Am J Cancer Res 2026;16(7):2861-2872
www.ajcr.us /ISSN:2156-6976/ajcr0173779

Original Article

Analysis of the relationship between immunological microenvironment and prognosis in pancreatic cancer

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Received May 5, 2026; Accepted June 27, 2026; Epub July 15, 2026; Published July 30, 2026

Abstract: Immune cell infiltration in tumors can serve as a prognostic marker in many cancers; however, its role in pancreatic ductal adenocarcinoma (PDAC) remains understudied. In this retrospective study, we aimed to evaluate the prognostic significance of the immunological microenvironment and immune cell infiltration in PDAC, considering both preoperative clinicophysiological and postoperative clinicopathological factors. Furthermore, we evaluated the same analyses after excluding patients who had received neoadjuvant treatment for the subgroup analysis. We included 189 patients who underwent radical resection for pancreatic cancer between October 2007 and October 2015 at our center and an affiliated university hospital. Immunohistochemical staining for clusters of differentiation (CD3, CD8, CD19, and CD20) was performed on the largest cross-sectional area of the tumor specimens to quantify immune cell infiltration. Disease-free survival (DFS) and overall survival (OS) were analyzed for each antigen. Preoperative clinicophysiological and postoperative clinicopathological factors were compared based on positively labeled cell counts. Among the 189 patients, 114 were men (average age, 69.3 years). CD20- and CD8-positive cell counts were significantly associated with DFS (P=0.027) and OS (P=0.033), respectively. No significant differences were observed between groups in terms of T factor for CD8, tumor location, biliary decompression, albumin level, C-reactive protein level, hemoglobin level, N factor, or disease stage for CD20. Multivariate analyses identified disease stage (P=0.04) and CD20 expression (P=0.043) as independent predictors of DFS, while CD8 expression (P=0.016) was the only independent predictor of OS. These results indicate that high CD20 expression, reflecting B-cell infiltration, predicts DFS, whereas high CD8 expression, reflecting T-cell infiltration, predicts OS. Collectively, our findings suggest that the immunological microenvironment represents a potential prognostic factor in patients with PDAC.

Keywords: CD20, CD8, immunological microenvironment, pancreatic cancer, prognostic factor

Introduction

Pancreatic cancer is characterized by aggressive locoregional invasion, frequently involving major vascular systems adjacent to the pancreas; consequently, pancreatic adenocarcinoma (PDAC) has a poor prognosis [1-3]. Furthermore, the 5-year survival rate of patients with PDAC following radical resection in multidisciplinary therapeutic regimens involving surgery, chemotherapy, and radiotherapy remains low at 21%-44% [4-6]. Therefore, alternative approaches are required to improve survival outcomes in patients with PDAC, with cancer immunotherapy being a valuable therapeutic option.

Given the critical role of the immune system in cancer development and progression, analyzing the immune components of the tumor microenvironment can provide valuable insights into how the host immune response interacts with cancer cells [7]. The immunological microenvironment in cancers comprises various components, including blood vessels, fibroblasts, immune cells, and the extracellular matrix. Recent evidence suggests that tumor-stroma interactions within the cancer immuno-

https://doi.org/10.62347/WYLC8512

logical microenvironment enhance malignant biological behavior, playing a pivotal role in determining the outcomes of various cancers [8-10]. Therefore, the histological products of desmoplastic reactions in curatively resected colorectal cancer have been evaluated to assess their prognostic value [11, 12]. Desmoplastic reactions in colorectal cancer have been reported to be associated with prognosis. Similarly, pancreatic cancer is well known to exhibit desmoplasia, characterized by fibrotic hyperplasia of the tumor stroma.

PDAC tumors are enriched in stromal components due to desmoplasia. The desmoplastic tumor microenvironment, including cancer-associated fibroblasts, consists of pancreatic stellate cells, immune cells, and extracellular matrix. Additionally, the fibrotic overexpression of the stroma has been intensively investigated [13, 14]. Solid tumors comprise a wide variety of cell types derived from the host immune system, with specific immune cell subtypes linked to prognostic and predictive features [15, 16]. PDAC has traditionally been regarded as a "non-immunogenic" tumor, and preclinical studies indicate that it employs multiple mechanisms to evade immune surveillance [17]. Therefore, identifying predictive biomarkers to select appropriate patient subsets is crucial for studies aimed at reducing PDAC mortality, particularly in clinical trials evaluating diverse therapeutic strategies [18].

