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Original Article

The Prognostic Impact of High Soluble Programmed Death Ligand 1 Levels in Patients with Hepato-Biliary-Pancreatic Cancer

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ABSTRACT

Introduction: Several reports revealed that the high soluble programmed death-ligand 1(sPD-L1) level was a risk factor for poor prognosis in various tumors. To date, the clinicopathologic and prognostic impact of the sPD-L1 level in patients with hepatobiliary-pancreatic cancer have not been determined.

Methods: A total of 119 patients (66 patients with hepatocellular carcinoma, 23 patients with cholangiocarcinoma, and 30 patients with pancreatic cancer) who were treated at the Toho University Omori Hospital (Tokyo, Japan) from 2008 to 2016 were retrospectively analyzed. The sPD-L1 levels were measured using an enzyme-linked immunosorbent assay for PD-L1 to evaluate the clinicopathologic and prognostic impact.

Results: The sPD-L1 levels were significantly higher in the low-albumin group than in the normal-albumin group. According to the stages in hepatocellular carcinoma and cholangiocarcinoma, no significant differences were observed in the sPD-L1 levels, which gradually increased according to the stage in pancreatic cancer. Using a cut-off value of 81.6 pg/mL for the sPD-L1 level, the high sPD-L1 group showed a significantly worse prognosis compared with the low-sPD-L1 group in patients with pancreatic cancer. Multivariate analysis identified sPD-L1 level $\geq$ 81.6 mg/dL ($p = 0.047$) as an independent predictor of poor overall survival in patients with pancreatic cancer.

Conclusions: Using a cut-off value of 81.6 pg/mL for the sPD-L1 level, high sPD-L1 levels were independently associated with poor prognosis in patients with pancreatic cancer. However, this association in hepatocellular carcinoma or cholangiocarcinoma was not clear.

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KEYWORDS: programmed death-ligand 1, hepato-biliary-pancreatic cancer, prognosis

Introduction

Programmed death-ligand 1 (PD-L1) is an immune checkpoint protein within the cancer-immunity cycle expressed on the surface of tumor cells and tumor-infiltrating immune cells to downregulate T-cell function.[1]

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Table 1 Characteristics and demographics of the patients

Variables		Number of patients (n = 119) (%)
Gender	Male	76 (64)
	Female	43 (36)
Age (year)	＜65	32 (27)
	≥65	87 (73)
Stage	I, II	71 (60)
	III, IV	48 (40)
AFP (ng/mL)	≤10	37 (56)
(n = 66)	＞10	29 (44)
PIVKA-II (ng/mL)	≤40	33 (50)
(n = 66)	＞40	33 (50)
CEA (ng/mL)	≤5	36 (68)
(n = 53)	＞5	17 (32)
CA19-9 (ng/mL)	≤37	22 (42)
(n = 53)	＞37	31 (58)
Treatment	Liver resection	65 (55)
	Pancreaticoduodenectomy	32 (27)
	Distal pancreatectomy	7 (6)
	Exploratory laparotomy	15 (12)

Recently, soluble PD-L1 (sPD-L1) has been detected in the blood of cancer patients.[2] Several clinical studies have evaluated the prognostic values of sPD-L1 in patients with various cancers and explored the associations of the sPD-L1 levels with clinicopathologic factors.[3–5] As a result, some reports revealed that high sPD-L1 level was a risk factor for poor prognosis in various tumors, including non-small-cell lung cancer, gastric cancer, hepatocellular carcinoma (HCC), and renal cell cancer.[2, 3, 5–9]

In patients with HCC, high sPD-L1 level and high PD-L1 expression in tumors have been identified as poor prognostic factors.[5, 10] Moreover, in patients with cholangiocarcinoma (CCA), high PD-L1 expression in tumors has been identified as a poor prognostic factor.[11] Conversely, the clinicopathologic significance of high sPD-L1 level has not been reported. In patients with pancreatic cancer (PC), high sPD-L1 level was not significantly associated with the overall survival (OS).[4] To date, most of the studies analyzing the clinicopathological and prognostic impact of sPD-L1 levels on hepatobiliary-pancreatic (HBP) cancer have focused on unresectable cases; few have analyzed resectable cases.[4, 5, 12]

Based on the reports of various carcinomas, high sPD-L1 level may be a poor prognosis factor in HBP cancer, either resectable or unresectable. Therefore, we aimed to conduct a cross-sectional analysis of the sPD-L1 levels of patients with HBP cancer and evaluate the relationship with clinicopathologic factors and prognosis. To appropriately assess the prognosis in a preoperative manner from stage I to stage IV, we decided to evaluate the prognosis of all enrolled patients, either resectable or unresectable.

