Original paper

Investigation of PD-L1 (CD274), PD-L2 (PDCD1LG2), and CTLA-4 expressions in malignant pleural mesothelioma by immunohistochemistry and real-time polymerase chain reaction methods

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Malignant pleural mesothelioma (MPM) is an aggressive malignant disease with a poor prognosis, which affects the surface mesothelium of the pleural cavity. Immune checkpoints are responsible for controlling the immune system to avoid autoimmunity and prevent tissue damage.

In this study, we aimed to investigate the expression of cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death ligand 1 (PD-L1), and programmed death ligand 2 (PD-L2) immuno-control receptors in MPM patients and the relationship of the expression with tumour types and prognostic parameters.

In this study, we evaluated 50 MPM cases. Immunohistochemically CTLA-4, PD-L1, and PD-L2 were detected by using monoclonal anti-CTLA-4, anti-PD-L1, and anti-PD-L2. Real-time polymerase chain reaction (RT-PCR) analysis was performed with the primers CTLA-4, PD-L1, and PD-L2.

Statistically, no significant relation was determined between the PD-L1, PD-L2, and CTLA-4 expressions (immunohistochemical and RT-PCR methods) and the MPM histological type. Interestingly significant correlation was observed between the mean survival time and immunohistochemical PD-L2 expression; thus, long-term survival was observed in cases with PD-L2 expression.

Programmed death ligand 1, PD-L2, and CTLA-4 expression were observed in some MPM cases, suggesting that treatments targeting immune checkpoints may be effective. Because immunohistochemical expression of PD-L2 is associated with better prognosis, it may provide useful clues in the follow-up of patients.

Key words: CTLA-4, immune checkpoints, PD-L1 (CD274), PD-L2 (PDCD1LG2), programmed cell death ligand 1, programmed cell death ligand 2, malignant pleural mesothelioma.

Introduction

Malignant pleural mesothelioma (MPM) is the most common primary malignant tumour of the pleura, originating from the mesothelial cells lining the pleura [1]. Its incidence is between 1 and 2.2 per million per year [1–3]. The most important aetiological factor for MPM is contact with asbestos or erionite fibres [3]. MPM is a disease with a poor prognosis. The median overall survival time in untreated patients is approximately 10 months [4, 5].

Multimodality treatment options are available for MPM, such as surgical resection, radiotherapy, and chemotherapy [6]. The only proven treatment modality for MPM is palliative platinum-antifolate chemotherapy, with a median survival time of approximately 12 months. This leads to the search new treatment modalities such as antibodies that block immune checkpoints which often have not been fully elucidated in MPM [7, 8].

Immune checkpoints are responsible for controlling the immune system to avoid autoimmunity and prevent tissue damage [9]. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is an immune inhibitory receptor found in T cells. It regulates T-cell activation by antigenic stimulation of T-cell receptor. Cytotoxic T lymphocyte antigen-4 suppresses T-cell function by means of blocking its interaction with the CD28 co-stimulatory molecule [10–16].

Programmed cell death protein 1 (PD-1, PDCD1) is a transmembrane protein of the B7/CD28 costimulatory receptor family. Programmed cell death protein 1 regulates T-cell activation through binding to programmed death ligand 1 (PD-L1, CD274) and programmed death ligand 2 (PD-L2, PDCD1LG2) [17–19]. Similarly to CTLA-4 signalling, PD-1 binding also decreases T-cell survival [19].

In view of the potential role of cancer-associated immunosuppression in the tumour microenvironment, targeting the PD-1/PD-L and CTLA-4 pathways seems to be an attractive therapeutic strategy. In this study, we investigate the expression of CTLA-4, PD-L1, and PD-L2 immuno-control receptors in MPM patients and the relationship of the expression with tumour types and prognostic parameters.

Material and methods

Tissue collection and examination

In our study, 50 MPM cases diagnosed among the pleural biopsy materials in the archives of the Pathology Department of Medical Faculty of Dicle University in the period 2010–2017 were evaluated. In all cases, diagnoses were confirmed by 2 pathologists with using haematoxylin-eosin stain and immunohistochemically: Calretinin, D2-40, BAP1, desmin, WT-1, EMA, CD68, and BerEp4. Cases showing calretinin, D2-40, EMA and WT-1 expression but not expressing BAP1, desmin, CD68, and BerEp4 were included in the study. Then we worked over all the cases with a light microscope (Olympus BX53, Tokyo, Japan) and subtyped them histopathologically (epithelioid, sarcomatoid, desmoplastic, and biphasic) according to the World Health Organization (WHO) classification guidelines. We documented the clinical records and the histopathological diagnosis of all patients. All the cases were staged clinically according to the tumour, node, and metastasis classification of pleural mesothelioma [2]. Local ethical approval was received from the Dicle University Medical Faculty Ethics Committee.

