



Prognostic Value of Immunohistochemical Expression of Programmed Death-Ligand 1 (PD-L1) in Pancreatic Ductal Adenocarcinoma

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Abstract:

Background: Pancreatic ductal adenocarcinoma is a highly aggressive malignancy with an unfavorable prognosis. Immunotherapy targeting immune checkpoints, particularly programmed death-ligand 1 (PD-L1), has demonstrated promising results in various cancer cases. However, its prognostic role in pancreatic ductal adenocarcinoma remains unclear. This study focused on evaluating PD-L1 expression in tumor cells and tumor-infiltrating lymphocytes using immunohistochemistry, and its association with clinicopathological features and survival outcomes.

Materials and methods: This study was retrospectively conducted on 40 patients confirmed to have pancreatic ductal adenocarcinoma. Immunohistochemistry was used to assess PD-L1 expression in both tumor cells and associated immune cells within the tumor microenvironment. Tumor-infiltrating lymphocytes were also evaluated. The correlations between PD-L1 expression and clinical, pathological, and survival outcomes were analyzed using chi-square and Fisher's exact tests. Overall survival and disease-free survival were analyzed using the Kaplan–Meier method and compared with the log-rank test.

Results: Expression of PD-L1 was observed in 27.5% of tumor cells and 40% of tumor-infiltrating lymphocytes. A significant association was found between PD-L1 expression in tumor cells and significantly correlated with poor histological differentiation ($p = 0.008$) and the presence of lymph node metastasis ($p = 0.03$). PD-L1 positivity in immune cells was significantly correlated with higher tumor grade ($p = 0.006$), advanced stage ($p = 0.04$), presence of lymphovascular invasion ($p = 0.02$), and lymph node involvement ($p = 0.02$). A strong association was observed between expression of PD-L1 in tumor cells and immune cells ($p < 0.0001$). Patients exhibiting PD-L1 expression in either compartment showed significantly reduced overall and disease-free survival ($p < 0.05$). Elevated tumor-infiltrating lymphocytes levels were also linked to adverse survival outcomes.

Conclusion: PD-L1 positivity in both tumor and immune cell populations is linked to aggressive pathological features and poorer survival in pancreatic ductal adenocarcinoma. These findings highlight PD-L1 as a potential prognostic biomarker and support its role in identifying candidates for immunotherapy. Further prospective studies are recommended to validate these findings.

Keywords: Pancreatic ductal adenocarcinoma · PD-L1 expression · Tumor-infiltrating lymphocytes · Immunohistochemistry · Prognostic biomarkers · Tumor microenvironment.

Received: 7 August 2025

Accepted: 12 October 2025

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Background:

Pancreatic cancer is a highly aggressive neoplasm; it is ranked sixth for overall global mortality from cancer,

with a five-year survival rate of 11%. Pancreatic cancer is related to more than forty thousand estimated mortality figures in the United States in 2015 [1].

Pancreatic ductal adenocarcinoma (PDAC) accounts for the majority of histologic subtype and accounts for about 85% of all pancreatic cancer cases [2]. In Egypt, PDAC constitutes 3.2% of all cancer cases according to the 2020 global cancer registry, with an annual rate exceeding 2702 patients [3].

The microenvironment of PDAC has immunosuppressive elements, like myeloid-derived suppressors, regulatory T cells, and tumor-associated macrophages, so it is considered a non-immunogenic tumor [4].

Immunotherapy is considered a paradigm shift in anti-cancer therapeutic approaches. It is a rapidly growing field with increasing interest. Targeting immune checkpoints, particularly the programmed cell death-1 protein (PD-1) and its ligand (PD-L1), has proven highly successful in the clinical setting, leading to a sustained response to treatment in certain malignancies. Recently, numerous clinical trials have been carried out to evaluate the efficacy of immunotherapeutic drugs in different cancer types including PDAC [5].

PD-1, commonly known as B7-1, is an immunoglobulin expressed in immune cells such as B cells, natural killer T cells, dendritic cells, and activated monocytes. Still, it is mainly expressed in T cells. PD-L1, also referred to as B7-H1, is the most crucial ligand of PD-1. PD1/PD-L1 serves an important inhibitory role on effector T cells function and promoting regulatory T cells [6].

PD1/PD-L1 checkpoint has regulatory mechanisms for the immune response [7]. Blockade of PD1/PD-L1 checkpoints mainly affects T cells, leading to apoptosis and increased immune response against tumor antigens of regulatory T cells and effector T cells, respectively. Tumor cells use this mechanism to avoid surveillance from the immune system through increasing PD-L1 expression, which interacts with PD-1, promoting T-cell exhaustion or programmed cell death [8]. Evaluating PD-1 and PD-L1 expression provides valuable prognostic and predictive indicator regarding the efficacy of immunotherapy using checkpoint inhibitors [9].

In this study, we focused on investigating the association between PD-L1 expressions in tumor cells and tumor-infiltrating lymphocytes using immunohistochemistry, and its association with clinicopathological features and survival outcomes in patients diagnosed with PDAC.