Methods

Patients

A total of 119 patients (66 patients with HCC, 23 patients with CCA, and 30 patients with PC) who were treated at the Toho University Omori Hospital (Tokyo, Japan) from 2008 to 2016 were retrospectively analyzed (Table 1). All patients were preoperatively proven to have their own carcinoma pathologically; patients with double cancer were excluded. Among the 66 patients with HCC, 19 had stage I, 14 had stage II, 28 had stage III, and 5 had stage IV. Among the 23 patients with CCA, 9 had stage I, 10 had stage II, 1 had stage III, and 3 had stage IV disease. Among the 30 patients with PC, 4 had stage I, 15 had stage II, 4 had stage III, and 7 had stage IV disease. All patients underwent liver resection (n = 65), pancreaticoduodenectomy (n = 32), distal pancreatectomy (n = 7), or exploratory laparotomy (n = 15). The patients who underwent exploratory laparotomy were unresected and determined to have stage IV disease. Among the 23 patients with CCA, 18 had distal CCA, and 5 had hilar CCA. Among the 15 patients with stage IV disease, 2 had distant lymph node metastasis, 4 had organ metastasis, and 9 had peritoneal metastasis or cancer cells on peritoneal cytology. Using the tumor-node-metastasis (TNM) classification system of malignant

Table 2 Comparison of soluble programmed death-ligand 1 according to clinicopathological factors and various laboratory data with hepatobiliary–pancreatic cancer

Variables		Number of patients (n = 119)	PDL1 level Mean ± SD (mg/dl)	P value[a]
WBC (/μl)	<7000	101	69.3 ± 30.4	0.452
	≥7000	18	77.5 ± 34.7	
Neutrophils (%)	<70	103	70.5 ± 31.6	0.723
	≥70	16	70.2 ± 28.3	
Lymphocytes (%)	<35	80	71.7 ± 33.6	0.995
	≥35	39	68.1 ± 25.3	
CRP (mg/dL)	<0.3	82	71.6 ± 32.6	0.464
	≥0.3	37	68.2 ± 27.8	
Albumin (g/dL)	<3.5	28	82.3 ± 34.9	0.035 *
	≥3.5	91	66.9 ± 29.1	
Bilirubin (mg/dL)	<1.5	108	70.4 ± 32.1	0.601
	≥1.5	11	71.4 ± 19.7	
Amylase (U/L)	<150	103	68.0 ± 27.4	0.227
	≥150	16	86.3 ± 46.7	

SD, Standard deviation; WBC, White blood cell; CRP, C-reactive protein
a. Mann–Whitney U test.
*P<0.05

tumors of the Union for International Cancer Control (UICC), 8th edition, the final stage of HBP cancer was pathologically evaluated.[13]

This retrospective study was approved by the Institutional Ethics Committee of the Toho University Omori Hospital (IRB no. M19213). All patients were followed up until the end of April 2020 or death.

Sample collection and enzyme-linked immunosorbent assay

All serum samples were collected within 1 month prior to surgery, and the sPD-L1 levels were measured using an enzyme-linked immunosorbent assay (ELISA) for PD-L1 (R&D Systems, Inc., Minneapolis, MN, USA) as previously described.[3]

Study design and serum biomarker analysis

To evaluate the relationship with the sPD-L1 level, C-reactive protein (CRP), albumin, and plasma fibrinogen, as well as white blood cell, neutrophil, and lymphocyte counts, were analyzed prior to surgery. The cut-off values were determined in accordance with the institutional standards for white blood cell count (7000 cells/mm^3), CRP levels (0.3 mg/mL), and albumin (3.5 mg/dL). Subsequently, we analyzed the association of the sPD-L1 level and clinicopathologic factors with HBP cancer.

Statistical analyses

Statistical analysis was conducted using the JMP statistical software (version 12; SAS Institute, Cary, NC, USA). The levels of serum biomarker were expressed as mean ± standard deviation. Comparisons between the unpaired groups for these variables were conducted using the Mann-Whitney U test. In addition, the survival rate was calculated using the Kaplan-Meier estimator. The differences between the groups with regard to survival were analyzed using the log-rank test. Multivariate analysis using the Cox proportional-hazards model was conducted to evaluate the significant predictors identified by univariate analysis. We considered $p < 0.05$ as statistically significant.