Immunohistochemical study

Four-micrometre sections were prepared from routinely processed paraffin blocks. The sections were mounted on positively charged slides and were incubated at 57°C for 60 minutes to remove the paraffin. Immunohistochemical staining was performed with the automated BenchMark XT immunohistochemical system (Ventana Medical Systems, Tucson, AZ, USA). Cytotoxic T lymphocyte antigen-4, PD-L1, and PD-L2 were detected by using monoclonal anti-CTLA-4, anti-PD-L1, and anti-PD-L2. Detailed technical information on the immunohistochemical antibodies is given in Table I.

Cytotoxic T lymphocyte antigen-4, PD-L1, and PD-L2 immunoreactivity were evaluated by 2 pathologists (FSKD and UA).

Immunohistochemical staining assessment was modelled from the study by Alabalik *et al*. [20]. The staining intensity and extent of stained cells were evaluated and scored for each sample. The distribution of CTLA-4, PD-L1, and PD-L2 immuno-

Table I. Antibodies and conditions for immunohistochemistry staining

Antibody	Clone	Dilution	Incubation	Antigen retrieval	Cellular localization	Control tissue	Brand
PD-L1	E1L3N	1/400	1.5 hours	Ventana CC1-EDTA, 64 minute	Membranous	Placenta	Cell Signalling Technology, USA
PD-L2	D7U8C	1/100	1.5 hours	Ventana CC1-EDTA, 64 minute	Cytoplasmic	Tonsil	Cell Signalling Technology, USA
CTLA-4	F8	1/500	2 hours	Ventana CC2- citrate, 68 minute	Cytoplasmic	Tonsil	Santa Cruz Biotechnology, USA

CTLA-4 – cytotoxic T lymphocyte antigen-4, PD-L1 – programmed death ligand 1, PD-L2 – programmed death ligand 2

reactivity was semi-quantitatively scored by using a 0–4 scale for the percentage of stained cells. A score of 0 represented 0-5% of cells stained, 1+ was 6–25% of cells stained, 2+ was 26– 50% of cells stained, 3+ was 51–75% of cells stained, and 4+ was 76–100% of cells stained. The immunohistochemical staining intensity was graded 0–3, with 0 indicating no staining intensity, 1 being weak, 2 being moderate, and 3 being strong. The combined scores were calculated as a sum of the extent and intensity scores. Finally, the combined scores were graded as follows: negative (0) = 0, weak (1) = 1 or 2, moderate (2) = 3 or 4, and strong (3) = 5–7. In all cases, pathologists agreed on the same score for all markers.

Real-time polymerase chain reaction analysis

Blocks consisting of more than 90% MPM cells and free of inflammatory cells or containing only a few were selected. Four-micrometre sections were prepared from routinely processed paraffin blocks. To amplify the CTLA-4, PD-L1, and PD-L2 gene regions from the RNA by real-time polymerase chain reaction (RT-PCR), RNA isolation was performed with the Roche High Pure RNA Paraffin Kit (Roche, Germany, Ref no: 03270289001). The cDNA synthesis from total RNA was performed with Roche Evo Script Universal cDNA Master Kit (Roche, Germany, Refno: 07912439001).

In the RT-PCR study a RocheCobas Z 480 (Roche, Germany) device with a LightCycler 480 System was used. A Roche Fast Start Essential DNA Probes Master (Roche, Germany, Refno: 06 402 682 001) kit was used for RT-PCR analysis. As primer Roche CTLA-4 (Roche, Germany, Id: 113194), Roche PD-L1 (Roche, Germany, Id: 104030), Roche PD-L2 (Roche, Germany, Id: 117537) and Roche ACTB (Roche, Germany, Id: 143636) was also used as control primer.