Methods:

Study design and patient selection:

This retrospective study included 40 PDAC patients diagnosed between January 2017 and December 2022. All patients presented with either resectable or borderline resectable disease at the time of initial diagnosis, received neoadjuvant chemotherapy, and subsequently underwent surgical resection. The diagnosis was established pre-treatment through image-guided true-cut needle biopsy (TCNB). The study has been conducted at the Department of Pathology of our

center. The cases were retrospectively collected from the archived material of the Pathology Laboratory at two centers.

Ethical Approval

This study received ethical approval from the Research Ethics Committee (Registration number: Soh-Med-22-01-30). It was registered at ClinicalTrials.gov (Registration ID: NCT05228808; Registration Date: January 27, 2022).

Inclusion and Exclusion Criteria

We included patients who had resectable or borderline resectable (PDAC), who underwent surgical resection, either following neoadjuvant chemotherapy or as upfront surgery. Only cases with sufficient pre-treatment biopsy tissue available for analysis were selected.

Patients were excluded if they had recurrent disease, inadequate or mostly necrotic biopsy samples, or if their clinical or follow-up data were incomplete.

Data Collection and Radiological Staging

Epidemiological data, including age and sex, were extracted from patient records. Tumors were staged radiologically using contrast-enhanced computed tomography, magnetic resonance imaging, 18F-Fluorodeoxyglucose positron emission tomography-computed tomography, or combinations thereof. Tumor location was categorized as either in the pancreatic head or body. Resectability status (resectable or borderline resectable) was determined based on imaging findings at initial diagnosis, following standard radiological criteria.

In addition to baseline characteristics, detailed treatment information was collected, including chemotherapy regimens administered as neoadjuvant therapy, receipt of adjuvant chemotherapy and/or radiotherapy, and type of surgical procedure (pancreaticoduodenectomy or distal pancreatectomy).

Survival outcomes, namely overall survival (OS) and progression-free survival (PFS), were carefully extracted for all patients. OS was calculated from the date of diagnosis to either death due to any cause or the most recent follow-up visit. PFS was measured from the date of diagnosis to the earliest occurrence of documented disease progression, recurrence, or death from any cause.

Histopathological Assessment

Tissue sections stained with Haematoxylin and eosin (H&E) from pre-treatment biopsies were examined to confirm PDAC diagnosis and to assess tumor grade. Lymphovascular invasion (LVI), perineural invasion, regional lymph node metastasis (LNM), and tumor-infiltrating lymphocytes (TILs) were re-evaluated. Tumor staging were reported according to the 8th edition of the American Joint Committee on Cancer (AJCC) TNM classification [10].

Tumor-infiltrating lymphocytes (TILs)

The percentage of the stromal TILs was evaluated according to the international TIL Working Group Consensus guidelines and defined as the portion of the entire intra-tumoral stromal region that is inhabited by mononuclear inflammatory cells. According to the predefined criteria, it is scored as low and high, where > 50% is lymphocytic predominant [11].

Immunohistochemistry procedure:

Tissue blocks preserved in formalin embedded in paraffin were sectioned using microtome at 4µm-thick sections, mounted on coated glass slides, and prepared for immunohistochemistry (IHC). Xylol and decreasing alcohol grades were used to deparaffinize and rehydrate the sections.

Ready-to-use monoclonal rabbit PD-L1 antibody (7 ml, catalog number: API 3171 AA, clone: CAL10, Lab Vision Laboratories) was applied to the slides and incubated with tissue sections for 30 minutes at room temperature. Then, slides were rinsed in phosphate buffer solution (PBS) and incubated with Mach 2 Rabbit-HRP polymer for 30 min. DAB chromogen was applied to each tissue section for 5 min at room temperature. A hematoxylin stain was used to counterstain the nuclei. Slides were rewashed, dried, and covered with Eco Mount. Strong concordance with the other clones is the basis for selecting the antibody clone.

Positive and negative controls

Positive and negative tissue controls were included in each staining run to confirm that the staining system was working correctly and that positive signals were specific. Tonsil tissue sections served as positive controls. Negative controls were prepared from PDAC, but PBS was added instead of the primary antibody.

PD-L1 immunohistochemical analysis and scoring:

PD-L1 scoring was evaluated by two pathologists and assessed for both tumor cells (TCs) and immune cells (ICs). Membranous or cytoplasmic reactivity was interpreted as a positive result. The histochemical Score (H-Score) system was used to assess its expression within TCs. The final score was calculated based on the combination of staining intensity and the percentage of positive tumor cells. Staining intensity was graded on a scale from 0 to 3 (0=negative, 1=weak, 2=moderate, and 3=strong), and each score was multiplied by the percentage of positive cells (0-100%). To calculate the H-score, the following formula was applied: $(1 \times \% \text{ of weak staining}) + (2 \times \% \text{ of moderate staining}) + (3 \times \% \text{ of strong staining})$, yielding a score between 0 and 300 [12]. According to a cutoff score of 100, PD-L1 status was subdivided into two groups: scores of 0-99 were considered negative/low expression, while scores of 100-300 were classified as positive expression [13]. While PD-L1 expression in ICs was assessed by the percentage of positively stained cells, membranous or cytoplasmic staining in stromal immune cells, including lymphocytes and macrophages, was classified as positive if it exceeded 1% [14].