Results

Correlation and association of serum programmed death-ligand 1 level with serum biomarkers

The associations of the sPD-L1 levels with several blood tests were evaluated (Table 2). The sPD-L1 level was significantly higher in the low-albumin group than in the normal-albumin group (p = 0.035, Table 2). No significant differences were observed between the sPD-L1 levels and serum biomarkers in the other parameters: white blood cells, neutrophils, lymphocytes, CRP, total bilirubin, and amylase.

Comparison between the soluble programmed death-ligand 1 levels of hepatobiliary-pancreatic cancer and each TNM stage

The mean sPD-L1 levels for 66 patients with HCC, 23 patients with CCA, and 30 patients with PC were 70.9 ± 35.1,

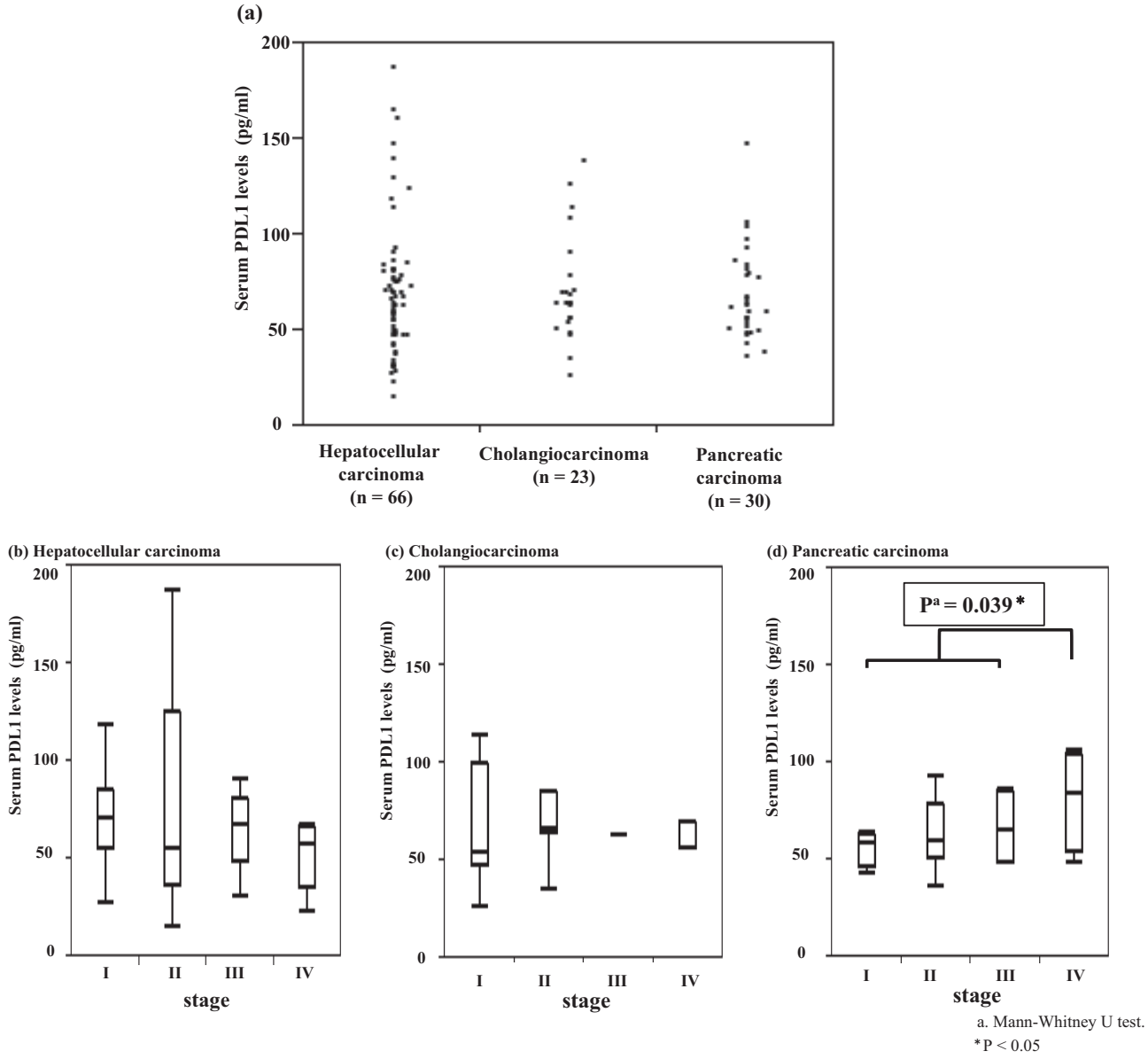

Fig. 1 The scatter plot of the sPD-L1 level of 66 patients with hepatocellular carcinoma, 23 patients with cholangiocarcinoma, and 30 patients with pancreatic cancer (a). Comparison of the sPD-L1 levels according to UICC stages in hepatocellular carcinoma (b), cholangiocarcinoma (c) and pancreatic cancer (d).

71.2 ± 27.7, and 69.1 ± 24.2 pg/mL (Fig. 1a), respectively. No significant differences were observed in the sPD-L1 levels among HBP cancer (HCC vs CCA p = 0.895, HCC vs PC p = 0.818, CCA vs PC p = 0.686). Moreover, there were no significant differences in the sPD-L1 levels according to the UICC stages in HCC and CCA (Fig. 1b, 1c). Conversely, the sPD-L1 levels gradually increased according to the UICC stage in PC (Fig. 1d). The sPD-L1 levels of stage IV PC were significantly higher than those of stage I/II/III PC (Fig. 1d, p = 0.039).

Comparisons of soluble programmed death-ligand 1 levels according to clinicopathologic factors and various biomarkers with HBP cancer