Statistical analysis

SPSS version 18.0 (Statistical Package for the Social Sciences) was used for statistical analysis. The correlation between CTLA-4, PD-L1, and PD-L2 expressions and the clinical and pathological characteristics of the patients was analysed by using the chi-square test. The effects of CTLA-4, PD-L1, and PD-L2 expressions and clinical and pathological characteristics on general survival time were evaluated with the help of the Kaplan–Meier method in univariate analysis and the Cox regression method in multivariate analysis. The results were considered statistically significant when the p value was less than 0.05 ($p < 0.05$).

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Results

Cohort of patients

According to the WHO Classification, 44 of the cases were evaluated as epithelioid type (Fig. 1A), one as sarcomatoid type, and 5 as biphasic type MPM.

Of the 50 cases in our study, 37 were male and 13 were female. No statistically significant relation was observed between the immunohistochemical and molecular expressions of PD-L1, PD-L2, and CTLA-4 according to gender ($p > 0.05$).

The patients were between 37 and 83 years old, with a mean age of 61.52 ±11.4 years. No statistically significant difference was observed between immunohistochemical PD-L1, PD-L2, and CTLA-4 expression and molecular PD-L1 and CTLA-4 expressions according to age ($p > 0.05$). A statistically significant correlation was observed between molecular PD-L2 expression according to age ($p < 0.05$).

The mean survival time of the cases was 12.98 ±1.33 months. There was no statistically significant relationship between survival time and immunohistochemical expression of PD-L1 and CTLA-4 ($p > 0.05$). However, a statistically significant relationship between the immunohistochemical expression of PD-L2 and survival time ($p < 0.05$) was observed.

Immunohistochemical results

The immunohistochemical staining characteristics are shown in Table I.

The membranous staining for PD-L1 was detected as strong in 2 (4%) cases, moderate in 6 (12%) cases, and weak in 7 (14%) cases (Fig. 1B). Staining with PD-L1 was not observed in 35 (70%) cases. No statistically significant relationship was determined between the PD-L1 expression level and the MPM histological type ($p > 0.05$).

The cytoplasmic staining for PD-L2 was detected as weak in 4 (8%) cases (Fig. 1C). In 46 (92%) cases, no staining with PD-L2 was observed. No statistically significant relationship was determined between the PD-L2 expression level and the MPM histological type ($p > 0.05$).

The cytoplasmic staining for CTLA-4 was detected as weak in 5 (10%) cases (Fig. 1D). In 45 (90%) cases, staining with CTLA-4 was not observed. No statistically significant relationship was determined between the CTLA-4 expression level and the MPM histological type ($p > 0.05$).

A statistically significant correlation was observed between PD-L1 and PD-L2 ($p < 0.001$), PD-L1 and CTLA-4 ($p < 0.001$), and PD-L2 and CTLA-4 ($p < 0.001$) immunohistochemical expressions.

A statistically significant relationship was observed between the PD-L2 expressions and the survival time ($p < 0.05$) (Fig. 2A). However, no significant relation