Statistical analysis:

Statistical analyses were conducted using SPSS software, version 22. Quantitative variables were expressed as mean $\pm$ standard deviation (SD) along with the range, while categorical variables were reported as frequencies and percentages. The Chi-square test was applied to evaluate the association between PD-L1 expression and various clinicopathological features. Survival outcomes, including OS and PFS, were performed using the Kaplan–Meier method, and differences between survival curves were assessed using the log-rank test. A p-value of less than 0.05 was considered statistically significant.

Results:

40 patients who had been diagnosed with PDAC were included in this study. The patients' ages ranged between 43 and 75; the mean $\pm$ SD is 58.7 $\pm$ 8.9 years, respectively, with predominant male affection (28 cases). Histologically, moderately differentiated tumors were the most frequent, representing 50% of all included cases (Figure 1). Preoperative clinical and radiological assessments showed that 23 (57.5%) patients had Stage II tumors. The clinicopathological aspects of the included cases are shown in (Table 1).

IHC expression of PD-L1 on tumor cells (TCs):

Positive PD-L1 expression was observed in the neoplastic cells' cell membranes, cytoplasm, or both (Figure 2). We examined the PD-L1 expression status in PDAC and its correlation with established prognostic factors, including sex, age, tumor size, degree of differentiation, LNM, stage, perineural invasion, LVI, and TILs (Table 2). Using the H-score, PD-L1 positivity (cutoff point of ≥ 100) was detected in 11/40 (27.5%) of the included cases. PD-L1 expression revealed a significant association with tumor differentiation ($p=0.008$), LNM ($p=0.03$), and TILs ($p=0.04$). It has been noted that PD-L1 positivity increases with the presence of vascular tumor emboli, but it doesn't reach a significant level ($p=0.08$). No significant correlations were observed between PD-L1 expression and age, sex, tumor size, tumor stage, tumor location, or perineural invasion (Table 2).

IHC expression of PD-L1 in TILs:

Positive PD-L1 expression in TILs was observed as punctuate cytoplasmic brown immunostaining (Figure 3). Positive PD-L1 reactivity in TILs was positive in 16/40 (40%) of the included cases. Its expression revealed a significant link with the degree of differentiation ($p=0.006$), tumor stage ($p=0.04$), LNM ($p=0.02$), LVI ($p=0.02$), and TILs ($p=0.01$). A strong correlation between PD-L1 expression in immune and tumor cells ($p<0.0001$) was identified. However, the statistical analysis of PD-L1 IHC expression in TILs concerning patients' age, sex, tumor size, tumor site within the pancreas, and presence of perineural invasion showed no statistical significance (Table 3).

Survival analysis:

Survival outcomes were measured by calculating both DFS and OS across the entire patient cohort. The median DFS was 16 months, with the range of 1 to 54 months. Meanwhile, median OS was 19.5 months (range: 3-58 months).

In univariate analysis, patients exhibiting positive PD-L1 expression in tumor cells demonstrated significantly shorter DFS when relative to those with negative expression (13 vs. 30.8 months; $p = 0.005$) (Table 4).

In the multivariate Cox regression model including variables with $p < 0.10$ in univariate analysis, only pathological tumor size remained an independent predictor of DFS (HR = 1.9; 95% CI: 1.1–3.6; $p = 0.03$). Tumor PD-L1 expression showed a near-significant association (HR = 2.5; 95% CI: 0.9–6.6; $p = 0.08$), while differentiation demonstrated only a trend towards worse DFS (HR = 2.3; 95% CI: 0.9–5.8; $p = 0.09$). Other variables, including immune PD-L1, TILs, and LNM, did not retain statistical significance after adjustment.

Similarly, OS was markedly lower in the tumor PD-L1 positive group (19.4 vs. 45.3 months; $p = 0.01$) (Table 5). These results suggest that tumor PD-L1 positivity is associated with both early recurrence and shorter life expectancy (figure 4).

A comparable pattern was noted regarding PD-L1 expression in immune cells. Patients with immune PD-L1 positivity had a significantly reduced DFS in comparison to those with negative expression (15.1 vs. 32 months; $p = 0.01$). Moreover, OS was also significantly reduced in immune PD-L1 positive

patients (21.2 vs. 48.8 months; $p = 0.006$). These results further support the adverse prognostic impact of PD-L1 expression within the tumor microenvironment (figure 5).

TILs showed a significant correlation with survival outcomes. Patients with high TIL levels had a markedly shorter DFS (13.1 months) compared to those with low TILs (30.4 months; $p = 0.006$). Similarly, patients with higher TILs levels experienced a significantly shorter overall survival, with a mean OS of 19 months versus 46.2 months in the low TIL group ($p = 0.006$). These results suggest that a higher presence of TILs in the tumor microenvironment may reflect a more aggressive disease course and poorer clinical outcomes.

Patients diagnosed with stage III disease had significantly poorer DFS (14.9 months) and OS (19.7 months) compared to those with stage I disease, who had a median DFS of 47.6 months and OS of 58 months, respectively, with statistically significant results ($p = 0.01$ and $p = 0.007$). Tumor size was also relevant, with larger tumors (T3/T4) associated with poorer DFS (14.2–19.5 months) compared to smaller tumors (T1: 47.6 months; $p = 0.004$).