The sPD-L1 levels were not associated with any of the clinicopathologic factors, such as gender, age, stage, tumor size, and conventional tumor markers, in patients with HCC and CCA (Table 3a, 3b, 3c). However, the sPD-L1 levels were significantly higher in PC patients with high CA19-9 levels (p = 0.028, Table 3c).

Table 3 Comparison of soluble programmed death-ligand 1 according to clinicopathological factors and various biomarkers with hepatobiliary–pancreatic cancer

(a) Hepatocellular carcinoma

Variables		Number of patients ($n = 66$)	PDL1 level: mean ± SD (mg/dl)	P value[a]
Gender	Male	46	65.4 ± 26.6	0.201
	Female	20	83.7 ± 47.9	
Age (year)	<65	15	79.2 ± 36.4	0.351
	≥65	51	68.5 ± 34.7	
Liver damage	A	53	72.3 ± 37.0	0.578
	B	13	65.5 ± 26.6	
Number of tumor	1	54	68.9 ± 29.9	0.907
	≥2	12	80.0 ± 53.4	
Tumor size (mm)	<20	20	70.2 ± 33.5	0.775
	≥20	46	71.3 ± 36.3	
Background liver	Normal	16	64.8 ± 16.7	0.958
	Other	50	72.8 ± 39.1	
Vascular invasion	Vp0	43	71.1 ± 38.6	0.59
	Vp1-	23	70.6 ± 28.2	
	Vv0	46	73.0 ± 37.0	0.36
	Vv1-	20	65.9 ± 30.7	
Stage	I, II	33	74.8 ± 39.4	0.533
	III, IV	33	67.1 ± 30.4	
AFP (ng/mL)	≤10	37	70.5 ± 33.7	0.969
	>10	29	71.5 ± 37.5	
PIVKA-II (ng/mL)	≤40	33	72.7 ± 41.9	0.695
	>40	33	69.1 ± 27.3	

(b) Cholangiocarcinoma

Variables		Number of patients ($n = 23$)	PDL1 level: mean ± SD (mg/dl)	P value[a]
Gender	Male	16	73.6 ± 32.6	0.688
	Female	7	65.6 ± 10.5	
Age (year)	<65	5	67.4 ± 26.9	0.371
	≥65	18	72.2 ± 28.6	
Tumor location	Bp	5	64.2 ± 14.9	0.419
	Bd	18	74.9 ± 32.5	
Tumor size (mm)	<30	13	79.6 ± 34.6	0.128
	≥30	10	60.6 ± 7.6	
UICC T class	1, 2	9	69.1 ± 30.4	0.488
	3, 4	14	72.5 ± 26.9	
UICC N class	0	19	68.4 ± 25.9	0.239
	1, 2	4	84.3 ± 36.2	
UICC M class	0	20	72.7 ± 29.4	0.615
	1	3	61.1 ± 7.5	
Stage	I, II	19	73.1 ± 30.1	0.49
	III, IV	4	61.7 ± 6.2	
CEA (ng/mL)	≤5	19	70.9 ± 30.1	0.35
	>5	4	72.3 ± 14.0	
CA19-9 (ng/mL)	≤37	13	63.3 ± 25.5	0.062
	>37	10	81.3 ± 28.4	