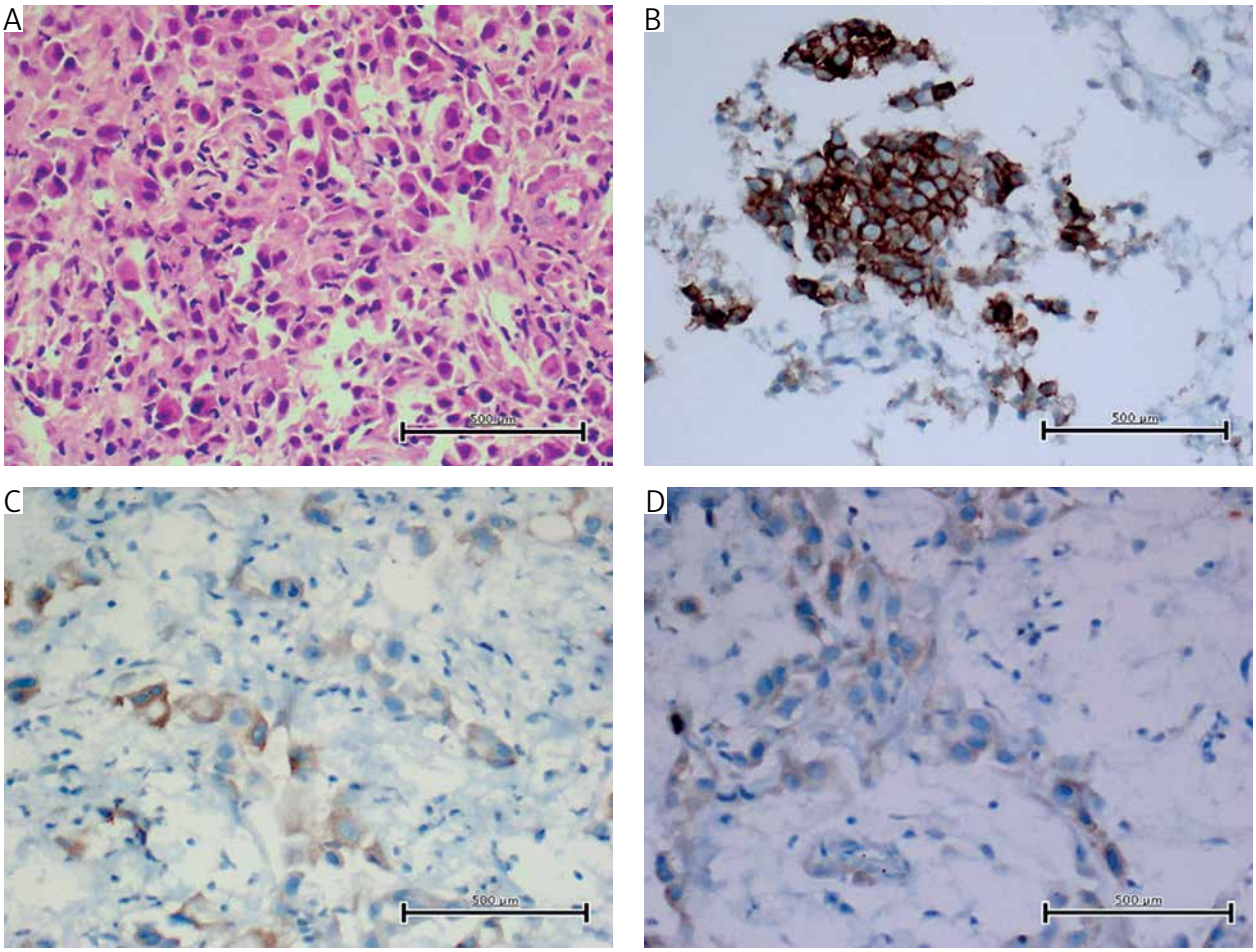

Fig. 1. A) Histomorphological appearance of malignant pleural mesothelioma (H and E, 400×). B) Strong expression of programmed death ligand 1 in malignant pleural mesothelioma (Immunoperoxidase, 400×). C) Weak expression of programmed death ligand 2 in malignant pleural mesothelioma (Immunoperoxidase, 400×). D) Weak expression of cytotoxic T lymphocyte antigen-4 in malignant pleural mesothelioma (Immunoperoxidase, 400×)

was determined between the PD-L1 and CTLA-4 expressions and the survival time ($p > 0.05$) (Figs. 2B, C).

Real-time polymerase chain reaction results

Molecular analysis results of the cases of our study are shown in Tables II and III.

Programmed death ligand 1 gene expression was detected in 5 (10%) of 50 cases by PCR. Of the cases with PD-L1 gene expression, 4 were diagnosed with epithelioid type MPM, and 1 was diagnosed with biphasic type MPM. No statistically significant difference was observed between histopathological subtype and PD-L1 gene expression ($p > 0.05$). Among all cases with PD-L1 gene expression, one weak, 3 moderate, and one strong also showed PD-L1 expression immunohistochemically. A statistically significant correlation was obtained between PD-L1 gene expression and immunohistochemical PD-L1 expression ($p = 0.001$).

Programmed death ligand 2 gene expression was detected in 11 (22%) of 50 cases with RT-PCR. Of the cases with PD-L2 gene expression, 9 were epithelioid type MPM and 2 were diagnosed with biphasic type MPM. No statistically significant difference was observed between histopathological subtype and PD-L2 gene expression ($p > 0.05$). Three of the cases with PD-L2 gene expression showed weak PD-L2 expression immunohistochemically. Programmed death ligand 2 gene expression was not observed in one case that showed PD-L2 expression immunohistochemically. Statistically significant correlation was obtained between PD-L2 gene expression and immunohistochemical PD-L2 expression ($p < 0.05$).