Immune cell infiltration in tumors has been identified as a prognostic marker in various types of carcinomas. However, in PDAC, relatively few studies have evaluated the tumor immune microenvironment using histological assessment of immune cell infiltration as a prognostic biomarker. Therefore, in this retrospective study, we aimed to evaluate the prognostic significance of the immunological microenvironment and immune cell infiltration in patients with PDAC using preoperative clinicophysiological and postoperative clinicopathological factors.

Materials and methods

Patient selection and ethical approval

A total of 189 consecutive patients who underwent curative resection for PDAC at Ibaraki Medical Center, Tokyo Medical University, and Tokyo Medical University Hospital between October 2007 and October 2015 and survived the hospitalization period were retrospectively enrolled. The mean age of the patients was 69.3 years (range, 36-88 years), and the cohort comprised 114 men and 75 women. The primary tumor locations were the pancreatic head (n=128), tail (n=55), and total pancreas (n=6).

The median follow-up period was 20.8 months. The relatively short median follow-up period was mainly due to the inclusion of recently treated patients and the predefined study cut-off date. In addition, the aggressive clinical course of PDAC resulted in early recurrence or death in a proportion of patients.

Patients were censored because they were alive without the event of interest at the last follow-up, the study period ended, or they were lost to follow-up.

Patients were followed up every 3-6 months after surgery with physical examination, laboratory testing including serum CEA and CA19-9 measurements, and contrast-enhanced computed tomography. Recurrence and survival data were obtained from outpatient medical records and referring physicians. Follow-up was continued until death or the last clinical contact.

The protocol for this study was approved by the Research Ethics Committee of Tokyo Medical University (approval number: IB1833). The requirement for patient consent was waived because of the retrospective nature of the study. All procedures were performed in accordance with the ethical standards of the Institutional Research Committee and the Declaration of Helsinki (2024).

Immunohistochemistry

Immunohistochemical staining for clusters of differentiation 3, 8, 19, and 20 (CD3, CD8, CD19, and CD20) was performed on the largest cross-sectional area of tumor specimens to assess immune cell infiltration. Formalin-fixed, paraffin-embedded tissue sections were stained with the following antibodies according to the manufacturer's instructions: CD3 (polyclonal; Dako, Glostrup, Denmark; 1:1 dilution), CD8 (4B11; Leica Biosystems, Nussloch, Germany; 1:50 dilution), CD19 (BT51E; Leica

Biosystems, Nussloch, Germany; 1:50 dilution), and CD20 (L26; Dako, Glostrup, Denmark; 1:1 dilution). The number of positively stained cells was quantified using ImageJ (National Institutes of Health, United States). All images were acquired using the Nikon NIS-Elements BR software, with a field of view measuring 234 μm in length and 312 μm in width (73,008 μm^2).

Positive immunostaining was defined as the presence of unequivocal membranous staining of the cell membrane for each antibody. Non-neoplastic lymphocytes located outside the tumor-infiltrating region served as internal positive controls. The selection of representative fields and image acquisition were performed by an independent pathologist. Quantification of positive cells was subsequently conducted using an automated image analysis system. The independent pathologist responsible for selecting representative fields and acquiring images was blinded to all clinicopathological data for each case. As the quantification of immunohistochemical staining was conducted using an automated image analysis system, no assessment of interobserver agreement was required or performed.

Clinical and pathological variables

Preoperative clinicophysiological factors obtained immediately prior to surgery evaluated in this study included sex, age, tumor location, body mass index, biliary decompression status, neoadjuvant treatment, and laboratory parameters such as total bilirubin, albumin, creatinine, hemoglobin A1C, amylase, C-reactive protein (CRP), white blood cell count, carbohydrate antigen 19-9, carcinoembryonic antigen, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, prognostic nutritional index, and CRP/albumin ratio. Postoperative clinicopathological factors, including histological findings, tumor/node/metastasis classification of malignant tumors (TNM classification, 8th ed.), cancer stage, and adjuvant treatment, were also assessed. The correlations between the median counts of immunohistochemically positive cells for each antigen and recurrence patterns (distant metastasis, local recurrence, and disease-free status) were subsequently analyzed. Patients who received neoadjuvant treatment were not excluded because preoperative treatment has become an integral component of contemporary PDAC management, and excluding these patients would reduce the generalizability of the study findings. In addition, neoadjuvant treatment may reflect tumor biology and treatment responsiveness and can influence the tumor microenvironment, including immune cell infiltration. Therefore, patients who received preoperative therapy were included in the analysis, and the potential confounding effect of preoperative treatment was adjusted for as a covariate.

