Original Article

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Correlation between the Expression of PD-L1 and Clinicopathological Parameters in Triple Negative Breast Cancer Patients

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ABSTRACT

Objective: Triple-negative breast cancer (TNBC) is a heterogenous group of tumors with no estrogen receptor (ER), progesterone receptor (PR) and Cerb-B2/HER2 expression. Programmed death ligand-1 (PD-L1) is a transmembrane protein located on both non-tumor and tumor cells and it has been shown to be associated with the escape of tumor cells from the immune system. PD-L1-targeted therapy alone or in combination is now an alternative strategy in several aggressive tumor types. In this respect, TNBC is a potential candidate having limited treatment options and poor outcome.

Material and Methods: Sixty-one breast cancers with no expression of ER, PR and Cerb-B2/HER2 were chosen to study PD-L1 immunohistochemistry. PD-L1 staining and its correlation with main clinicopathological parameters were evaluated.

Results: The percentage of PD-L1 positivity was 37.7% and 47.5% in tumor and tumor microenvironment, respectively. The positivity rate was higher in breast carcinomas with medullary features (83.3%) and metaplastic carcinoma (66.6%) subgroups. PD-L1 expression of tumors was positively correlated with their Ki-67 score and PD-L1 positivity of the tumor microenvironment. No significant relationship was found between the other variables.

Conclusion: PD-L1 expression rate was remarkable both in the tumor and the tumor microenvironment of TNBCs. Larger cohorts of TNBC are required to further describe their PD-L1 expression characteristics and help standardize PD-L1 immunohistochemistry assays in these tumors.

Keywords: PD-L1, breast cancer, triple-negative breast cancers, immunohistochemistry, monoclonal antibody

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Introduction

Breast cancer is the most common malignancy and the second most common cause of cancer-related death in women (1). The widespread use of mammographic screening in recent years has increased the awareness of breast cancer (1). Targeted therapies against the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) have provided significant improvement in breast cancer prognosis (2). However, tumors lacking ER, PR and HER2 expression, called triple-negative breast cancers (TNBC), have a poor prognosis and unsatisfactory treatment options (3).

Programmed death ligand-1 (PD-L1) encoded by the CD274 gene on the chromosome 9 is a 40 kDa transmembrane protein found in a number of normal tissue cells such as natural killer cells, macrophages, myeloid dendritic cells, B-cells, epithelial cells and vascular endothelial cells (4). Recent studies on a wide variety of epithelial tumors have shown that tumoral escape from the host immune system is enhanced by the PD-1 (Programmed Death Receptor 1)/PD-L1 signal pathway by the interaction of the PD-1 expressed on tumor-infiltrating lymphocytes (TIL) and the PD-L1 expressed on tumor cells (4).

Expression of PD-L1 in tumor cells is one of the most important mechanisms associated with tumors' defense against immune system attacks (4). Studies have demonstrated that PD-L1 expression is evident in malignant melanoma, renal cell carcinoma, non-small cell lung cancer, colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, some breast carcinomas and various hematological malignancies (5). These tumors are potential targets for PD-1/PD-L1 inhibitor therapies (5). However, data on PD-L1 expression of breast cancers has been limited. There is conflicting data on the possible effect of PD-L1 expression on breast cancer prognosis; some reports indicate PD-L1 to be a favorable factor (6-8), while others consider it unfavorable (2, 4, 9) or of no effect (10, 11).

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In this study, we analyzed PD-L1 expression of 61 TNBC cases and correlated them with major clinicopathological parameters.

Materials and Methods

Case selection and patient data

Triple negative breast cancers diagnosed in our Pathology Department between January 2009 and July 2017 were retrieved from pathology archives. Sixty one cases had paraffin blocks available for the study. The grades and histotypes of tumors were reviewed by two pathologists using American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) breast cancer guidelines. The slides with hematoxylin and eosin (H&E), ER, PR, HER2 and Ki-67 stainings were evaluated. Clinicopathological information including patient age, tumor size, TNM stage, type of surgery, date of the last follow-up and date of recurrence were collected from the medical records retrospectively. The Şişli Hamidiye Etfal Training and Research Center, University of Health Sciences Ethics Committee approval has been received beforehand. Patient consent forms were deemed nonessential.