Cytotoxic T lymphocyte antigen-4 gene expression was detected in 10 (20%) of 50 cases with RT-PCR. Of the cases with CTLA-4 gene expression, 8 were epithelioid type MPM and 2 were diagnosed with biphasic type MPM. No statistically significant difference was observed between histopathological subtype and CTLA-4 gene expression ($p > 0.05$). Four of the cases with CTLA-4 gene expression, showed weak CTLA-4 expression immunohistochemically. Cytotoxic T lymphocyte antigen-4 gene expression was not observed in one case that showed CTLA-4 expression immunohistochemically. A statistically

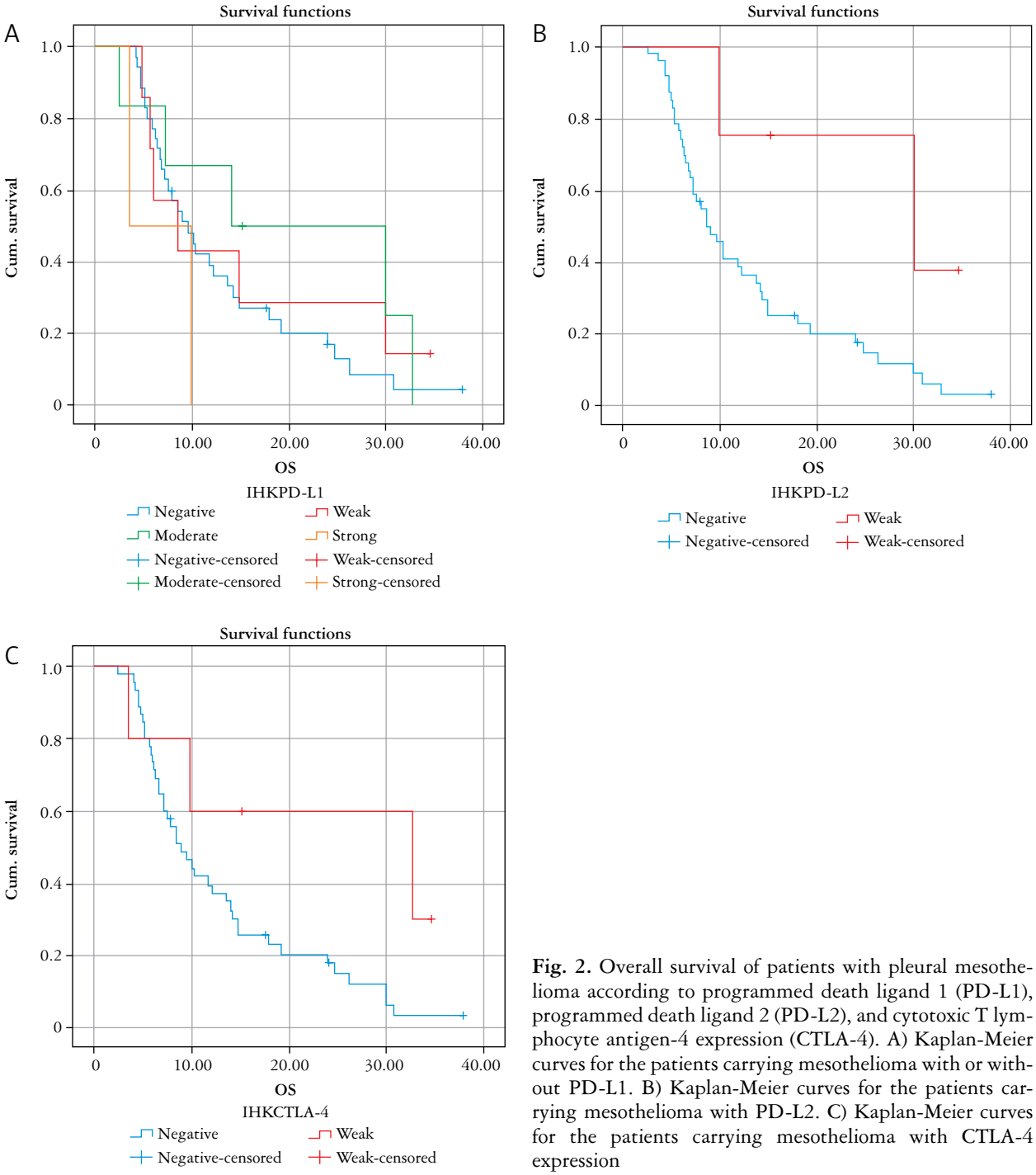

Fig. 2. Overall survival of patients with pleural mesothelioma according to programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), and cytotoxic T lymphocyte antigen-4 expression (CTLA-4). A) Kaplan-Meier curves for the patients carrying mesothelioma with or without PD-L1. B) Kaplan-Meier curves for the patients carrying mesothelioma with PD-L2. C) Kaplan-Meier curves for the patients carrying mesothelioma with CTLA-4 expression

significant correlation was found between CTLA-4 gene expression and immunohistochemical CTLA-4 expression ($p < 0.01$).