Lymphovascular invasion and lymph node metastasis were linked to worse outcomes. Although not statistically significant, patients with LVI had shorter DFS (19.5 vs. 29.9 months; $p = 0.1$) and OS (29.1 vs. 44.8 months; $p = 0.14$). Likewise, lymph node-positive cases had reduced DFS (16.9 vs. 30.3 months; $p = 0.06$) and OS (25.2 vs. 42.8 months; $p = 0.46$). Perineural invasion was common but did not affect survival outcomes.

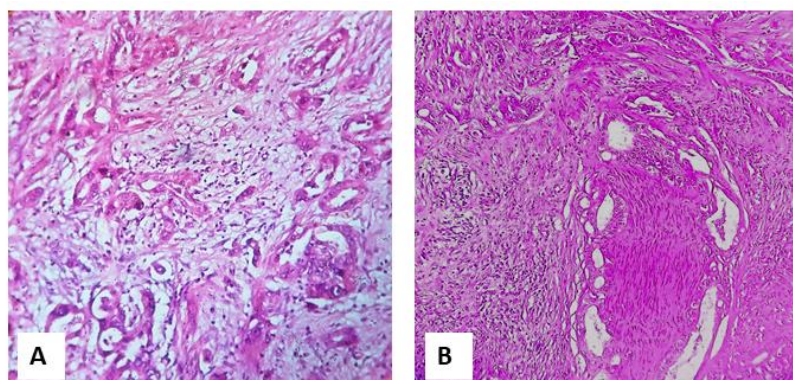


Figure 1. (A) Moderately differentiated PDAC with lymphocytic infiltrate (H&E, X400), (B) Moderately differentiated PDAC with perineural invasion (H&E, X200).

Table 1. The clinicopathological aspects of the included cases

Parameter	Number (n=40)	Percentage
Age: Mean $\pm$ SD	58.7 $\pm$ 8.9	
<60	19	47.5
$\geq$ 60	21	52.5
Gender:		
Male	28	70
Female	12	30
Tumor location:		
Pancreatic head	34	85
Pancreatic body	6	15
Pathological tumor size (pT)		
T ₁	5	12.5
T ₂	13	32.5
T ₃	18	45
T ₄	4	10
Degree of differentiation:		
Well	9	22.5
Moderate	20	50
Poor	11	27.5
TNM staging:		
I	5	12.5
II	23	57.5
III	12	30
LNM:		
Absent	26	65
Present	14	35
LVI:		
Positive	16	40
Negative	24	60
Perineural invasion:		
Present	25	62.5
Absent	15	37.5
TILs:		
High	11	27.5
Low	29	72.5
Tumor PD-L1:		
Positive	11	27.5
Negative	29	72.5
Immune PD-L1:		
Positive	16	40
Negative	24	60
Chemotherapy:		
5-FU based	19	47.5
Gemcitabine-based	21	52.5
Radiotherapy:		
Yes	17	42.5
No	23	57.5
Outcome:		
Free	14	35
Local recurrence	16	40
Distant recurrence	10	25

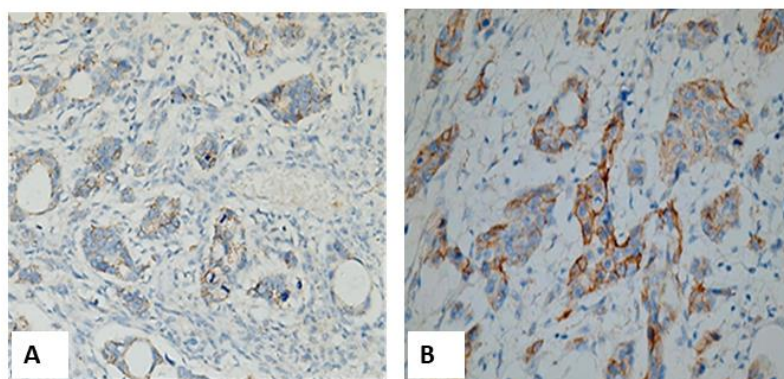


Figure 2. (A) -ve/low IHC expression of PD-L1 within the tumor cells in well-differentiated PDAC, (B) Positive expression of PD-L1 within the tumor cells in moderately-differentiated PDAC (IHC X400).

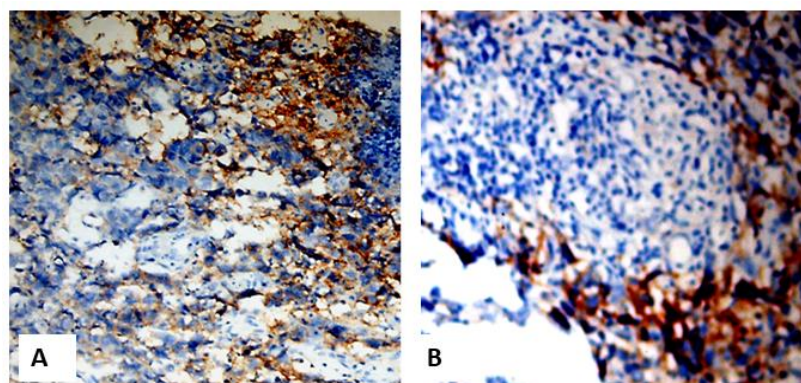


Figure 3. Punctuate brown cytoplasmic PD-L1 immunostaining in TILs (IHC X400)

Table 2. Association between IHC expression of PD-L1 within the tumor cells and the clinicopathological characteristics