Table 3 continued

(c) Pancreatic carcinoma

Variables		Number of patients ($n = 30$)	PDL1 level: mean ± SD (mg/dl)	P value[a]
Gender	Male	14	68.8 ± 20.0	0.901
	Female	16	69.3 ± 28.0	
Age (year)	＜65	12	63.3 ± 18.6	0.271
	≥65	18	73.0 ± 27.1	
Tumor location	Head	17	67.2 ± 19.8	0.851
	Other	13	71.6 ± 27.9	
Tumor size (mm)	＜40	23	69.6 ± 25.3	0.825
	≥40	7	67.3 ± 21.5	
UICC T class	1, 2	4	56.5 ± 9.5	0.329
	3, 4	26	71.0 ± 25.3	
UICC N class	0	20	67.0 ± 25.3	0.378
	1, 2	10	73.3 ± 22.3	
UICC M class	0	23	65.1 ± 23.5	0.081
	1	7	82.4 ± 23.0	
Stage	I, II	19	64.7 ± 24.6	0.149
	III, IV	11	76.7 ± 22.5	
CEA (ng/mL)	≤5	17	63.6 ± 15.9	0.346
	＞5	13	76.2 ± 31.2	
CA19-9 (ng/mL)	≤37	9	55.4 ± 12.4	0.028 *
	＞37	21	74.9 ± 25.8	

SD, Standard deviation
a. Mann–Whitney U test.
*P＜0.05

Overall survival curves according to soluble programmed death-ligand 1 levels in patients with HBP cancer

All 119 cases were divided by quartiles according to the sPD-L1 levels as follows: the range of the sPD-L1 levels with Q1 was 15.8 to 49.3 pg/mL; Q2, 49.8 to 64.3 pg/mL; Q3, 64.5 to 81.5 pg/mL; and Q4, 81.7 to 188.0 pg/mL. Although there were no significant differences in the OS between each group ($p = 0.878$), the Q4 group revealed the worst survival rate (Fig. 2a). Therefore, we decided to set the cut-off value to 81.6 pg/mL for the sPD-L1 level in order to evaluate the prognostic significance of sPD-L1 in further analyses. Although the Q4 group revealed worse prognosis than the other groups (Q1Q2Q3), the difference was not statistically significant ($p = 0.333$, Fig. 2b).

To evaluate the prognostic significance of sPD-L1 in each cancer type, the OS was compared between the Q1Q2Q3 group and the Q4 group in each cancer type (Fig. 3).

When the number of each cancer type was divided by quartiles according to each sPD-L1 level, compared between the Q1Q2Q3 group and the Q4 group in each cancer type, the cut-off value for sPD-L1 was also 81.6 pg/mL in common (Fig. 3a-f). No prognostic significance was observed in HCC ($p = 0.977$, hazard ratio (HR) = 1.017, Fig. 3b) and CCA ($p = 0.665$, HR = 0.717, Fig. 3d). Conversely, the Q4 group showed a significantly worse prognosis than the Q1Q2Q3 group in patients with PC ($p = 0.005$, HR = 5.059, Fig. 3f).

Univariate and multivariate analysis of risk factors for overall survival with pancreatic cancer

Several prognostic factors, including the sPD-L1 levels, were evaluated in patients with PC (Table 4). The univariate analysis identified the UICC M1 and sPD-L1 level ≥ 81.6 mg/dL as significant predictors of poor OS. Moreover, multivariate analysis conducted using the Cox proportional-hazard regression model identified the UICC M1 ($p = 0.017$; HR = 3.997; 95% CI = 1.779-12.03) and sPD-L1 level ≥ 81.6 mg/dL ($p = 0.047$; HR = 3.588; 95% CI = 1.419-11.19) as independent predictors of OS.

