterogeneous staining of PD-L1. We herein found a correspondence between better OS/DFS and PD-L1 expression with an insignificant p-value. However, we couldn't compare the treatments in detail. Our results might be affected due to the presence of any difference in treatments or accompanied diseases of the patients. In our series, there were some limitations due to lower numbers. The number of rare conditions such as male BC, synchronous tumors, and examined tumors with close metastasis within a couple of years were limited. Also, even if we found a contradiction between old paraffin age and PD-L1 expression, the number of older age paraffin blocks was lower than recent blocks. These conditions with lower numbers should be examined with larger series.

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CONCLUSION

In our study, PD-L1 expression was correlated with ER/PR negativity, HER2 positivity, Ki-67 value, mitosis, high grade, negative LN, HER2-expressing type, and TNBCs. PD-L1 expression increased as immune cells' density increased.

We couldn't show any significant effect of PD-L1 expression on survival. Many parameters may cause this result. Decreasing PD-L1 positivity in materials belonging to older years suggests that the material should be examined more up-to-date and fresh. Differences between treatments, IHC antibodies, and evaluation methods may cause false negative or positive results. PD-L1 assessment needs standardization.

Although immunotherapy prolongs the survival of cancer patients, it would be an ideal treatment when it could only target the tumor and not be systemic. Further studies and finding other target points are needed.

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