

CLINICAL STUDY

The value of maspin and PD-L1 expression and peritumoral lymphocytic infiltration in breast tumors

Tuncel F¹, Bozkurt F¹, Berkesoglu M²Mersin University Medical Faculty, Medical Pathology Department, Mersin, Turkey. ferahdaloglu@hotmail.com**ABSTRACT**

OBJECTIVE: Breast cancer is the most common type of cancer in women worldwide. Our aim is to evaluate the importance of immunotherapy in breast cancer cases by using PD-L1 expression and to determine the effect of Maspin expression and lymphocytic density on predicting the prognosis.

METHODS: A total of 200 breast cancer cases with different histological types were included in the study. The expression of PD-L1 and Maspin in these tumors were evaluated, and the lymphocytic infiltration density was examined.

RESULTS: Expression of PD-L1 in tumor cells was significantly higher in "medullary carcinoma" and grade 3 tumors. Expression of PD-L1 in inflammatory cells was increased in the group of "other types" and grade 3 tumors. Maspin expression was observed in 93 cases and was higher in "lobular carcinoma" and high grade tumors. In our study, a significant correlation was found between lymphocytic density score and histological grade and PD-L1 expression.

CONCLUSION: In our study, PD-L1 expression was detected in breast cancer, and was associated with triple negativity and high grade. Our results show that the Maspin expression and lymphocyte density are associated with the prognosis (Tab. 5, Fig. 4, Ref. 44). Text in PDF www.elis.sk

KEY WORDS: Breast carcinoma, PD-L1, Maspin, lymphocyte infiltration.

Introduction

Breast cancer is the most common cancer type in women worldwide and is the leading cause of cancer death (1). The use of screening methods, innovations in diagnosis and treatment reduce the mortality rates related to breast cancer. However, the heterogeneous nature of breast cancer affects the response to treatment (2). The importance of the immune system in the progression of breast cancer was emphasized by showing the effect of tumor microenvironment on survival and response to treatment (3). In recent years, immunotherapies that target immune control point molecules such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein-1 (PD-1) have emerged as new treatment modalities (2).

PD-1 is an immune suppressive molecule from the B7-CD28 family that regulates T cell activation (4). PD-1 is expressed on tumor infiltrating lymphocytes (TIL), CD4+ T cells, B cells, natural killer cells, monocytes and dendritic cells. PD-1 has two ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) (5). PD-L1 is expressed in hematopoietic cells and some tumor cells (6).

Expression of PD-L1 in tumor cells is explained by two mechanisms. In the primary mechanism, tumor cells activate PD-L1 expression with various oncogenic signal pathways such as phosphoinositide 3-kinase / protein kinase B (PI3K / AKT), extracellular-signal-regulator protein kinase / mitogen-activating kinase (Erk / MAPK), Janus kinase (JAK2) / STAT (7). The second mechanism (adaptive immune resistance) leads to PD-L1 expression on tumor cells in response to specific cytokines, especially IFN- γ . IFN-induced expression of PD-L1 is more common in most cancer histologies than in structural expression. This expression occurs mostly in T cell-rich and invasive areas of the tumor. In some tumors, there is a significant correlation between PD-L1 expression, T-cell infiltration and IFN- γ levels in intrinsic immune resistance (8).

PD-1 reduces anti-tumor T cell activity after binding to its ligands and facilitates tumor escape from the immune system (9). In many solid tumors such as melanoma, renal cell carcinoma and lung cancer, blockade of the PD-1 / PD-L1 axis has been found to increase survival (10–12).

Maspin is a member of the serine protease inhibitor family. It was first described in normal breast tissue in myoepithelial cells and later in breast cancers (13). Studies have shown that maspin plays a role in the inhibition of metastasis and invasion of cancer cells. In addition, maspin protein has an inhibitory effect on angiogenesis in cancers. However, the mechanism of Maspin function and its role in cancer is still uncertain and there is insufficient information about it (14).

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In studies conducted on the role and importance of maspin protein in breast cancer, conflicting results have been observed (14, 15). Studies have shown that nuclear maspin expression is associated with well-differentiated tumors and good prognosis, while cytoplasmic maspin expression is associated with poor prognosis in many cancers (16, 17).