Prognostic factors associated with disease-free survival (DFS) and overall survival (OS) were assessed. For each antigen assessed using immunostaining, the cutoff values for positive cell counts using the median values of each group were determined based on the median DFS and OS. Given the relatively limited sample size of this retrospective cohort, median-based dichotomization was considered the most robust and unbiased method for stratifying patients. Furthermore, we evaluated the same analyses after excluding patients who had received neoadjuvant treatment for the subgroup analysis. For antigens showing significant immunostaining, associations with preoperative clinicophysiological and postoperative clinicopathological factors were compared between groups stratified by the number of positively labeled cells.

Statistical analysis

Potential prognostic factors that exhibited significant differences between the clinical, physiological, and pathological factors were assessed using univariate and multivariate regression analyses. The cutoff values for the number of CD3-, CD8-, CD19-, and CD20-positive cells were defined using the median values of each group. Continuous and categorical variables were compared using the Fisher exact test and the Kruskal-Wallis test, respectively. Kaplan-Meier survival curves for DFS and OS were generated for each immunostained antigen and compared using the log-rank test. The date of surgery was used as the starting point for survival analyses. Univariate and multivariate analyses were performed using Cox regression, with the multivariate analysis incorporating significant factors identified in the univariate analysis. Statistical Package for the

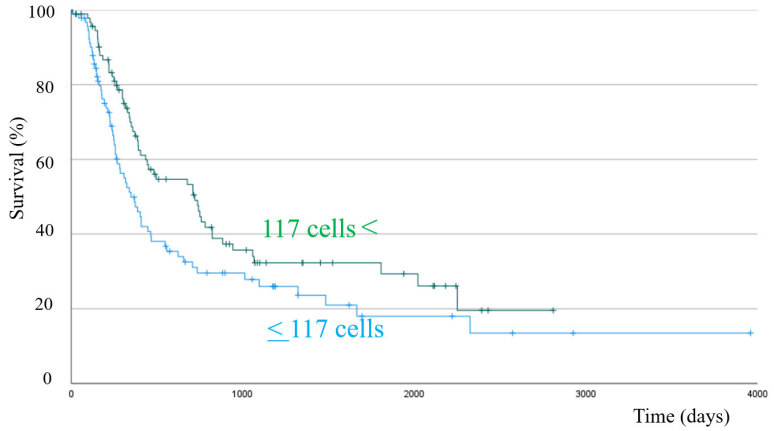

Figure 1. Comparison of patient survival over time based on median DFS stratified by CD20-positive cell count. DFS: Disease-Free Survival; CD20: Cluster of Differentiation 20.

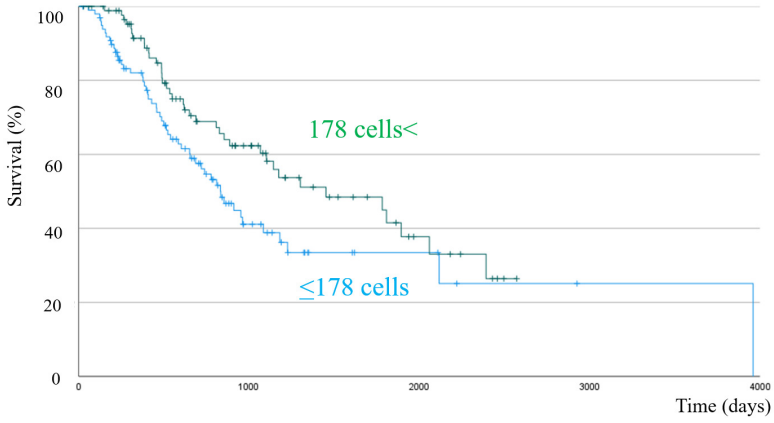

Figure 2. Comparison of patient survival over time based on median OS according to CD8-positive cell counts. OS: Overall Survival; CD8: Cluster of Differentiation 8.

Social Sciences (SPSS; version 24.0; IBM Corp., Armonk, NY, USA) was used for all statistical analyses, with statistical significance set at $P < 0.05$.