Parameter	Tumor PF-L1 expression Cutoff point ≥ 100		<i>p</i> value
	+ve PD-L1	-ve/low PD-L1	
Age:			
<60	6 (31.58)	13 (68.42)	0.73 [†]
≥ 60	5 (23.81)	16 (76.19)	
Gender:			
Male	9 (32.1)	19 (67.9)	0.45 [†]
Female	2 (16.7)	10 (83.3)	
Tumor location:			
Pancreatic head	9 (27.3)	24 (72.7)	1 [†]
Pancreatic body	2 (28.6)	5 (71.4)	
Pathological tumor size: (pT)			
T ₁	0 (0)	5 (100)	0.15 [‡]
T ₂	2 (15.38)	11 (84.62)	
T ₃	8 (44.44)	10 (55.56)	
T ₄	1 (25)	3 (75)	
TNM staging:			
I	0 (0)	5 (100)	0.08 [‡]
II	5 (21.74)	18 (78.26)	
III	6 (50)	6 (50)	
Degree of differentiation:			
Well	1 (11.1)	8 (88.9)	0.008 ^{*‡}
Moderate	3 (15)	17 (85)	
Poor	7 (63.6)	4 (36.4)	
LNM:			
Absent	4 (15.4)	22 (84.6)	0.03 ^{*†}
Present	7 (50)	7 (50)	
LVI:			
Positive	7 (43.8)	9 (56.3)	0.08 [†]
Negative	4 (16.7)	20 (83.3)	
Perineural invasion:			
Present	9 (36)	16 (64)	0.12 [†]
Absent	2 (13.3)	13 (86.7)	
TILs:			
High	6 (54.5)	5 (45.5)	0.04 ^{*†}
Low	5 (17.2)	24 (82.8)	

† Fisher's exact test ‡ Freeman-Halton test * Significant at *p* value <0.05

Table 3. Association between IHC expression of PD-L1 in TILs and the clinicopathological characteristics

Immune PD-L1 expression			
Parameter	Cutoff point ≥ 1		<i>p</i> value
	+ve PD-L1	-ve/low PD-L1	
Age:			
<60	10 (52.6)	9 (47.4)	0.11 [§]
≥ 60	6 (28.6)	15 (71.4)	
Gender:			
Male	12 (42.9)	16 (57.1)	0.73 [†]
Female	4 (33.3)	8 (66.7)	
Tumor location:			
Pancreatic head	13 (38.2)	21 (61.8)	0.67 [†]
Pancreatic body	3 (50)	3 (50)	
Pathological tumor size (pT)			
T ₁	0 (0)	5 (100)	0.09 [‡]
T ₂	5(38.5)	8 (61.5)	
T ₃	9 (50)	9 (50)	
T ₄	2(50)	2(50)	
TNM staging:			
I	0 (0)	5 (100)	0.04 ^{*‡}
II	9 (39.1)	14 (60.9)	
III	7 (58.3)	5 (41.7)	
Degree of differentiation:			
Well	2 (22.2)	7 (77.8)	0.006 ^{*‡}
Moderate	5 (25)	15 (75)	
Poor	9 (81.8)	2 (18.2)	
LNM:			
Absent	7 (26.9)	19 (73.1)	0.02 ^{*§}
Present	9 (64.3)	5 (35.7)	
LVI:			
Positive	10(62.5)	6 (73.1)	0.02 ^{*§}
Negative	6 (25)	18 (75)	
Perineural invasion:			
Present	12 (48)	13 (52)	0.18 [§]
Absent	4 (26.7)	11 (73)	
TILs:			
High	8 (72.7)	3 (27.3)	0.01 ^{*†}
Low	8 (27.6)	21 (72.4)	

§ Chi-squared test † Fisher's exact test ‡ Freeman-Halton test * Significant at p value <0.05

Table 4. Prognostic Factors for Disease-Free Survival in PDAC: Univariate and Multivariate Analyses.

Parameter	Univariate analysis		Multivariate analysis	
	Median DFS (months) (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Age:				
<60	25 (11.4-38.6)	0.66	—	—
≥60	18 (13.9-22.1)			
Gender:				
Male	17 (13.3-20.7)	0.32	—	—
Female	25 (4.2-45.8)			
Tumor location:				
Pancreatic head	19 (12.2-25.8)	0.98	—	—
Pancreatic body	27 (11.7-42.3)			
Degree of differentiation:				
Well	49.0 (38.6-59.4)	0.03*	2.3 (0.9-5.8)	0.09
Moderate	17 (13.9-20.2)			
Poor	16 (7.4-24.6)			
Pathological tumor size (pT)				
T ₁	47.6 (39.4-55.7)	0.004*	1.9 (1.1-3.6)	0.03*
T ₂	30.8 (19.8-41.8)			
T ₃	16.5 (20.2-32.8)			
T ₄	14.2 (10.8-17.6)			
LNM:				
Absent	30.3 (22.0-38.6)	0.06	0.7 (0.2-2.8)	0.6
Present	16.9 (13.1-20.7)			
LVI:				
Positive	19.5 (13.1-25.9)	0.1	—	—
Negative	29.9 (21.3-38.5)			
Perineural invasion:				
Present	26.5 (18.6-34.4)	0.93	—	—
Absent	25.5 (15.6-35.3)			
TILs:				
High	13.1 (7.4-18.7)	0.006*	1.5 (0.9-2.5)	0.1
Low	30.4 (23.1-37.7)			
Tumor PDL-1				
Positive	13 (6.7-19.3)	0.005*	2.5 (0.9-6.6)	0.08
Negative	30.8 (23.7-38)			
Immune PDL-1				
Positive	15.1 (10.0-20.1)	0.01*	1.8 (0.6-5.7)	0.3
Negative	32 (24.2-39.8)			
Chemotherapy:				
5 Fluorouracil-based	28.9 (19.5-38.4)	0.5	—	
Gemcitabine-based	22.5 (15.9-29.2)			
Radiotherapy:				
Yes	24.2 (15.4-32.9)	0.6		
No	27.3 (19.2-35.3)			