Immunohistochemical studies

Immunohistochemical staining for PD-L1 antibody (rabbit monoclonal antibody, #13684, clone: E1L3N, cell signalling technologies, USA, 1:400) was performed using the DAB peroxidase method on a (Leica Bond III) device. Other primary antibodies used for immunohistochemical assays are as follows: ER, PR, HER2 and Ki-67. Three-micron thick sections were taken from the paraffin embedded blocks for immunohistochemical assays.

Immunostaining procedure was performed on a (Leica Bond III) device after slides were incubated at 80°C for 3 hours. Briefly, Bond-Dewak solution was applied for 10 minutes at 60°C, slides were then deparaffinized and rehydrated through graded ethanol solutions. Antibody retrieval was carried out by applying ER1 at 96°C for 20 minutes, followed by H_2O_2 blocking for 13 minutes at room temperature. The primary antibody (PD-L1, rabbit monoclonal antibody, #13684, clone: E1L3N, cell signalling technologies, USA, 1:400) was applied for 30 minutes, then it was washed and secondary antibody was applied for 8 minutes at room temperature. DAB was used as a chromogen and hematoxylin was used for counterstaining. Coverslipping followed graded alcohols and xylene.

Immunohistochemical evaluation

Programmed death ligand-1 immunohistochemical staining was evaluated both in the tumor and the peritumoral microenvironment. Tumoral PD-L1 staining was designated as positive when clear membranous or cytoplasmic staining was present in at least 1% of tumor cells. The extent of tumor staining was further classified into the following subcategories: <1%: score 0, 1% to 5%: score 1, 6% to 50%: score 2 and >50%: score 3 (Figure 1). Scores 1 to 3 were considered as posi-