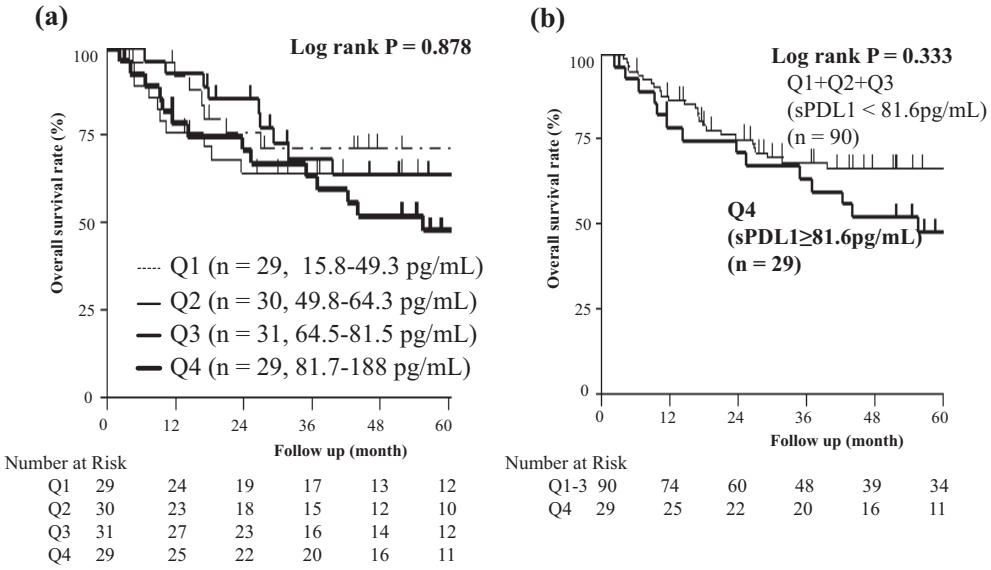

Fig. 2 (a) Comparison of the overall survival of the patients with hepatobiliary-pancreatic cancer according to soluble PD-L1 levels classified into four groups (Q1Q2Q3Q4). (b) Comparison of the overall survivals according to soluble PD-L1 levels classified into two groups (Q1 + Q2 + Q3 vs Q4). Statistical analyses were conducted using the log-rank test.

Discussion

In the present study, preoperative sPD-L1 levels were analyzed in a total of 119 patients with HCC, CCA, or PC. High sPD-L1 level was associated with a low level of albumin in all HBP cancer patients and not in the specific cancer type. In patients with PC, high sPD-L1 levels were associated with high CA19-9 levels but not with tumor stage. Using a cut-off value of 81.6 pg/mL, high sPD-L1 level was found to be an independent risk factor for poor OS of patients with PC.

sPD-L1 levels have been reported to increase in patients with cancer and/or systemic inflammation in HCC, gastric cancer, and PC.[3–5] Previous studies demonstrated the significant association among sPD-L1, CRP, and albumin. There was a significant relationship between sPD-L1 and albumin but none between sPD-L1 and CRP in our current study. Such a discrepancy could be partly attributed to the significant difference between the ratio of stage IV in previous studies and the ratio in our present study: stage IV cases accounted for 14%-85% in previous studies and 12% in our study. In stage IV disease, the tumor microenvironment contains numerous cells producing inflammatory cytokines and promoting metastatic disease. Although Xu et al. have reported that high interleukin-6 level in patients with prostate cancer was associated with the expression of PD-L1 in prostate cancer tissues, the mechanism of high serum PD-L1 levels is unclear.[14]

Although previous reports did not demonstrate that high sPD-L1 levels had significant negative effect on the OS in PC, our present data indicated that high sPD-L1 level was a significant risk factor that reduces patients' OS. This discrepancy could be explained by the aforementioned ratio of stage IV disease in previous reports being higher than that in our present study. When analyzed in subjects with a high proportion of stage IV disease, the effects of PD-L1 expression on prognosis and the effects of checkpoint inhibitors in PC may be masked.[15, 16] PD-L1 inhibitors may be effective in suppressing postoperative recurrence in patients with high PD-L1 expression, even if they are resectable PC. This means that PD-L1 inhibitors may effectively improve the prognosis of PC with high serum PD-L1 levels. Given the small number of patients included in our study, further studies are needed to confirm this observation.