* Significant at *p* value <0.05

Table 5. Prognostic Factors for Overall survival in PDAC: Univariate and Multivariate Analyses.

Parameter	Univariate analysis		Multivariate analysis	
	Median DFS (months) (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Age:				
<60	34.1 (23.0-45.2)	0.09	0.4 (0.1-1.2)	0.1
≥60	47.5 (39.2-55.9)			
Gender:				
Male	37.9 (26.4-48.3)	0.28	—	—
Female	40.2 (30.4-47.6)			
Tumor location:				
Pancreatic head	40.8 (32.4-49.3)	0.9	—	—
Pancreatic body	32.2 (18.7-45.6)			
Degree of differentiation:				
Well	53.9 (43.2-64.5)	0.08	1.9 (0.8-4.3)	0.1
Moderate	40 (29.9-50.2)			
Poor	23.2 (16.5-29.7)			
Pathological tumor size (pT)				
T1	58 (42.5-63.4)	0.13	—	—
T2	41.6 (29.2-53.9)			
T3	26 (18.8-33.2)			
T4	21.1 (17.1-25.2)			
LNM:				
Absent	42.8 (33.5-52.1)	0.46	—	—
Present	25.2 (19.8-30.6)			
LVI:				
Positive	29.1 (19.1-39.0)	0.14	—	—
Negative	44.8 (34.9-54.6)			
Perineural invasion:				
Present	37.7 (32.0-52.8)	0.56	—	—
Absent	42.4 (26.1-49.3)			
TILs:				
High	19 (12.2-25.8)	0.006*	1.7 (0.9-3.1)	0.09
Low	46.2 (38.0-54.4)			
Tumor PDL-1				
Positive	13 (11.4-27.4)	0.01*	3.9 (1.2-12.9)	0.02*
Negative	45.3 (37.3-53.3)			
Immune PDL-1				
Positive	21.2 (15.3-27.1)	0.006*	3.3 (0.9-11.1)	0.05
Negative	48.8 (40.9-56.7)			
Chemotherapy:				
5 Fluorouracil-based	44.9 (34.3-55.7)	0.43	—	—
Gemcitabine-based	32.7 (24.1-41.4)			
Radiotherapy:				
Yes	39.9 (25.5-54.3)	0.82	—	—
No	39.8 (30.3-49.3)			

* Significant at *p* value <0.05

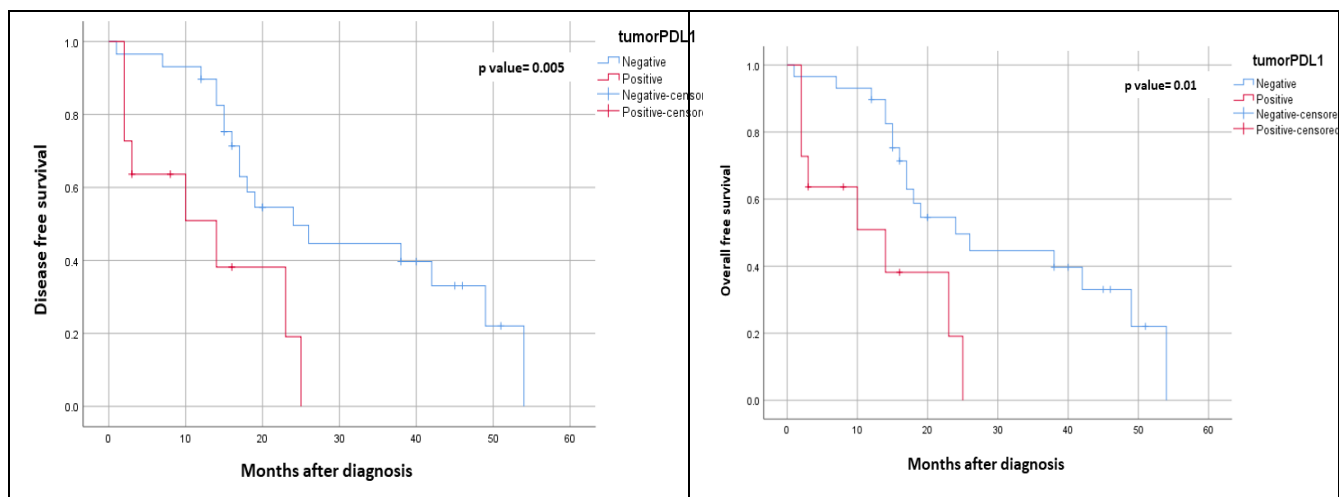


Figure 4: Kaplan–Meier curve illustrating disease-free survival (DFS) overall survival (OS) in patients with pancreatic ductal adenocarcinoma (PDAC) according to PD-L1 expression in tumor cells. Patients with positive PD-L1 expression are expressed in red line. Patients with negative PD-L1 expression are expressed in blue line.