Results

Survival analyses

No significant differences in DFS were observed according to CD3-, CD8-, or CD19-positive cell counts; however, DFS differed significantly with the number of CD20-positive cells (P=0.027) (**Figure 1**). CD3-, CD19-, and CD20-positive cell counts did not significantly affect OS, whereas OS differed significantly by the number of CD8-positive cells (P=0.033) (**Figure 2**). Correlations between the median counts of positive cells for each antigen and the recurrence patterns were not significant.

Associations with clinicophysiological and clinicopathological factors

Comparisons of immunostaining results across preoperative and postoperative factors revealed significant associations as follows:

CD8-positive cells: significant differences with T factor (**Table 1**).

CD20-positive cells: significant differences with tumor location, biliary decompression status, albumin, CRP, hemoglobin levels, N factor, and disease stage (**Table 2**).

Multivariate analyses

In multivariate analyses, disease stage (P=0.04) and CD20-positive cell counts (P=0.043) were independent prognostic factors for DFS (**Table 3**). CD8-positive cell count (P=0.016) was the only independent prognostic factor associated with OS (**Table 4**).

Subgroup analysis excluding patients who had received neoadjuvant treatment

In the analysis excluding patients who had undergone neoadjuvant treatment, a significant difference in DFS was observed only in CD20-positive cell counts (P=0.024) (**Figure 3**). Similarly, for OS, only CD8-positive cell counts demonstrated a significant difference, even after excluding patients who had received neoadjuvant treatment (P=0.049) (**Figure 4**).

In associations with clinicophysiological and clinicopathological factors, similarly, CD8-positive cells: were significant differences with only T factor (**Table 5**). CD20-positive cells: were significant differences with tumor loca-

Table 1. Comparison of clinical, physiological, and pathological factors according to $CD8^+$ cells level

CD8		≤ 178 cells (n=99)	178 cells < (n=90)	*P*-value
Sex	Men	63	51	0.328
	Women	36	39	
Location	Head	72	56	0.291
	Body/tail	24	31	
	Total	3	3	
Biliary decompression	+	51	54	0.241
	-	48	36	
Neoadjuvant treatment	+	3	0	0.31
	-	136	47	
Age		70.02±0.97	68.33±1.02	0.186
Total Bilirubin	mg/dl	1.43±0.17	1.51±0.22	0.78
Albumin	mg/dl	3.74±0.04	3.78±0.05	0.537
Creatinine	mg/dl	0.74±0.03	0.74±0.03	0.997
HbA1C	%	6.47±0.22	6.96±0.20	0.097
Amylase	IU/L	99.82±6.89	114.33±16.93	0.429
CRP	mg/dl	1.21±0.31	0.84±0.20	0.326
WBC	/μl	5783.84±163.81	5551.11±178.70	0.338
Hemoglobin	g/dl	12.25±0.15	12.31±0.17	0.792
Platelet	$\times 10^4$/μl	15.18±1.23	17.13±1.02	0.223
CA19-9	U/ml	630.65±149.57	374.26±77.89	0.139
CEA	mg/nl	5.05±0.64	4.19±0.44	0.272
NLR		2.89±0.20	2.61±0.16	0.275
PNI		44.90±0.57	44.86±0.76	0.964
PLR		107.64±8.72	128.19±9.72	0.117
CAR		0.371±0.103	0.259±0.068	0.366
T	is	1	0	0.044
	1	4	2	
	2	0	6	
	3	94	82	
N	0	23	24	0.585
	1	76	66	
R	0	74	75	0.344
	1	21	13	
	2	4	2	
Stage	0	1	0	0.46
	1a	2	1	
	1b	0	3	
	2a	20	20	
	2b	73	63	
	4	3	3	
Adjuvant treatment	+	94	31	0.833
	-	45	16	

Note: CD8: Cluster of Differentiation 8; HbA1C: Hemoglobin A1C; CRP: C-Reactive Protein; WBC: White Blood Cell Count; CA19-9: Carbohydrate Antigen 19-9; CEA: Carcinoembryonic Antigen; NLR: Neutrophil-to-Lymphocyte Ratio; PNI: Prognostic Nutritional Index; PLR: Platelet-to-Lymphocyte Ratio; CAR: C-Reactive Protein/Albumin ratio.

tion, biliary decompression status, albumin, CRP, N factor, and disease stage (**Table 6**). In multivariate analyses, similarly, disease stage (*P*=0.044) and CD20-positive cell counts