Although the sPD-L1 level was significantly associated with poor prognosis in previous reports in HCC, our present series did not demonstrate such association.[5, 10] Two previous reports used an ELISA Kit different from that used in our present analysis. Moreover, the detection limits and cut-off values were completely different from those in our series. Such differences might partly explain these discrepancies. With regard to the correlation between high sPD-L1 level and high CRP, two previous reports re-

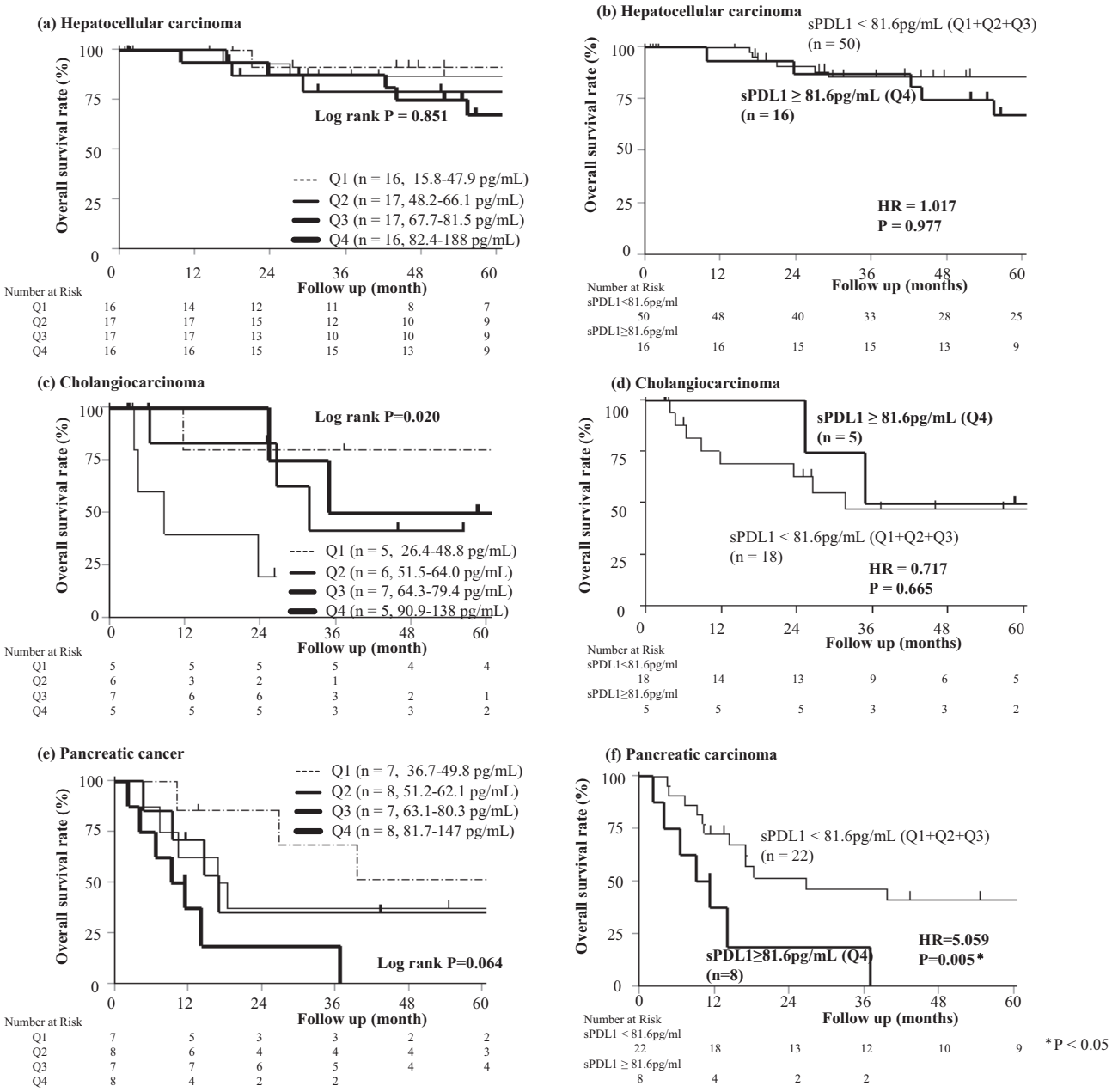

Fig. 3 Comparison of the overall survival of the patients according to soluble PD-L1 levels classified into four groups (Q1Q2Q3Q4) with hepatocellular carcinoma (a), cholangiocarcinoma (c), and pancreatic cancer (e). Comparison of the overall survival according to soluble PD-L1 levels classified into two groups (Q1 + Q2 + Q3 vs Q4) with hepatocellular carcinoma (b), cholangiocarcinoma (d), and pancreatic cancer (f). Statistical analyses were conducted using the log-rank test.