Maspin is a tumor suppressor protein found in normal breast myoepithelial cells. Its expression is known to decrease in breast cancer progression, and decreased expression is associated with a higher risk of distant metastasis (18, 19).

Immune system and tumor microenvironment play an important role in the progression of breast cancer (3). Medullary carcinoma, which is one of the triple negative breast cancers, has a relatively good prognosis that is partially attributed to the lymphocytic response (20). The vast majority of malignant breast tumors contain varying degrees of lymphocytic infiltration in their stroma (21).

Our aim in this study is to detect PD-L1 and maspin protein expressions in different types of breast cancers, to evaluate the lymphocytic density of different types of tumors comparatively and to investigate their relationship with clinicopathological parameters. Thus, the importance of immunotherapy in cases with breast cancer will be evaluated and the effect of maspin expression and lymphocytic density on predicting prognosis will be determined.

Materials and methods

Case selection

200 cases diagnosed with breast cancer in mastectomy specimens evaluated between 2015–2019 in Mersin University Medical Faculty Medical Pathology Department were included in the study. 115 of the cases were invasive ductal carcinoma (25 histological grade 1, 40 histological grade 2, 50 histological grade 3), 35 were invasive lobular carcinoma, and 25 were medullary carcinoma/ invasive carcinoma with medullary features. 25 cases included other histological types (tubular, cribriform, micropapillary, mucinous carcinoma). Age, gender, tumor size, lymph node metastasis, lymphovascular invasion, pathological stage information of the cases were obtained from the hospital information system and corresponding pathology reports.

This study was approved by Mersin University Clinical Research Ethics Committee with the decision numbered 2019/34 on 23/01/2019.

Immunohistochemical staining

Sections were taken from the prepared paraffin blocks and staining was performed with PD-L1 and Maspin antibody. This staining was done in Ventana Benchmark-XT automatic staining device (Ventana Medical Systems; Roche; USA) using anti-PD-L1 (SP142), anti-Maspin (ab182785) antibodies and ultraView Universal DAB Detection kit. Placental tissue for PD-L1 and esophageal squamous cell carcinoma tissue sample for Maspin antibody were used as positive external control.

Immunohistochemical assessment

Immunohistochemically stained slides were evaluated by 2

pathologists for each case. Expression of PD-L1 was evaluated separately in both tumor cells and inflammatory cell groups accompanying the tumor. Cytoplasmic or membranous staining was considered significant, and the percentage of positive stained tumor cells and inflammatory cells was determined.

PD-L1 expression is graded as 0 if tumor cells do not have staining or have staining below 1 %, score 1 if they show 1–5 % staining, score 2 if they have 5–50 % staining, score 3 if 50 % or more of tumor cells show staining. PD-L1 expression was considered positive for SP142 clone of tumor cells with scores 2 and 3 staining. In inflammatory cells, no staining or below 1 % staining were considered PD-L1 negative and, 1% or more staining is considered to be PD-L1 positive (22).

Expression was determined as positive in the presence of staining in more than 10 % of tumor cells with maspin antibody. Cytoplasmic, nuclear or cytoplasmic and nuclear staining were accepted as positive (23).

In the evaluation of the lymphocytic density of the tumors, the score was graded as 1 if the lymphocytic response was present in less than 10% of the area that contains tumor cells, graded as 2 if that area percentage was between 10–30 %, and graded as 3 for area percentage of 30 % and above.

Tab. 1. Clinicopathological features of breast cancer cases.

Clinicopathologic features	Total n=200 (100%)	
	n	(%)
Age Median (range)	55.96±13.09 (27–85)	
≤ 50 years	72	(36)
>50 years	128	(64)
Gender		
Female	197	(98.5)
Male	3	(1.5)
Tumor type		
Invasive ductal carcinoma (NST)	115	(57.5)
Invasive lobular carcinoma	35	(17.5)
Medullary carcinoma/invasive carcinoma with medullary features	25	(12.5)
Other types	25	(12.5)
Histologic grade		
Grade I	31	(15.5)
Grade II	80	(40)
Grade III	89	(44.5)
Tumor size		
≤ 2 cm	56	(28)
2–5 cm	123	(61.5)
>5 cm	21	(10.5)
Lymphnode metastasis		
Present	89	(44.5)
Absent	111	(55.5)
Lymphovascular invasion		
Present	89	(44.5)
Absent	111	(55.5)
Lymphocytic infiltration density score		
Score 1 (<10%)	114	(57)
Score 2 (10–30%)	51	(25.5)
Score 3 (≥30%)	35	(15.5)