Table 2. Comparison of clinical, physiological, and pathological factors according to $CD20^+$ cells level

CD20		≤ 117 cells (n=93)	117 cells < (n=93)	*P*-value
Sex	Men	61	52	0.177
	Women	32	41	
Location	Head	72	54	0.05
	Body/tail	17	37	
	Total	4	2	
Biliary decompression	+	42	61	0.05
	-	51	32	
Neoadjuvant treatment	+	3	1	0.705
	-	122	63	
Age		69.52±1.01	69.19±1.00	0.41
Total Bilirubin	mg/dl	1.76±0.21	1.08±0.15	0.09
Albumin	mg/dl	3.68±0.05	3.84±0.05	0.018
Creatinine	mg/dl	0.74±0.03	0.74±0.03	0.938
HbA1C	%	6.79±0.21	6.59±0.20	0.488
Amylase	IU/L	106.47±10.30	108.49±14.59	0.91
CRP	mg/dl	1.43±0.35	0.66±0.15	0.046
WBC	/μl	5534.41±158.83	5781.72±186.41	0.314
Hemoglobin	g/dl	12.03±0.15	12.54±0.17	0.025
Platelet	$\times 10^4$/μl	16.32±1.24	16.34±1.05	0.99
CA19-9	U/ml	619.77±144.33	393.09±99.32	0.198
CEA	mg/nl	4.69±0.61	4.65±0.52	0.955
NLR		2.92±0.21	2.63±0.16	0.264
PNI		44.04±0.62	45.65±0.71	0.089
PLR		122.34±9.46	115.90±9.14	0.625
CAR		0.437±0.115	0.202±0.052	0.158
T	is	0	1	0.202
	1	2	4	
	2	1	5	
	3	90	83	
N	0	16	31	0.011
	1	77	62	
R	0	69	78	0.75
	1	21	12	
	2	3	3	
Stage	0	0	1	0.036
	1a	2	1	
	1b	1	2	
	2a	13	27	
	2b	76	57	
	4	1	5	
Adjuvant treatment	+	80	46	0.277
	-	45	18	

Note: CD20: Cluster of Differentiation 20; HbA1C: Hemoglobin A1C; CRP: C-Reactive Protein; WBC: White Blood Cell Count; CA19-9: Carbohydrate Antigen 19-9; CEA: Carcinoembryonic Antigen; NLR: Neutrophil-to-Lymphocyte ratio; PNI: Prognostic Nutritional Index; PLR: Platelet-to-Lymphocyte Ratio; CAR: C-Reactive Protein/Albumin ratio.

(*P*=0.026) were independent prognostic factors for DFS (**Table 7**). CD8-positive cell count (*P*=0.024) was the only independent prognostic factor associated with OS (**Table 8**).

Table 3. Multivariate analyses of prognostic factors associated with DFS

	Hazard ratio	95% CI	*P*-value
CD20	0.667	0.450-0.988	0.043
Location	1.24	0.796-1.933	0.341
Biliary decompression	1.039	0.649-1.662	0.874
Total Bilirubin	1.067	0.954-1.193	0.259
Albumin	0.812	0.487-1.353	0.424
CRP	1.043	0.947-1.150	0.394
Hemoglobin	0.954	0.830-1.096	0.504
T	0.786	0.458-1.348	0.381
N	0.747	0.255-2.188	0.594
Stage	2.355	1.040-5.333	0.04

Note: DFS: Disease-Free Survival; 95% CI: 95% Confidence Interval; CD20: Cluster of Differentiation 20; CRP: C-Reactive Protein.

Table 4. Multivariate analyses of prognostic factors associated with OS

	Hazard ratio	95% CI	*P*-value
CD8	0.582	0.376-0.903	0.016
Location	1.064	0.641-1.766	0.811
Biliary decompression	0.898	0.511-1.577	0.708
Total Bilirubin	0.9	0.750-1.078	0.252
Albumin	0.567	0.313-1.029	0.062
CRP	1.033	0.899-1.187	0.646
Hemoglobin	1.102	0.935-1.299	0.246
T	1.065	0.504-2.251	0.869
N	0.934	0.283-3.078	0.91
Stage	2.342	0.924-5.936	0.073

Note: OS: Overall Survival; 95% CI: 95% Confidence Interval; CD8: Cluster of Differentiation 8; CRP: C-Reactive Protein.