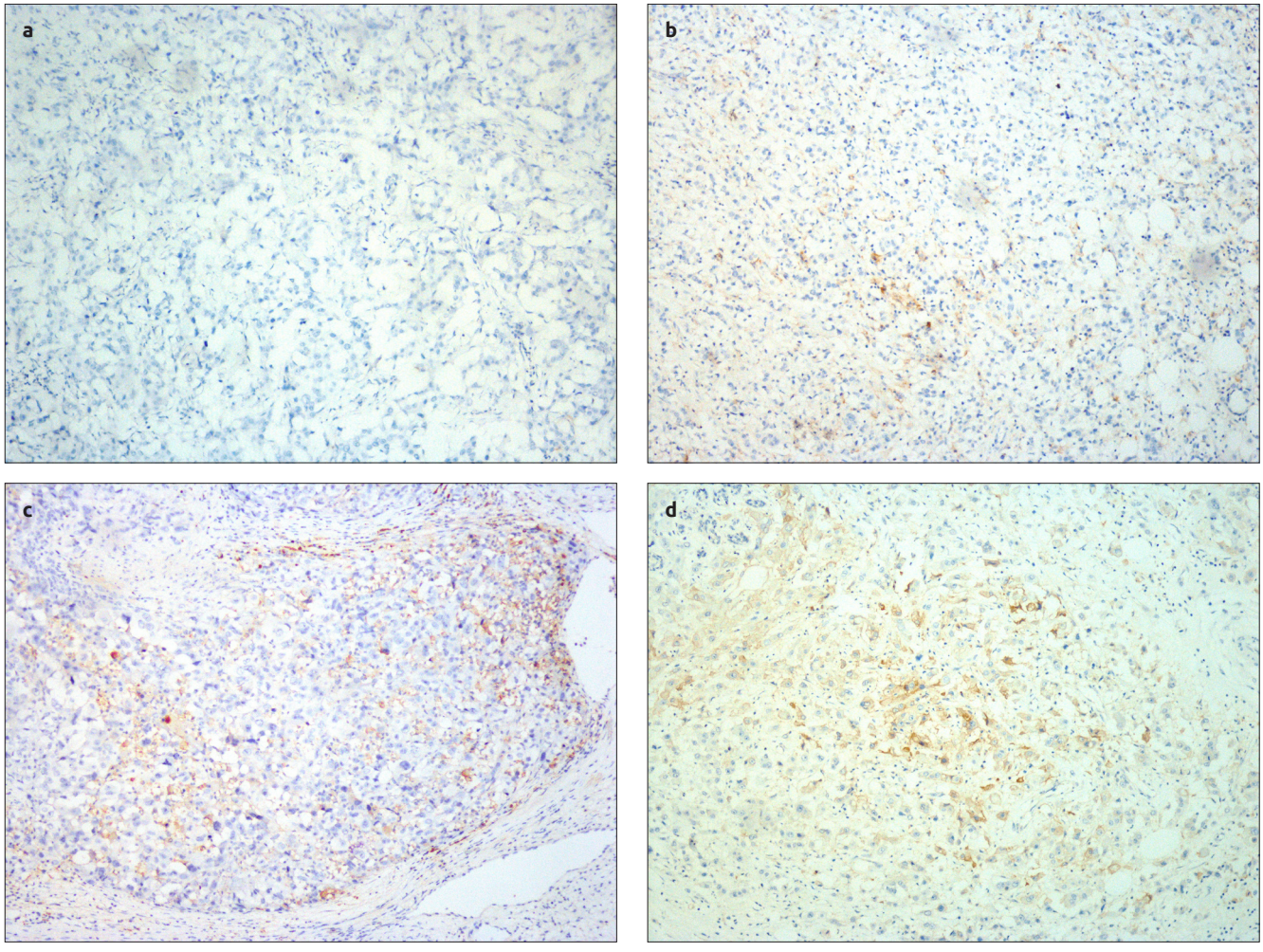

Figure 1. a-d. Tumoral PD-L1 scoring. Score 0: no staining, x100 (a). Score 1: 1-5% tumoral staining, x100 (b). Score 2: 6-50% tumoral staining, x100 (c). Score 3: >50% tumoral staining (d)

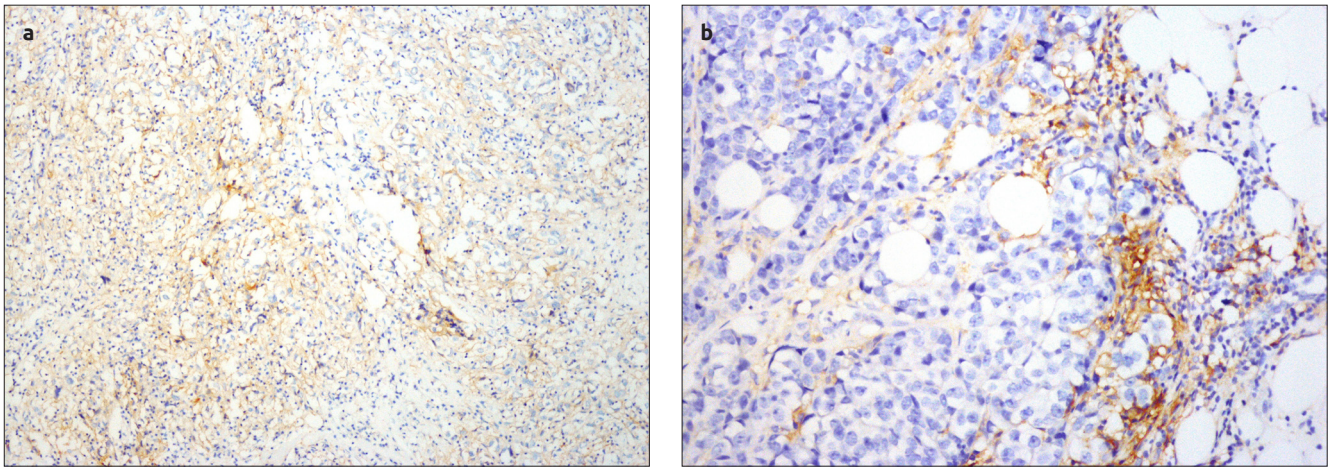

Figure 2. a, b. PD-L1 positivity of tumor and tumor microenvironment. PD-L1 immunostaining in tumor and tumor microenvironment, x100 (a). Marked PD-L1 expression in tumor microenvironment, x200 (b)