n – number of patients

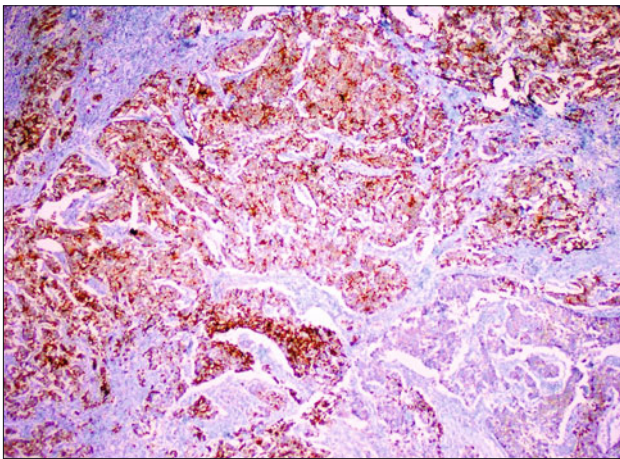


Fig. 1. PD-L1 expression in tumor cells (x 40).

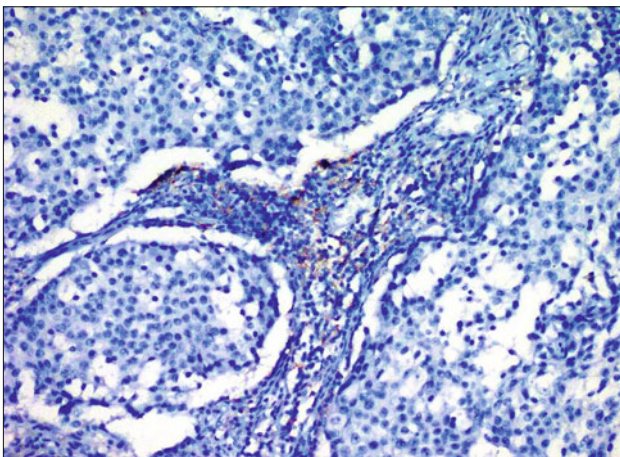


Fig. 2. PD-L1 expression in inflammatory cells(x 200).

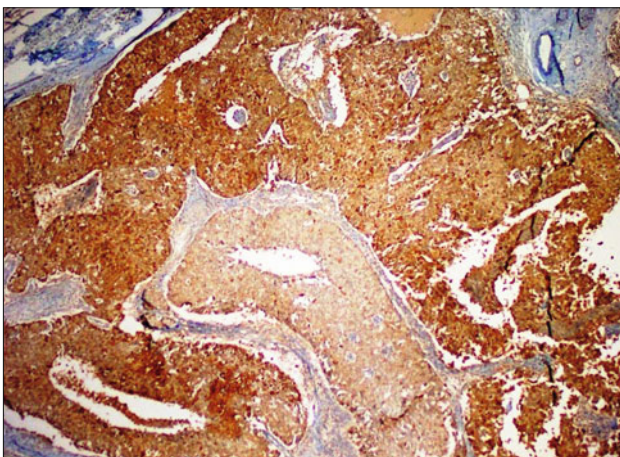


Fig. 3. Cytoplasmic maspin expression(x 40).

Statistical analysis

All statistical analyses were done with STATISTICA Version 13.5.0.17 17 (TIBCO Software Inc. (2017)) program. The normal distribution assumption was checked by the Shapiro Wilk test. Age,