Neoadjuvant treatment was not identified as an independent prognostic factor in the multivariate analysis.

Discussion

The characteristics of immune cell infiltration vary by cancer type, and the infiltration profile is recognized as a prognostic factor in many cancers [19]. Immunotherapy has become a cornerstone of cancer treatment with the advent of immune checkpoint inhibitors (ICIs). Consequently, understanding the immune microenvironment at local tumor sites in humans has become increasingly important in translational research. However, compared with other cancers, PDAC exhibits a powerful immunosuppressive environment and paucity of tumor-infiltrating lymphocytes, making it difficult to treat with immunotherapies, such as those involving ICIs [20]. Numerous studies have investigated tumor-infiltrating immune cells and their clinicopathological significance in various cancers; however, reports concerning PDAC are limited. Therefore, in this study, we assessed the clinicopathological significance of the immune microenvironment in PDAC by examining its immunohistological characteristics and their correlation with prognosis.

The cancer immunological microenvironment comprises various components, including blood vessels, fibroblasts, immune cells, and extracellular matrix. However, tumor-infiltrating immune cells are also heterogeneous owing to variability in their density, quantity, and distribution. Matsugi et al. reported intratumoral heterogeneity in the distribution of $CD8^+$ cells, observing substantial differences in cell density between the tumor center and margin in PDAC tissues. They further demonstrated the prognostic significance of $CD8^+$ cell density in the tumor center, where infiltration was unevenly distributed [21]. As PDAC is often accompanied by peritumoral pancreatitis at the invasive front, immune cell infiltration near this region may be influenced by inflammatory responses. Therefore, immune cell infiltration into the tumor core is considered to have a greater prognostic impact. In the present study, immune cell infiltration was examined by immunostaining the largest cross-sectional areas of the tumor specimens.

Stromal responses in PDAC contribute to tumor progression through various mechanisms involving activated pancreatic stellate cells, myeloid-derived suppressor cells, and regulatory T cells. Moreover, dysregulated signaling by

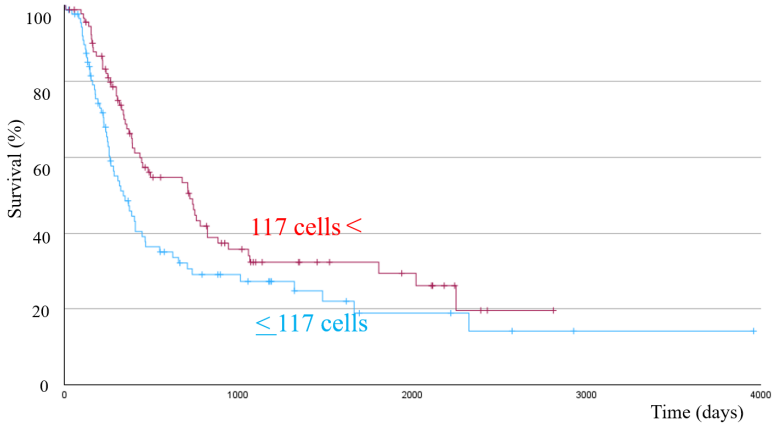

Figure 3. Comparison of patient survival over time based on median DFS stratified by CD20-positive cell count excluding patients who had received neoadjuvant treatment. DFS: Disease-Free Survival; CD20: Cluster of Differentiation 20.

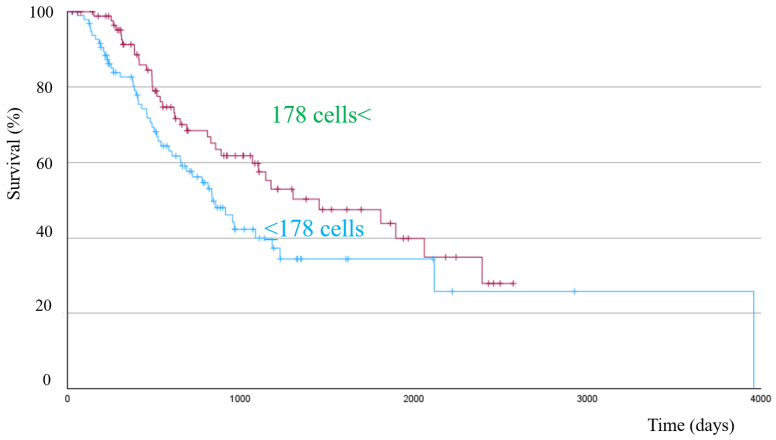

Figure 4. Comparison of patient survival over time based on median OS according to CD8-positive cell counts. OS excluding patients who had received neoadjuvant treatment. OS: Overall Survival; CD8: Cluster of Differentiation 8.