Table 1. Clinicopathological features of triple negative breast cancer cases

		Mean±SD (Min-Max)	
Age		50.2±12.0 (26-95)	
Mean Ki-67 score (%)		38.6±23.8 (5-80)	
Tumor diameter (cm)		4.2±3.3 (0.7-15)	
		n	%
Tumor site	UOQ	28	45.9
	UIQ	13	21.3
	LOQ	8	13.1
	Multiple quadrants	5	8.2
	LIQ	4	6.6
	Retroareolar	3	4.9
Histologic type	Invasive carcinoma, NST	42	68.9
	Invasive carcinoma with medullary features	6	9.8
	Metaplastic carcinoma	6	9.8
	Apocrine carcinoma	3	4.9
	Invasive lobular carcinoma	2	3.3
	Mixed carcinoma	1	1.6
	Secretory carcinoma	1	1.6
Histologic grade	1	3	4.9
	2	8	13.1
	3	50	82.0
Nuclear grade	1	1	1.6
	2	11	18.0
	3	49	80.3
Pathologic stage	1	13	21.3
	2	33	54.1
	3	9	14.8
	4	6	9.8
Lymph node metastasis		31	50.8
Lymphovascular invasion		30	49.2
DCIS		22	36.1
Neoadjuvant therapy		16	26.2
Recurrence/distant metastasis		18	31.0

SD: Standard deviation; UOQ: Upper outer quadrant; UIQ: Upper inner quadrant; LOQ: Lower outer quadrant; LIQ: Lower inner quadrant; NST: No special type; DCIS: Ductal carcinoma in-situ

Table 2. PD-L1 expression in tumor and tumor microenvironment

		n	%
Tumoral positivity of PD-L1		23	37.7
Tumoral PD-L1 score	0 (-)	38	62.3
	1 (1-5%)	6	9.8
	2 (5-50%)	11	18.0
	3 (>50%)	6	9.8
Microenvironment positivity of PD-L1		29	47.5
Tumoral or microenvironment positivity of PD-L1		36	59

tive and score 0 as negative. Peritumoral PD-L1 expression was scored as positive or negative where "positive" noted ≥5% PD-L1 staining (Figure 2).

Expressions of ER and PR were considered negative when less than 1% of tumor cells were stained (12). HER2 staining of the tumors were evaluated according to ASCO/CAP recommendations (13). HER2 slides were scored as 0, no staining or faintly seen incomplete membranous staining within <10% of tumor cells; 1+, faintly seen incomplete membranous staining within >10% of tumor cells; 2+, weak/moderate incomplete membraneous staining within >10% of tumor cells or complete circumferential membranous staining within <10% of tumor cells; and 3+, complete circumferential membranous staining within >10% of tumor cells (13). HER2 expression was regarded negative if the score was 1 or lower. Microscopic evaluation of the immunohistochemically stained slides were made by two pathologists (RU, CT).