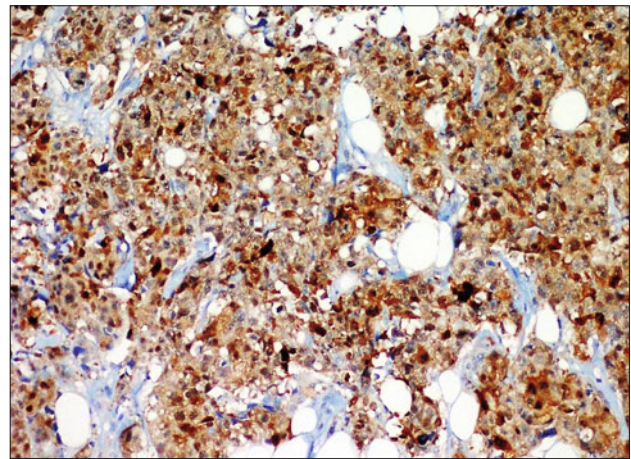


Fig. 4. Cytoplasmic and nuclear maspin expression(x200).

minimum, maximum and mean \pm standard deviation values matching normal distribution and continuous variables that do not show normal distribution are summarized, with the median and quarterly differences (Q3-Q1), the categorical variance of clinicopathological features, PD-L1 expressions, maspin expressions and lymphocytic density scores (summarized as frequencies [n] and percentage rates (%)). To investigate the relationships between categorical variables, Chi-Square analysis was performed. As a result of this analysis, taking into account the minimum expected values and their rates; the p values of Pearson Chi-square test, Fisher's Exact test or Likelihood Ratio test were used. The ratios of variables with statistically significant relationship in cross tables with more than two rows or columns were compared with two ratios Z test. Kruskal-Wallis test, one of the non-parametric methods, was used to compare the medians of more than two independent groups.

Statistical significance level of $(p) \leq 0.05$ was accepted for all comparisons.

Results

This study includes 200 cases diagnosed with breast carcinoma. The age of the patients ranged from 27 to 85 (55.96 ± 13.09). The clinicopathological features of the cases are summarized in Table 1.

PD-L1 expression

PD-L1 expression was evaluated in tumor cells and inflammatory cells that infiltrate the tumor. Patients with a score of +2 and +3 according to the percentage of staining in tumor cells and 1% or more staining in inflammatory cells were considered expression positive.

PD-L1 expression in tumor cells and its relationship with clinicopathological parameters

Expression of PD-L1 (clone sp142) in tumor cells was detected in 12 (6%) cases (Fig. 1). Five of the cases with expression were diagnosed with invasive ductal carcinoma, 1 with invasive lobular carcinoma and 6 with medullary carcinoma / invasive carcinoma

Tab. 2. PD-L1 expression in tumor cells and its relationship with clinicopathological parameters.

Clinicopathologic parameters	PD-L1 negative n=188	PD-L1 positive n=12	p
Age			
≤ 50 years	69	3	0.543
>50 years	119	9	
Gender			
Female	185	12	1.0
Male	3	0	
Tumor type			
Invasive ductal carcinoma (NST)	110	5	0.005*
Invasive lobular carcinoma	34	1	
Medullary carcinoma/invasive carcinoma with medullary features	19	6	
Other types	25	0	
Histologic grade			
Grade I	30	1	0.002*
Grade II	80	0	
Grade III	78	11	
Tumor size			
≤ 2 cm	54	2	0.812
2–5 cm	114	9	
>5 cm	20	1	
Lymphnode metastasis			
Present	85	4	0.422
Absent	103	8	
Lymphovascular invasion			
Present	85	4	0.422
Absent	103	8	
Lymphocytic infiltration density score			
Score 1 (<10%)	110	4	0.033*
Score 2 (10–30%)	44	7	
Score 3 (≥30%)	34	1	

n – number of patients. * Significant (p < 0.05)

with medullary features. In our study, the PD-L1 expression rate in patients with medullary carcinoma / invasive carcinoma with medullary features was significantly higher than in others (p = 0.0001).

11 of PD-L1 positive tumors were histological grade 3, 1 was histological grade 1. Grade 3 tumor cases had significantly higher percentage of PD-L1 expression positivity (p = 0.0007).

A statistically significant relationship was found between PD-L1 expression in tumor cells and lymphocytic density score (p ≤ 0.05). In cases with lymphocytic density between 10–30 % (score 2), PD-L1 expression positivity rate (58.3 %) was higher than negative (24.3 %), and this difference between the rates was statistically significant (p = 0.007). In cases where the lymphocytic response was score 1 and score 3, there was no significant difference in terms of PD-L1 expression in tumor cells.