A statistically significant correlation was observed between PD-L1 and PD-L2 ($p = 0.000$), PD-L1 and CTLA-4 ($p = 0.000$), and PD-L2 and CTLA-4 ($p = 0.000$) gene expressions.

A statistically significant correlation was detected between immunohistochemical expressions and gene expressions for each of PD-L1, PD-L2, and CTLA-4 (Table IV). However, no significant relation was determined between PD-L1, PD-L2, and CTLA-4 gene expressions and the survival time ($p > 0.05$).

Discussion

Malignant pleural mesothelioma is an aggressive malignant disease affecting the surface mesothelium of the pleural cavity, particularly associated with exposure to asbestos fibres [21]. The incidence of MPM is predicted to increase in the coming years, due to the long period between asbestos exposure and di-

Table II. Pathologic and immunohistochemical features of tumours according to programmed death ligand 1, programmed death ligand 2, and cytotoxic T lymphocyte antigen-4 expression

Histological subtype	Immunohistochemical Expression	PD-L1		PD-L2		CTLA-4	
		Frequency	%	Frequency	%	Frequency	%
Epithelioid	Negative	30	60	41	82	40	80
	Weak	7	14	3	6	4	8
	Moderate	6	12	0	0	0	0
	Strong	1	2	0	0	0	0
Sarcomatoid	Negative	1	2	1	2	1	2
	Weak	0	0	0	0	0	0
	Moderate	0	0	0	0	0	0
	Strong	0	0	0	0	0	0
Biphasic	Negative	4	8	4	8	4	8
	Weak	0	0	1	2	1	2
	Moderate	0	0	0	0	0	0
	Strong	1	2	0	0	0	0
p		0.474^{ns}		0.563^{ns}		0.702^{ns}	

CTLA-4 – cytotoxic T lymphocyte antigen-4, PD-L1 – programmed death ligand 1, PD-L2 – programmed death ligand 2

Table III. Pathologic and real-time polymerase chain reaction assay features of tumours according to programmed death ligand 1, programmed death ligand 2, and cytotoxic T lymphocyte antigen-4 expression

Histological subtype	Gene expression	PD-L1		PD-L2		CTLA-4	
		Frequency	%	Frequency	%	Frequency	%
Epithelioid	Negative	40	80	35	70	36	72
	Positive	4	8	9	18	8	16
Sarcomatoid	Negative	1	2	1	2	1	2
	Positive	0	0	0	0	0	0
Biphasic	Negative	4	8	3	6	3	6
	Positive	1	2	2	4	2	4
p		0.702^{ns}		0.525^{ns}		0.451^{ns}	

CTLA-4 – cytotoxic T lymphocyte antigen-4, PD-L1 – programmed death ligand 1, PD-L2 – programmed death ligand 2

Table IV. Correlation table for immunohistochemical expression and gene expression results

Parameters	*R*	*P*
IHKPD-L1- MPD-L1	0.588	0.000
IHKPD-L2-MPD-L2	0.377	0.007
IHKCTLA-4-MCTLA-4	1.00	0.000

İHKPD-L1 – immunohistochemical PD-L1 expression, İHKD-L2 – immunohistochemical PD-L2 expression, İHKCTLA-4 – immunohistochemical CTLA-4 expression, MPD-L1 – PD-L1 gene expression, MPD-L2 – PD-L2 gene expression, MCTLA-4 – cytotoxic T lymphocyte antigen-4 gene expression

agnosis, in addition to the continued use of asbestos in developing countries [5].