Table 3. Correlation of tumoral PD-L1 positivity with clinicopathologic parameters

		Tumoral PD-L1 positivity				
		Positive (n=23)		Negative (n=38)		
		n	%	n	%	p
Histologic type	Invasive carcinoma, NST	10	43.5	32	84.2	0.004
	Invasive carcinoma with medullary features	5	21.7	1	2.6	
	Metaplastic carcinoma	4	17.4	2	5.3	
	Invasive lobular carcinoma	1	4.3	1	2.6	
	Apocrine carcinoma	1	4.3	2	5.3	
	Mixed carcinoma	1	4.3	0	0.0	
	Secretory carcinoma	1	4.3	0	0.0	
Histologic grade	1	0	0.0	3	7.9	0.440
	2	4	17.4	4	10.5	
	3	19	82.6	31	81.6	
Nuclear grade	1	0	0.0	1	2.6	1.000
	2	4	17.4	7	18.4	
	3	19	82.6	30	78.9	
Pathologic stage	1	4	17.4	9	23.7	0.545
	2	11	47.8	22	57.9	
	3	5	21.7	4	10.5	
	4	3	13.0	3	7.9	
Lymph node metastasis	present	11	47.8	20	52.6	0.716
	absent	12	52.2	18	47.4	
Lymphovascular invasion	present	13	56.5	17	44.7	0.372
	absent	10	43.5	21	55.3	
DCIS	present	10	43.5	12	31.6	0.348
	absent	13	56.5	26	68.4	
Microenvironment positivity of PD-L1	present	16	69.6	13	34.2	0.007
	absent	7	30.4	25	65.8	
		Mean±SD (Median)		Mean±SD (Median)		p
Mean Ki-67 score (%)		47.8±26.4 (50)		33.0±20.4 (30)		0.017

SD: Standard deviation; NST: No special type; DCIS: Ductal carcinoma in-situ

Statistical Analysis

The software Statistical Package for the Social Sciences version 15.0 (SPSS Inc.; Chicago, IL, USA) was used for the statistical analysis. Independent two-group comparisons were made by Student's t test when the variables provided normal distribution and Mann Whitney U test was used when the variables did not display a normal distribution. Comparisons of ratios in independent groups were performed with Chi-Square Analysis. P values lower than 0.05 were considered statistically significant.

Disease-free survival (DFS) was measured as the time between the date of the initial diagnosis and the date of metastasis or relapse whichever was earlier. The duration of follow-up was the period between the date of diagnosis to the the last follow-up date.

Results

Clinical and histopathological findings

Sixty one TNBCs were included in the study. The mean age was 50.2±12.0 years (range 26-95 years). The mean tumor size was 4.2±3.3 cm (range 0.7–15.0 cm). Tumors consisted of 42 (68.9%) invasive carcinoma, NST, 6 (9.8%) breast carcinomas with medullary features, 6 (9.8%) metaplastic carcinomas, 3 (4.9%) apocrine carcinomas, 2 (3.3%) invasive lobular carcinomas (pleomorphic variant), 1

Table 4. Correlation of microenvironment positivity of PD-L1 with clinicopathologic parameters

		Microenvironment positivity of PD-L1				
		Positive (n=29)		Negative (n=32)		
		n	%	n	%	p
Histologic type	Invasive carcinoma, NST	17	58.6	25	78.1	0.252
	Invasive carcinoma with medullary features	5	17.2	1	3.1	
	Metaplastic carcinoma	4	13.8	2	6.3	
	Invasive lobular carcinoma	1	3.4	1	3.1	
	Apocrine carcinoma	1	3.4	2	6.3	
	Mixed carcinoma	0	0.0	1	3.1	
	Secretory carcinoma	1	3.4	0	0.0	
Histologic grade	1	0	0.0	3	9.4	0.277
	2	5	17.2	3	9.4	
	3	24	82.8	26	81.3	
Nuclear grade	1	0	0.0	1	3.1	1.000
	2	5	17.2	6	18.8	
	3	24	82.8	25	78.1	
Pathologic stage	1	1	3.4	12	37.5	0.001
	2	23	79.3	10	31.3	
	3	3	10.3	6	18.8	
	4	2	6.9	4	12.5	
Lymph node metastasis	present	14	48.3	17	53.1	0.705
	absent	15	51.7	15	46.9	
Lymphovascular invasion	present	15	51.7	15	46.9	0.705
	absent	14	48.3	17	53.1	
DCIS	present	9	31.0	13	40.6	0.436
	absent	20	69.0	19	59.4	
Tumoral PD-L1 score	0	13	44.8	25	78.1	0.033
	1-5%	3	10.3	3	9.4	
	5-50%	9	31.0	2	6.3	
	>50%	4	13.8	2	6.3	
		Mean±SD (Median)		Mean±SD (Median)		p
Mean Ki-67 score (%)		44.5±25.9 (40)		33.3±20.7 (32.5)		0.066