vealed positive correlations in HCC and in PC.[4,5] They suspected that an increased activation of innate immunity is part of the immunosuppressive environment hampering the activity of the adaptive anti-tumor response. We could not confirm such correlations in our present study. The impact of sPD-L1 levels might be independent of the activity of inflammatory cytokines in HCC.

One of the limitations of the present study was its retrospective nature. Also, the number of patients with each cancer type was too small to draw any solid conclusions. Second, this study lacks healthy controls or patients with inflammatory diseases, such as pancreatitis and liver inflammation, whose sPD-L1 levels can be compared with those of patients with HBP cancer. To conclude and validate this observation without doubt, a larger cohort with a control group is needed. Using the same ELISA Kit used in the present study, Chen et al. reported that the median serum PD-L1 level in healthy controls was 48.15 pg/mL,[17]

Table 4 Univariate and multivariate analyses of the risk factors for overall survival with pancreatic cancer

Variables		Univariate	Multivariate		
		P value[a]	H.R.[b]	95%CI[c]	*P* value[d]
Gender	Male	0.429			
	Female				
Age (year)	<65	0.643			
	≥65				
mGPS (score)	0	0.776			
	1, 2				
NLR	<2.0	0.853			
	≥2.0				
Tumor location	Head	0.410			
	Body-tail				
Tumor size (mm)	<40	0.342			
	≥40				
UICC T class	1, 2	0.811			
	3, 4				
UICC N class	0	0.217			
	1, 2				
UICC M class	0	<0.001	1		0.017 *
	1		3.997	1.779–12.03	
sPDL1 (pg/mL)	<81.6	0.001	1		0.047 *
	≥81.6		3.588	1.419–11.19	
CEA (ng/mL)	≤5	0.799			
	>5				
CA19-9 (ng/mL)	≤37	0.137			
	>37				

mGPS: Modified Glasgow prognostic score
NLR: Neutrophil/lymphocyte ratio
a. Log-rank test
b. Adjusted hazard ratio
c. Adjusted 95% confidence interval
d. Cox regression analysis
*P<0.05

which is lower than the median serum PD-L1 level reported in our present study. Furthermore, no immunohistochemical analysis was conducted to evaluate the impact of tissue PD-L1 expression on serum PD-L1 levels. In cancer, the relationship between sPD-L1 and PD-L1 expression in tissues remains unclear. sPD-L1 may be produced by multiple sources *via* the distinct mechanisms from both the tumor and immune cells.[18, 19] Another limitation was that the blood samples were taken only once prior to surgery. Postoperative blood samples are also needed to analyze the correlation with prognosis. In the next study, comparison of the samples before and after surgery as well as long-term monitoring would be conducted.

Thus far, the association of the expression of PD-L1 in the treatment response of anti-PD-L1 antibody in cancer tissues was not evident in previous clinical trials. One possible explanation for such confusing results is that the circulating PD-L1, rather than the tissue PD-L1 protein, may have interacted with the anti-PD-L1 antibody. Further prospective observation study is required to monitor the fluctuations in the serum PD-L1 level during anti-PD-L1 antibody treatment, which will further elucidate the clinical significance of the serum PD-L1 level in patients with HBP cancer. In conclusion, high sPD-L1 levels might be associated with tumor progression in PC. Moreover, high sPD-L1 levels were independently associated with poor patient survival in the PC group. However, these associations in HCC and CCA were limited.

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Authors' contribution: R.O., and H.S. designed the study; R.O., Y. O., M.T., T.M., J.I., Y.M., and Y.I. performed the experiments and analyzed the data; K.F. and H.S. supervised the experiments; R.O. and H. S. wrote the manuscript.

Ethics states: This study was approved by the Institutional Ethics Committee of Toho University Omori Medical Center (Tokyo, Japan). All patients provided written informed consent for the sampling, analyses, and publications.

Conflicts of interest: None declared.

Informed consent: Written informed consent was obtained from the patients and legal guardians for the publication of these reports.

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