There was no significant correlation between PD-L1 expression in tumor cells and other clinicopathological parameters (lymph node metastasis, lymphovascular invasion, tumor size) (p > 0.05) (Tab. 2).

PD-L1 expression in inflammatory cells and its relationship with clinicopathological parameters

Expression of PD-L1 (clone sp142) in inflammatory cells was positive in 45 (22.5 %) cases, and no expression was observed in

Tab. 3. PD-L1 expression in inflammatory cells and its relationship with clinicopathological parameters.

Clinicopathologic parameters	PD-L1 negative n=155	PD-L1 positive n=45	p
Age			
≤ 50 years	54	18	0.525
>50 years	101	27	
Gender			
Female	154	43	0.250
Male	1	2	
Tumor type			
Invasive ductal carcinoma (NST)	98	17	<0.0001*
Invasive lobular carcinoma	29	6	
Medullary carcinoma/invasive carcinoma with medullary features	16	9	
Other types	12	13	
Histologic grade			
Grade I	27	4	0.023*
Grade II	67	13	
Grade III	61	28	
Tumor size			
≤ 2 cm	46	10	0.617
2–5 cm	93	30	
>5 cm	16	5	
Lymphnode metastasis			
Present	69	20	0.993
Absent	86	25	
Lymphovascular invasion			
Present	70	19	0.727
Absent	85	26	
Lymphocytic infiltration density score			
Score 1 (<10 %)	101	13	<0.0001*
Score 2 (10–30 %)	36	15	
Score 3 (≥30 %)	18	17	

n – number of patients. * Significant (p < 0.05)

155 cases (Fig. 2). Considering the relationship between tumor types and PD-L1 (clone sp142) expression in inflammatory cells, the rate of negative cases in invasive ductal carcinoma was significantly higher than positive (p = 0.0002), while the group that contained other tumor types had significantly higher PD-L1 expression positivity rate (p = 0.0002).

In our study, a statistically significant relationship was found between tumor grade and PD-L1 expression in inflammatory cells (p ≤ 0.05). In Grade 3 tumors, PD-L1 positivity in inflammatory cells was found to be significantly higher (p = 0.006).

In tumors with a lymphocytic density of 30 % or more (score 3), the positivity of PD-L1 expression in inflammatory cells was found to be significantly higher than score 1 and score 2 tumors (p = 0.00001). There was no significant correlation between PD-L1 expression in inflammatory cells and other clinicopathological parameters (p > 0.05) (Tab. 3).

Maspin expression

In our study, maspin expression positivity was found in 93 of 200 cases (46.5 %). In 73 (78.5 %) of 93 cases with positive maspin expression, staining was detected only in the cytoplasm (Fig. 3), and 20 (21.5 %) in both the cytoplasm and nucleus (Fig. 4). There was no case with only nuclear maspin positivity.

Tab. 4. Relationship between Maspin expression and clinicopathological parameters.

Clinicopathologic parameters	Maspin negative n=107	Maspin positive n=93	p
Age			
≤50 years	37	35	0.653
>50 years	70	58	
Gender			
Female	104	93	0.297
Male	3	0	
Tumor type			
Invasive ductal carcinoma (NST)	67	48	0.033*
Invasive lobular carcinoma	13	22	
Medullary carcinoma/invasive carcinoma with medullary features	10	15	
Other types	17	8	
Histologic grade			
Grade I	20	11	0.252
Grade II	38	42	
Grade III	49	40	
Tumor size			
≤2 cm	33	23	0.263
2–5 cm	66	57	
>5 cm	8	13	
Lymphnode metastasis			
Present	48	41	0.913
Absent	59	52	
Lymphovascular invasion			
Present	52	37	0.211
Absent	55	56	
Lymphocytic infiltration density score			
Score 1 (<10 %)	56	58	0.341
Score 2 (10–30 %)	31	20	
Score 3 (≥30 %)	20	15	

n – number of patients. * Significant (p < 0.05)