Malignant pleural mesothelioma is a disease with a poor prognosis; the median overall survival time in untreated patients is approximately 10 months, and the 5-year survival rate is less than 5% [4, 5]. Palliative platinum-antifolate chemotherapy is the only proven treatment in MPM, resulting in a median survival time of approximately 1 year. Therefore, new therapeutic strategies are needed. Antibodies that block immune checkpoints in MPM patients have recently been investigated [7, 8]. In our study, we have investigated the expression of immune checkpoint receptors such as CTLA-4, PD-L1, and PD-L2, which may be targets for treatment strategies in MPM patients.

In the literature, MPM is generally seen in patients over 60 years of age, and the male/female ratio is approximately 4/1 [1, 16, 22]. In our study, the mean age of the patients was found to be 61.52 ±11.4 years, consistent with the literature, and male predominance was observed.

Because MPM typically presents at a late clinical stage, the median survival time after diagnosis is

10–12 months in the literature, and the tumour nearly always causes death within a few years [4, 5, 23, 24]. The mean survival time of the patients in our study was 12.98 ±1.33 months, which is consistent with the literature.

There are some studies in the literature researching PD-L1 expression immunohistochemically in MPM. Cedres *et al*. [25] showed that 6 (22.2%) of 27 patients had a positive immunoreaction with PD-L1 (clone E1L3N), and no immune reaction was observed in 20 patients (77.7%). In the same study, more PD-L1 positivity was found in the patient group with non-epithelioid histology than in the group with epithelioid histology. Mansfield *et al*. [26] detected PD-L1 (clone 5H1-A3) expression in 42 patients in a study they conducted on 106 patients with MPM – 14 of them (33%) were epithelioid type and 28 (67%) were non-epithelioid type. In the same study, all MPM cases with sarcomatoid differentiation showed PD-L1 expression, except for one case with desmoplastic subtype, which is a valuable result. Combaz-Lair *et al*. [27] investigated immunohistochemical PD-L1 expression analysis with both clone E1L3N and clone SP142 in 58 MPM patients. E1L3N clone was expressed in 8 (23.5%) of 34 epithelioid type MPM, one (9%) of 11 biphasic type MPM, and 8 (62%) of 13 sarcomatoid type MPM. With the SP142 clone, expression was observed in 4 (12%) epithelioid, one (9%) biphasic, and 5 (38%) sarcomatoid type MPM cases. Brosseau *et al*. [28] showed that PD-L1 positivity in the sarcomatoid/biphasic subtype was higher than in the epithelioid subtype. In our study, PD-L1 expression was found immunohistochemically in 15 patients (30%) with a diagnosis of MPM, 7 of whom were weak (14%), 6 were moderate (12%), and 2 were strong (4%). Of the 15 cases with PD-L1 expression, 14 were diagnosed as epithelioid type, and one case had a diagnosis of biphasic MPM. There was no statistically significant difference between histological subtype and immunohistochemical PD-L1 expression. In our opinion, this situation was due to the low number of patients with sarcomatoid features, because only one (2%) of the 50 patients in our series was sarcomatoid subtype and 5 (10%) were biphasic.

In the study of Cedres *et al*. [25], patients with PD-L1 clone E1L3N expression (median 5 months) showed shorter survival time than negative patients (median 20 months). In the study of Mansfield *et al*. [26], patients with PD-L1 clone 5H1-A3 expression (median 5 months) had a shorter survival time than those without PD-L1 expression (median 14.5 months). Combaz-Lair *et al*. [27] showed that PD-L1 clone SP142 expression was associated with poor survival, but PD-L1 clone E1L3N expression was non associated with survival time. In the study of Nguyen *et al*. [29], patients with PD-L1 clone SP263 expression (median 6 months) demonstrated shorter survival time than negative patients (median 15.5 months). Inaguma *et al*. found that PD-L1 clone E1L3N expression was related with poor prognosis [30]. In our study, the mean survival time was obtained as 12.98 ±1.33 months. There was no significant difference in survival times between the group with PD-L1 expression and the group with no PD-L1 expression. Although our result is inconsistent with other studies, it is consistent with the result of Combaz-Lair *et al*. In our opinion this result is due to an insufficient number of MPM patients (6/50) with sarcomatoid features.

There are very few studies in the literature investigating PD-L1 expression in MPM patients other than the immunohistochemical method [31]. In our study, PD-L1 expression was detected by RT-PCR method in 5 of our 50 MPM cases. No significant relationship was observed between PD-L1 gene expression and both the mean survival time and histological subtype. Five cases showing PD-L1 expression with the RT-PCR method also showed PD-L1 expression immunohistochemically. In this way, a significant correlation was obtained between immunohistochemical PD-L1 expression and PD-L1 gene expression by RT-PCR method.