pancreatic stellate cells activated within the tumor microenvironment can reduce the migration of CD8-positive T cells, thereby preventing them from accessing tumor cells [22]. Generally, PDAC has an immunosuppressive microenvironment in which antitumor T-cell infiltration rarely occurs [23], and immunohistochemical studies have demonstrated that higher levels of $CD3^+$ and $CD8^+$ T-cell infiltration are associated with an improved prognosis in PDAC [24]. Both tumor-infiltrating $CD4^-$ and CD8 T cells have been identified as independent prognostic factors; however, enhanced infiltration of both $CD4^+$ and $CD8^+$ T cells is a prerequisite for prolonged survival [25]. In the present study, T cells were one of the main components of antitumor immunity, and high levels of T-cell infiltration were associated with a favorable prognosis in patients with PDAC.

Macrophages are the most abundant immune cells that infiltrate the tumor stroma. Unlike T cells, which diffuse throughout the stroma, B cells are typically organized into clusters, such as lymphoid follicles, and contribute to the regulation of antitumor immunity through humoral immune mechanisms [26]. Ino et al. reported that a high density of infiltrating $CD163^+$ M2 tumor-associated macrophages in the tumor stroma is associated with poor prognosis, whereas a higher proportion of $CD68^+$ human leukocyte antigen DR isotype macrophages correlates with a better prognosis [25]. Therefore, the latter was identified as an independent prognostic factor. In this study, high CD20 expression was associated with a favorable DFS prognosis. As $CD20^+$ cells are functional B cells with antigen-recognition capabilities similar to those of $CD19^+$ cells, these findings suggest that cellular immune responses mediated by humoral immunity play a role in regulating antitumor immune defense against PDAC.

The presence of intratumoral B cells reflects the activation of local antitumor immunity and contributes to the maintenance of localized immune responses. Furthermore, B cells are involved in the control of minimal residual disease and suppression of tumor recurrence, thereby showing a closer association with DFS. By contrast, T cells exert direct cytotoxic effects on tumor cells and contribute to tumor control through the maintenance of immune surveillance, making them more closely associated with OS.

Table 5. Comparison of clinical, physiological, and pathological factors according to $CD8^+$ cells level excluding patients who had received neoadjuvant treatment

		≤ 178 (n=97)	178 < (n=89)	*P*-value
Sex	Men	63	50	0.221
	Women	34	39	
Location	Head	72	56	0.236
	Body/tail	22	30	
	Total	3	3	
Jaundice decompression	+	49	53	0.216
	-	48	36	
Age		70.22±0.99	68.31±1.03	0.185
Total Bilirubin	mg/dl	1.45±0.18	1.52±0.22	0.798
Albumin	mg/dl	3.74±0.05	3.79±0.05	0.473
Creatinine	mg/dl	0.75±0.03	0.74±0.03	0.861
HbA1C	%	6.47±0.22	6.87±0.18	0.157
Amylase	IU/L	101.21±6.96	114.59±17.13	0.47
CRP	mg/dl	1.23±0.32	0.85±0.21	0.317
WBC	/μl	5831.96±162.21	5560.67±180.46	0.265
Hemoglobin	g/dl	12.30±0.15	12.34±0.17	0.869
Platelet	$\times 10^4$/μl	15.11±1.25	17.30±1.02	0.176
CA19-9	U/ml	641.39±152.56	378.34±78.67	0.128
CEA	mg/nl	5.11±0.65	4.22±0.44	0.262
NLR		2.94±0.20	2.62±0.16	0.224
PNI		44.92±0.57	44.95±0.76	0.975
PLR		107.15±8.89	129.46±9.74	0.093
CAR		0.377±0.105	0.261±0.069	0.357
T	is	1	0	0.045
	1	4	2	
	2	0	6	
	3	92	81	
N	0	22	24	0.499
	1	75	65	
R	0	72	74	0.327
	1	21	13	
	2	4	2	
Stage	0	1	0	0.572
	1a	2	1	
	1b	0	2	
	2a	19	21	
	2b	72	63	
	4	3	3	
Adjuvant treatment	+	37	26	0.199
	-	60	63	

Together, B cells primarily function in the prevention of recurrence, whereas T cells are mainly involved in tumor control. In addition, T cells may influence treatment responsiveness after recurrence, which may explain the differential associations observed between immune cell subsets and DFS versus OS.