Maspin expression and its relationship with clinicopathological parameters

48 of 93 cases with maspin expression were diagnosed as invasive ductal carcinoma, 22 were invasive lobular carcinoma, 15 were medullary carcinoma / invasive carcinoma with medullary features, 8 were other types of carcinoma. In our study, a significant relationship was found between tumor types and maspin expression, and it was found that maspin expression was significantly higher in invasive lobular carcinoma (p = 0.03). No significant relationship was observed with other clinicopathological parameters (p > 0.05) (Tab. 4). However, when the median value of maspin expression percentage and tumor grade were compared, it was found that grade 3 tumors had higher maspin expression percentage than grade 1 tumors (p = 0.031). Grade 3 tumors had a median maspin expression value of 5, Q1 value of 0 and Q3 value of 55; meanwhile for grade 1 tumors, these values were 0, 0 and 10 respectively.

When the maspin staining method was evaluated, cytoplasmic staining was found to be significantly higher in medullary carcinoma / invasive carcinoma with medullary features cases (p = 0.001). In our study, there was no significant relationship between maspin expression and PD-L1 expression in tumor cells and inflammatory cells (p > 0.05).

Tab. 5. Relationship between lymphocytic density score and clinicopathological parameters.

Clinicopathologic parameters	Lymphocytic density score			p
	Score 1 n=114	Score 2 n=51	Score 3 n=35	
Age				
≤50 years	40	17	15	0.634
>50 years	74	34	20	
Gender				
Female	113	50	34	0.683
Male	1	1	1	
Tumor type				
Invasive ductal carcinoma	62	38	15	<0.0001*
Invasive lobular carcinoma	33	1	1	
Medullary carcinoma/invasive carcinoma with medullary features	12	8	5	
Other types	7	4	14	
Histologic grade				
Grade I	22	6	3	0.014*
Grade II	52	13	15	
Grade III	40	32	17	
Tumor size				
≤2 cm	34	15	7	0.553
2–5 cm	67	30	26	
>5 cm	13	6	2	
Lymphnode metastasis				
Present	45	27	17	0.238
Absent	69	24	18	
Lymphovascular invasion				
Present	45	29	15	0.113
Absent	69	22	20	

n – number of patients. * Significant (p < 0.05)

Lymphocytic density score and its relationship with clinicopathological parameters

In our study, our lymphocytic density scoring detected 114 cases as score 1, 51 cases as score 2, and 35 cases as score 3.

In invasive ductal carcinoma cases, the number of cases with lymphocytic density score 1 was significantly higher than score 2, and the number of score 2 cases was significantly higher than score 3 (p = 0.01, p = 0.003). In lobular carcinoma, the number of score 1 cases was found to be much higher than the number of score 2 and 3 cases, and this difference was statistically significant (p = 0.0001, p = 0.0001). The number of cases with lymphocytic density score of 3 in the group containing other carcinomas was significantly higher than the other 2 groups (p = 0.0001, p = 0.0003). There was no statistically significant difference between the lymphocytic density score groups in medullary carcinoma (p > 0.05).

A statistically significant relationship was found between lymphocytic density score and grade (p ≤ 0.05). The rate of score 1 cases in Grade 2 tumors is higher than score 2 cases (p = 0.001). In Grade 3 tumors, the rate of score 2 cases was higher than score 1 cases (p = 0.001). No relationship was found with other clinicopathological parameters (p > 0.05) (Tab. 5).

Discussion

PD-L1 is a type I transmembrane protein with 290 amino acids encoded by the CD272 gene on the 9th chromosome (24). It is

known that PD-L1 is expressed in tumor cells and immune cells that infiltrate the tumor (25). Tumor cell expressing PD-L1 inhibits T cell function and prevents tumor eradication. This situation can be prevented by anti-PD-1 / PD-L1 immunotherapy (26). The blockade of the PD-1 / PD-L1 pathway shows significant improvements with new agents used in lung cancer, renal cell carcinoma and melanoma (27). In immunotherapy, not only PD-L1 expression in tumor cells but also intratumoral and peritumoral immune cell density and PD-L1 expression in those immune cells are important (28).

In the literature, studies on breast cancer have shown that PD-L1 expression is more common in lymphocytes that infiltrate the tumor rather than tumor cells. Its expression in lymphocytes infiltrating the tumor has been shown to be associated with prognosis (29–30). In our study, PD-L1 expression was detected in tumor cells in 12 (6 %) cases and in inflammatory cells in 45 (22.5 %) cases. A wide variety of positivity rates were found in the literature studies. This may be due to the differences in the antibody clones, scoring systems, sample size, and the positively evaluated cell type (tumor cell, inflammatory cell) (31–35).