In the literature there is no comprehensive study investigating PD-L2 expression with any method in MPM. In our study, we detected immunohistochemically weak PD-L2 expression in 4 (8%) of our 50 patients with MPM. Significant correlation was not obtained between histological subtype and immunohistochemical PD-L2 expression.

In our study, the median survival time was 39.50 months in 4 cases with immunohistochemical expression of PD-L2, and 24.28 months in 46 negative cases. A statistically significant correlation was observed between the mean survival time and immunohistochemical PD-L2 expression, and a long survival time was observed in cases with PD-L2 expression. These results suggest that PD-L2 expression may be associated with good prognosis. Our result is in contrast with that of PD-L1, which is associated with poor prognosis in many studies, so we think that it is a remarkable situation that can be used to predict patient survival. However, the relationship between PD-L2 expression and survival time should be investigated in further research with a higher number of MPM patients.

In addition, PD-L2 expression was detected by RT-PCR method in 11 of the 50 MPM cases in our study. Of the cases with PD-L2 gene expression, 9 were epithelioid type and 2 were biphasic. Significant correlation was not found between PD-L2 gene expression and both mean survival time and histological subtypes. Immunohistochemically, PD-L2 expression was detected in 3 of the cases with PD-L2 gene

expression. A statistically significant correlation was obtained between PD-L2 gene expression and immunohistochemical PD-L2 expression. In our opinion, the difference between the expressions with immunohistochemistry and RT-PCR is caused by the fact that the detection of PD-L2 protein is more difficult, and it has a smaller structure (30 kDa) than the PD-L1 protein molecule (approximately 40 kDa). Also, this molecule is vulnerable to damage during routine tissue follow-up procedures, and this may cause protein denaturation.

There have only been a few immunohistochemical studies in the literature on CTLA-4 expression in MPM and its relationship with prognostic parameters. Roncella *et al*. [16] found no significant correlation between CTLA-4 expression and both mean survival time and histological subtype. In our study, CTLA-4 expression was detected immunohistochemically in 5 of 50 MPM cases. There was no significant relationship between immunohistochemical CTLA-4 expression and both mean survival time and histological subtype. There are no studies in the literature investigating CTLA-4 expression in MPM except using the immunohistochemical method. In our study, CTLA-4 expression was detected in 10 of 50 cases by RT-PCR method. No significant correlation was obtained between CTLA-4 gene expression and both mean survival time and histological subtypes. Significant correlation was obtained between immunohistochemical CTLA-4 expression and CTLA-4 expression by RT-PCR method. Similarly to the data in our PD-L2 study, in our opinion the difference between the expressions with immunohistochemistry and RT-PCR is caused by the fact that the detection of CTLA-4 protein is more difficult, and it has a smaller structure (24 kDa) than the PD-L1 protein molecule (approximately 40 kDa). Also, this molecule is vulnerable to damage during routine tissue follow-up procedures, and this may cause protein denaturation. Some further studies with a higher number of MPM patients investigating the relationship between the prognosis and CTLA-4 expression are necessary.

It was determined that 4 cases with immunohistochemical PD-L2 expression and all 5 cases with CTLA-4 expression also showed PD-L1 expression. PD-L2 expression was also observed in 3 of 5 cases with CTLA-4 expression. It was found that all 5 cases with PD-L1 gene expression also showed both PD-L2 and CTLA-4 gene expression. In 9 of 10 cases showing CTLA-4 gene expression, PD-L2 gene expression was also observed.

Conclusions

Because MPM is a disease with poor prognosis and treatment options are limited, new and more effective treatments are necessary. Immunohistochemical expression of PD-L2 is associated with better prognosis, so it may provide useful clues in the follow-up of patients. However, studies in larger series are needed. PD-L1, PD-L2, and CTLA-4 expression were seen in some MPM cases, which suggests that treatments targeting the immune checkpoints may be effective. Because therapies targeting immune checkpoints are promising new treatment options, clinical trials of agents targeting the immune checkpoints such as PD-L1, PD-L2, and CTLA-4 should be supported in MPM patients. The relationship between the presence of expression and the treatment response should be demonstrated by clinical studies with further research including larger numbers of patients.

The authors declare no conflict of interest.

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