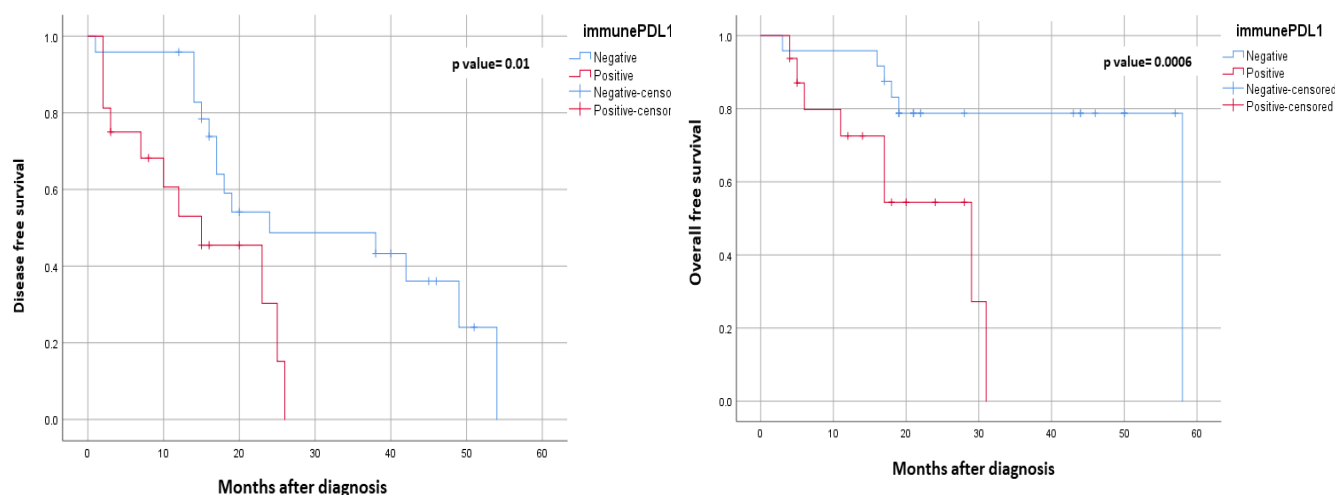


Figure 5: Kaplan–Meier curve illustrating disease-free survival (DFS) overall survival (OS) in patients with pancreatic ductal adenocarcinoma (PDAC) according to PD-L1 expression in immune cells. Patients with positive PD-L1 expression are expressed in red line. Patients with negative PD-L1 expression are expressed in blue line.

Discussion:

Pancreatic adenocarcinoma has the most unfavorable prognosis among all solid malignancies. It is highly resistant to chemotherapy and radiotherapy [15]. TILs were reported to effectively predict the outcome of various malignant tumors [16]. TILs are essential to the host immunological response against the cancer. High levels of TILs have been shown to positively affect survival in several human cancers. Usually, T cells are lacking in pancreatic tissue. However, they increase in precancerous lesions as well as in invasive malignancies. A few studies reported that higher TILs infiltration in PDAC, especially by CD8+ TILs, is linked to better survival [17]. Our findings showed that higher TIL levels were actually associated

with worse survival in PDAC, possibly reflecting an exhausted or ineffective immune response.

Immunotherapy is an emerging therapeutic strategy in the oncology field. Its role is based mainly on inhibition of the immunosuppressive effect of tumor cells. PD-L1 belongs to immune checkpoint proteins. It is expressed in both TCs and stromal ICs. PD-1/PD-L1 binding results in apoptosis of ICs, especially T cells, followed by further tumor progression. Immune checkpoint inhibitors, such as PD-L1 inhibitors, can regulate the blockage of this reaction. PD-1/PD-L1 pathway blockade has significant clinical responses in patients with various neoplasms, such as lung cancer, colorectal carcinoma, renal cell carcinoma, melanoma, and urothelial carcinoma [18]. Patients that have

positive PD-L1 expression frequently experience considerably worse outcomes in clinical settings [19]

In this study, we assessed PD-L1 expression in tumor as well as in immune cells in PDAC tissue. Tumor cell PD-L1 expression was detected in 27.5% of cases, a range consistent with prior studies (4–49%) that vary based on the antibody clone, scoring method, and cutoff values used [20]. We found that PD-L1 expression in TCs was significantly correlated with poor differentiation, higher stage, and positive lymph node metastasis, echoing published data by Wang et al. and Hu et al. [19, 21]. These findings suggest that PD-L1 overexpression may play a role in aggressive tumor behavior. In contrast, Yamaki et al. [22] reported no such correlations, highlighting variability in the literature.

There was a statistically significant correlation between tumoral PD-L1 expression and histologic grade ($p = 0.008$), and LNM (0.03). These results are in agreement with data reported by Wang et al. and Hu et al. [19, 21]. This may provide an additional indication for anti-PDL1 therapeutic agents in poorly differentiated and advanced PDAC. However, these results disagreed with those of Yamaki et al. [22], who reported no significant association between PD-L1 immunostaining in TCs and tumor grade, stage, or LNM.