SD: Standard deviation; NST: No special type; DCIS: Ductal carcinoma in-situ

(1.6%) secretory carcinoma, and 1 (1.6%) mixed carcinoma. Three cases (4.9%) were grade I, 8 cases (13.1%) were grade II, and 50 cases (82.0%) were grade III. According to the AJCC's 8th Edition of Cancer Staging Manual, 13 (21.3%) of the pathologically staged tumors were pT1, 33 (54.1%) were pT2, 9 were (14.8%) pT3 and 8 were pT4. At the time of diagnosis, 31 (50.8%) cases were positive and 30 (49.2%) cases were negative for lymph node metastasis (Table 1).

PD-L1 expression

Thirty six cases (59%) displayed PD-L1 expression in either the tumor or the tumor microenvironment. Twenty three cases (37.7%) showed tumoral positivity (score 1-3) with PD-L1. Six (9.8%) of these positive cases were score 1, 11 cases (18.0%) were score 2 and 6 cases (9.8%) were score 3. No tumoral staining (score 0) was observed in 38 cases (62.3%). Twenty nine cases (47.5%) showed PD-L1 positivity (>5%) in tumor microenvironment (Table 2).

Tumor PD-L1 positivity rate was relatively low in patients with invasive carcinoma, NST (23.8%) and high in patients with breast carcinomas with medullary features (83.3%) and metaplastic carcinoma (66.6%) (Table 3).

Programmed death ligand-1 positivity rate in the microenvironment was higher in cases where tumoral PD-L1 was also positive (p=0.007). Similarly, tumoral PD-L1 positivity of the cases with a positive microenvironment staining was statistically significantly high as well (p=0.033). Sixteen cases were PD-L1 positive in both the tumor and the microenvironment. There was no statistically significant relationship between tumoral or microenvironmental PD-L1 expression status and main clinicopathological and survival parameters such as tumor type, tumor grade, lymph node metastasis, lymphovascular invasion (LVI), the presence of ductal carcinoma in-situ (DCIS), recurrence and/or metastatic status (Table 3, 4).

Discussion and Conclusion

Triple negative breast cancers are generally aggressive tumors that occur in a younger population than other breast cancers. They constitute approximately 10-20% of all breast carcinomas (14, 15). Due to their rapid growth, they are usually encountered in advanced stage at the time of diagnosis (3). TNBCs do not benefit from neither hormone therapy nor trastuzumab, due to their lack of responsive receptors (3). Anthracycline, taxane, ixabepilone and platinum-based chemotherapeutic agents are the current treatment strategies; yet there is no single effective agent for these tumors (16). The presence of PD-L1 in TNBCs can justify a potential treatment option and prove to be a prognostic and predictive marker as was demonstrated in other types of tumors (5).

There are significant differences in the method and evaluation of PD-L1 immunohistochemistry assays in the literature. H scores, percentage thresholds (1%) and tiered scoring systems (0-3) are the most common approaches to evaluate tumoral PD-L1 expression (4, 10, 17, 18). Threshold values of 1% and 5% have been applied to assess PD-L1 positivity in the tumor microenvironment (10, 17). Three different clones of PD-L1 (E1L3N, SP142, 28-8) have been used in different studies (4, 6, 10, 17, 18). In one study that compares these three clones, the staining rates in each of the three clones were found to be different from each other but their superiority was not specified (19). Further studies are recommended in larger groups to determine the gold standard antibody and the optimal cutoff value (19). E1L3N was the preferred clone in our study. We evaluated the PD-L1 response both in the tumor and the tumor microenvironment as was done by others (10, 20). We preferred a 0-3 scoring system for tumoral staining and a 5% cutoff for microenvironmental staining. The lack of validation among different PD-L1 clones limits our study. Besides, further analytic methods other than immunohistochemistry could enhance the value of our results.