Increased proliferative activity, high histological and nuclear grade and advanced stage are known to be poor prognostic criteria in breast cancer (36). In addition, PD-L1 expression in breast cancer is generally associated with the presence of tumor infiltrating lymphocytes and prognostic criteria such as high grade, triple negative histological type, and its expression is heterogeneous (37, 38). It is known that PD-L1 expression is detected as a negative prognostic factor in different tumors (39). In our study, PD-L1 expression in tumor cells was highest in medullary carcinoma / invasive carcinoma with medullary features. Also, PD-L1 expressions in both tumor cells and inflammatory cells were found to be significantly higher in histological grade 3 tumors. Similarly, in the study of Tawfik et al, PD-L1 expression was reported to be associated with higher grade and increased proliferative activity (31).

Maspin is a member of the serine protease family, which inhibits tumor invasion and angiogenesis, regulates apoptosis and shows tumor suppressive properties (13). In a study, it was reported that maspin expression decreased in the progression from in-situ ductal carcinoma to invasive carcinoma (40).

In our study, maspin expression positivity was found in 93 of 200 cases (46.5 %). Maspin positivity was found to be higher in invasive lobular carcinoma than other types of tumors. In some studies, it has been shown that there is a relationship between high tumor grade and maspin expression (23, 41).

Maspin expression is generally expected to be limited to cytoplasm. However, in a study by Pemberton et al., it was found to be expressed not only in the cytoplasm, but also in the nucleus (42). In our study, 73 (78.5 %) of 93 cases with positive maspin expression were detected only in cytoplasm and 20 (21.5 %) in both cytoplasm and nucleus. In addition, cytoplasmic maspin expression was significantly higher in medullary carcinoma / invasive carcinoma with medullary features cases ($p = 0.001$), but no relation was found between clinicopathological parameters and maspin staining. In a study, cytoplasmic staining was found in 21 (63.4 %) of 33 maspin positive cases and nuclear staining was found in 12 cases (36.6 %). Similar to our study, nuclear staining was always accompanied by

cytoplasmic staining. In this study; higher grade, lymph node metastasis and perineural invasion were found to be associated with cytoplasmic maspin expression. Therefore, cytoplasmic maspin expression is thought to be associated with poor prognosis (43). In a study of Gurzu et al, In colorectal carcinomas, tumors in which cytoplasmic maspin staining is dominant have a better prognosis and nuclear dominance is associated with high aggressiveness (44). In our study, maspin expression was detected in invasive breast carcinoma cases, and it was found that there was an expression difference between tumor types. Maspin expression percentage was found to be higher in grade 3 cases than grade 1 cases ($p = 0.031$).

The density of lymphoid cells accompanying the tumor is important in terms of prognosis and treatment response (3). In our study, varying degrees of lymphocytic density were observed in different types of breast cancer. As the histological grade progressed from grade 2 to grade 3, the lymphocytic density score increased. Our results also showed that there was a significant relationship between the degree of lymphocytic density and the expression of PD-L1 in tumor cells and inflammatory cells. However, more detailed studies are needed for the detection and importance of subtypes of these lymphoid cells.

Our results showed that there is PD-L1 expression in breast cancer. It was determined that PD-L1 expression was associated with prognostic parameters such as high grade, and triple negativity. This expression was detected in both tumor cells and inflammatory cells and was more intensely detected in inflammatory cells in accordance with the literature. Therefore, tumor cells should not be examined exclusively in the evaluation of PD-L1, and inflammatory cells should also be evaluated.

In conclusion, our study emphasizes the applicability and importance of immunotherapy based on the blockade of PD-1 / PD-L1 axis in the treatment of breast cancer. In our study, breast cancer types other than invasive ductal carcinoma were also examined. As a result of the study, it was determined that a significant amount of maspin expression occurred in tumor cells, and the lymphocytic density accompanying the tumor was graded and examined. The results obtained and the literature information show that these parameters will make important contributions in predicting prognosis.

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