Despite these positive findings, this study had certain limitations. First, the study design was

Table 6. Comparison of clinical, physiological, and pathological factors according to $CD20^+$ cells level excluding patients who had received neoadjuvant treatment

		≤ 117 (n=90)	117 < (n=93)	*P*-value
Sex	Men	60	52	0.136
	Women	30	41	
Location	Head	72	54	0.001
	Body/tail	14	37	
	Total	4	2	
Jaundice decompression	+	39	61	0.002
	-	51	32	
Age		69.51±1.04	69.19±1.00	0.826
Total Bilirubin	mg/dl	1.80±0.21	1.08±0.15	0.006
Albumin	mg/dl	3.69±0.05	3.84±0.05	0.027
Creatinine	mg/dl	0.75±0.03	0.74±0.03	0.94
HbA1C	%	6.69±0.19	6.59±0.20	0.718
Amylase	IU/L	108.30±10.58	108.49±14.59	0.992
CRP	mg/dl	1.47±0.37	0.66±0.15	0.043
WBC	/μl	5590±158.80	5781.72±186.41	0.436
Hemoglobin	g/dl	12.10±0.15	12.54±0.17	0.051
Platelet	$\times 10^4/\mu l$	16.42±1.27	16.34±1.05	0.959
CA19-9	U/ml	637.87±148.88	393.09±99.32	0.173
CEA	mg/nl	4.78±0.63	4.65±0.52	0.87
NLR		2.98±0.21	2.63±0.16	0.191
PNI		44.12±0.64	45.65±0.71	0.109
PLR		123.33±9.69	115.90±9.14	0.578
CAR		0.449±0.118	0.202±0.052	0.058
T	is	0	1	0.223
	1	2	4	
	2	1	5	
	3	87	83	
N	0	15	31	0.009
	1	75	62	
R	0	66	78	0.182
	1	21	12	
	2	3	3	
Stage	0	0	1	0.028
	1a	2	1	
	1b	1	1	
	2a	12	28	
	2b	74	57	
	4	1	5	
Adjuvant treatment	+	34	27	0.21
	-	56	66	

retrospective; however, the included PDAC cases were consecutive. Second, although lymphoid follicle areas were excluded when quantifying positive cells using immunohistochemistry, the reproducibility of these measurements is limited. Therefore, further multicenter investigations involving larger patient populations and additional specialists incorporating immu-

Table 7. Multivariate analyses of prognostic factors excluding patients who had received neoadjuvant treatment associated with DFS

	Hazard ratio	95% CI	*P*-value
CD20	0.634	0.424-0.946	0.026
Location	1.319	0.837-2.077	0.232
Jaundice decompression	1.01	0.630-1.618	0.967
Total Bilirubin	1.067	0.955-1.191	0.255
Albumin	0.687	0.426-1.110	0.125
CRP	1.035	0.938-1.141	0.498
T	0.796	0.463-1.367	0.408
N	0.792	0.271-2.312	0.67
Stage	2.306	1.023-5.201	0.044

Table 8. Multivariate analyses of prognostic factors excluding patients who had received neoadjuvant treatment associated with OS

	Hazard ratio	95% CI	*P*-value
CD8	0.6	0.385-0.936	0.024
Location	1.049	0.625-1.781	0.856
Jaundice decompression	0.883	0.502-1.554	0.667
Total Bilirubin	0.886	0.736-1.067	0.201
Albumin	0.636	0.354-1.144	0.131
CRP	1.031	0.895-1.188	0.675
T	1.088	0.517-2.291	0.825
N	1.031	0.306-3.474	0.961
Stage	2.199	0.863-5.604	0.099

nohistochemical staining for more T-cell surface markers are required before definitive conclusions can be established.

In conclusion, we demonstrated that patient prognosis in PDAC is associated with high CD20 expression, reflecting B-cell infiltration and linked to DFS, and high CD8 expression, reflecting T-cell infiltration and associated with OS. The tumor immune microenvironment, which influences cellular immune responses, may serve as a prognostic factor in PDAC.

Disclosure of conflict of interest

None.

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