Programmed death ligand-1 expression ranges between 8.3%-59% for the tumoral compartment and between 16.2%-93% for the microenvironment in the studies with different evaluation methods and clones (4, 6, 10, 17-21). We found a PD-L1 expression (score 1-3) rate of 37.7% (23 cases) in our 61 TNBC cases. Staining was negative (score 0) in 38 cases (62.3%). In detail, the numbers of cases with each score were 6 (9.8%), 11 (18.0%) and 6 (9.8%) for the score 1, 2 and 3 respectively. In two TNBC studies using the same clone, tumoral staining was reported as 21% and 33.2% (17, 21). Dill et al. (17) identified a subgroup of TNBC with high PD-L1 expression (>50%) which they named 'diffuse staining'; it constituted 5% of their cases. We named this pattern 'score 3' and 9.8% of our cases were in this subgroup. In our study, there was PD-L1 positivity in 36 cases (59%) in at least one compartment and there was a statistically significant positive correlation between the PD-L1 tumoral staining and the expression of the PD-L1 in tumor microenvironment (p=0.007).

The relationship between tumoral PD-L1 positivity and Ki-67 proliferation index was found to be statistically significant (p=0.017). This result should be supported by the data of further survival studies. There was no statistically significant relationship between PD-L1 expression in tumor or tumor microenvironment and parameters such as age, tumor size, tumor grade, lymph node metastasis, the presence of LVI or DCIS, recurrence and/or metastasis status. However, there are several studies in the literature that reported a significant relationship between some of these parameters and tumoral PD-L1 expression (4, 6, 10, 17, 18). There was a statistically significant difference in histopathological tumor types of the PD-L1 positive and negative cases in our study (p=0.004). Most of the 61 TNBC cases were invasive ductal carcinomas, NST and 84.2% of these showed no expression of PD-L1. However tumors with medullary-like features and metaplastic carcinomas showed high PD-L1 expression ratios; 83.3% and 66.6% respectively. Increased PD-L1 expression has been previously reported in breast carcinomas with medullary features, apocrine and metaplastic carcinoma subtypes of breast cancer (17).

Triple-negative breast cancers are tumors showing early and frequent recurrence and/or metastasis (22). The mean follow-up period in our study was 24.8 months (0-87 months) and recurrence and/or metastasis was seen in 31% of cases. The mean duration of disease-free follow-up was 22.6 months. However, the follow-up times of our cases were too short and the clinical data were mostly insufficient to build up a Kaplan-Meier plot. Several studies in the literature showed variable association between PD-L1 expression and overall or disease-free survival (10, 20, 21). Studies with larger series can clarify the relevance of PD-L1 with regards to survival.

In conclusion, PD-L1 expression rate was remarkable both in the tumor and the tumor microenvironment of TNBCs. There was a statistically significant association between the tumoral PD-L1 positivity and parameters such as histological type and Ki-67 index, but no relationship was found between PD-L1 expression and other prognostic factors. Data presented by other reports in the literature is highly variable on account of technical differences and use of several PD-L1 clones. Standardization should be provided with further studies.

Triple-negative breast cancers constitute a tumor category that has no specific targeted therapy and requires new therapeutic options. The expression of PD-L1 in and around these tumors may provide rationale for the use of anti-PD-L1 therapies (PD-L1 monoclonal antibodies) for these aggressive neoplasms. Larger cohorts of TNBC are required to further describe PD-L1 expression characteristics and help standardize PD-L1 immunohistochemistry use in these tumors.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Şişli Hamidiye Etfal Training and Research Center (Approval Date: 23.01.2018 / Approval Number: 1876).

Informed Consent: Informed consent was not received due to the retrospective nature of the study

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