However, IHC expression of PD-L1 within TCs revealed a non-significant correlation with other clinicopathological characteristics, including age, sex, and tumor location, and size, LVI, or perineural invasion, following a previous study by Liang et al. [23]

Previous studies suggested that tumor-infiltrating ICs influence PD-L1 expression levels in PDAC. However, such a correlation hasn't been elucidated [24]. We evaluated the relationship between PD-L1 expression level and TILs and found a significant positive correlation between PD-L1 expression and percentage of TILs ($p = 0.04$). Meanwhile, this result was in contrast to that of Zhao and Gao [25], who reported a negative correlation between PD-L1 and TILs. The microenvironment model demonstrated that tumors that exhibited PD-L1 and TILs positivity had a relatively high response to PD-1/PD-L1 blockade therapy. Patients with positive PD-L1 expression tended to resist PD-1/PD-L1 monotherapy, which is why TILs should be taken into account when using PD-1/PD-L1-based immunotherapy [26].

We also evaluated PD-L1 positivity in TILs, which was present in 40% of cases. Positive PD-L1 staining in TILs showed statistically significant correlations with tumor grade ($p = 0.006$), tumor stage ($p = 0.04$), presence of lymphovascular invasion ($p = 0.02$), and positive lymph node metastasis ($p = 0.02$). These findings suggest that immune cells throughout the tumor microenvironment may help the tumor escape immune detection and promote tumor progression. To our knowledge, this is among the first studies to examine these specific associations between PD-L1 positive TILs and clinicopathological parameters in PDAC.

PD-L1 expression on TCs showed a significant correlation with its positivity in ICs ($p < 0.0001$). This finding is consistent with the results reported by Zhang and his colleague [26], who reported that positive PD-L1 expression on TCs was more frequently detected among the samples, exhibited PD-L1 positivity in ICs.

In addition to its association with adverse clinicopathological features, PD-L1 expression significantly affected the survival outcomes in our cohort. Patients with positive PD-L1 expression in TCs showed worse overall survival (OS) and disease-free survival (DFS) compared to those with negative expression (DFS: 13.1 vs. 30.8 months, $p = 0.005$; OS: 19.4 vs. 45.3 months, $p = 0.01$). This observation aligns with several previous studies suggesting that PD-L1 overexpression contributes to immune evasion and tumor aggressiveness, ultimately leading to poor prognosis [19, 21]. Similarly, PD-L1 positivity in tumor-infiltrating lymphocytes (TILs) was significantly correlated with worse OS, suggesting that PD-L1 expression within the tumor microenvironment may suppress effective antitumor immune responses and facilitate disease progression. Our findings support the role of PD-L1 not only as a biomarker of tumor aggressiveness but also as a potential prognostic indicator in PDAC. These results highlight the possible therapeutic benefit of immune checkpoint blockade in selected PDAC patients with high PD-L1 expression, although additional prospective studies are required to validate these associations and optimize patient selection for immunotherapy.

This study is subject to several limitations. First, the sample size was relatively small ($n = 40$), which may limit the ability to generalize the findings to larger populations. Second is the retrospective design of the study, which may be subjected to selection bias and incomplete clinical data. Finally, immunohistochemical evaluation of PD-L1 expression can vary due to different techniques, and scoring systems,

Given the limitations mentioned, future prospective studies with greater number of patients, and extended follow-up period are recommended to assess the predictive value of PD-L1 for response to checkpoint inhibitor therapy, potentially leading to more personalized treatment strategies. Integrating PD-L1 evaluation with other immune parameters, such as CD8⁺ T-cell density and tumor mutational load, may better define immunological subtypes of PDAC.

Conclusion:

PD-L1 expression in both tumor and immune cells is associated with more aggressive tumor features and worse survival outcomes in PDAC. These findings suggest its possible utility as a prognostic marker and may aid in identifying patients who could benefit from immunotherapy.

List of abbreviations:

PD-L1: Programmed Death Ligand-1.

PDAC: Pancreatic Ductal Adenocarcinoma.

PD-1: Programmed Death-1.

TCNB: true-cut needle biopsy.
 OS: Overall Survival.
 DFS: Disease-Free Survival.
 H&E: Haematoxylin and eosin.
 LVI: Lymphovascular invasion.
 LNM: regional lymph node metastasis.
 TILs: tumor-infiltrating lymphocytes.
 IHC: immunohistochemistry.
 PBS: phosphate buffer solution.
 TCs: Tumor Cells.
 ICs: Immune Cells.
 H-Score: histochemical Score.

Acknowledgment:

The authors would like to thank the data management team who supported the collection of patients' records.

Conflict of interests:

The authors declare that they have no competing interests.

Ethical Approval and Consent to Participate

This retrospective study was approved by the Research Ethics Committee at our center (Registration number: Soh-Med-22-01-30). It was registered at ClinicalTrials.gov (Registration ID: NCT05228808; Registration date: January 27, 2022). The requirement for informed consent was waived due to the retrospective nature of the study and the use of anonymized archival data, in accordance with institutional guidelines and ethical standards.

Consent for Publication

Not applicable.

Funding

Not applicable.

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