Soluble PD-L1 reflects cachexia status in patients with gastric cancer and is an independent prognostic marker for relapse-free survival after radical surgery

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Abstract. Soluble programmed death-ligand 1 (sPD-L1) levels can be used as a biomarker for gastric cancer (GC). However, comprehensive information regarding the sPD-L1 expression profiles and their association with cachexia in GC is lacking. Therefore, the present study evaluated the association between clinicopathological findings and sPD-L1 levels in patients with GC. Serum samples were collected from patients with GC during their first visit to Department of Esophageal-Gastro-Intestinal Surgery, Chiba University Hospital, Chiba, Japan (January 2012-December 2017; n=173), and sPD-L1 levels were measured using an enzyme-linked immunosorbent assay. Survival rates among 116 patients, excluding cases with preoperative chemotherapy or no radical procedures, were analyzed. sPD-L1 levels were associated with factors such as neutrophil-to-lymphocyte ratio, hemoglobin (Hb) and albumin (Alb) levels, total cholesterol and C-reactive protein (CRP) levels, and related to inflammation and nutrition in patients. Notably, the higher the number of applicable indicators related to cachexia (Hb <12 g/dl, Alb <3.2 g/dl, CRP >0.5 mg/dl and low body mass index) was, the higher the sPD-L1 value was. However, the pathological stage did not significantly differ between the groups. Clinicopathologically, there was no association with tumor depth, lymph node metastasis or vascular invasion; however, patients with the intestinal type had significantly higher sPD-L1 levels than patients with the diffuse type (P=0.032; Wilcoxon test). The overall survival did not significantly differ between the groups with low and high sPD-L1 levels; however, among patients who received radical treatment, the relapse-free survival was significantly worse in the high-sPD-L1-level group than in the low-sPD-L1-level group (P=0.025; log-rank test). Multivariate Cox regression analysis revealed that a high sPD-L1 concentration was a sign of poor prognosis, independent of pathological stage and cancer antigen CA19-9 (P=0.0029). Therefore, the present findings suggest that sPD-L1 can reflect cachexia status in patients with GC and may serve as a prognostic marker for relapse-free survival after radical GC surgery.

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Key words: cachexia, gastric cancer, prognostic markers, programmed death-ligand 1, radical surgery, relapse-free survival

Introduction

Gastric cancer (GC) remains one of the most common and deadly cancers worldwide, with over 50% of GC cases prevalent in Eastern Asia. Based on GLOBOCAN 2020 data, stomach cancer is the fifth most common neoplasm and the fourth most deadly cancer, with an estimated 769,000 deaths in 2020 (1).

The invention of immune checkpoint inhibitors (ICIs) has paved the way for a new era in cancer immunotherapy. Notably, inhibition of the programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) axis with ICIs, such as nivolumab and pembrolizumab, in combination with cytotoxic drugs, is emerging as a new treatment strategy for advanced GC. PD-L1 plays a vital role in tumor cells evading anti-tumor immunity in various types of cancer. PD-L1 is widely expressed in various cells and tissues, including cancer and immune cells. It is upregulated by multiple inflammatory cytokines such as IL-6, TNF-α, and interferon-γ, and likely functions as a negative feedback loop during inflammation. These inflammatory cytokines are thought to play a major role in cancer cachexia (2).

The 2011 consensus definition described cachexia as a 'multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment' (3). Cachexia is observed in advanced malignancy as well as in the terminal course of many chronic diseases, such as cardiac or renal failure and chronic obstructive pulmonary disease. Common

clinical symptoms include muscle wasting, anemia, decreased caloric intake, and altered immune function, contributing to disability, fatigue, decreased quality of life, and decreased survival in patients with advanced GC (4-6).

Soluble PD-L1 (sPD-L1) is reportedly generated mainly by proteolysis of membrane-bound PD-L1 (7) and has recently been identified in blood samples of patients and is reportedly useful as a factor of poor prognosis in various cancers, such as hepatocellular carcinoma (8,9), lung cancer (10), and GC (11-14). sPD-L1 may impair host immunity and contribute to systemic immunosuppression, leading to cancer progression and poor clinical outcomes (15). However, there are only a few reports of sPD-L1 expression profiles in cancer and host in clinical practice for GC, especially in correlation to cancer cachexia.

Therefore, we explored the utility of sPD-L1 as a biomarker in patients with GC and its integrated analysis with clinicopathological factors, including cancer cachexia.

Materials and methods

Clinical samples. This study was approved by the Ethics Committee of the Graduate School of Medicine, Chiba University (assignment number 1103), and informed consent was obtained from all patients. Blood samples were collected from 173 patients with histologically proven gastric adenocarcinoma during their first visit to Department of Esophageal-Gastro-Intestinal Surgery, Chiba University Hospital, Chiba, Japan between January 2012 and December 2017. No inclusion criteria such as age or performance status were used. Exclusion criteria were defined as the coexistence of active other cancers.

sPD-L1 levels were measured using enzyme-linked immunosorbent assay (ELISA), as described under 'Measurement of sPD-L1 levels,' and integrated with clinicopathological factors such as age, sex, body mass index (BMI), stage, pathological findings, and blood test results. Clinical data were collected from the clinical database.

Among the 173 cases, 32 were pretreated, and 141 were preceded by surgical treatment. Twenty-five of the 141 patients underwent non-radical surgery, and 116 underwent radical surgery with no preoperative treatment. Patients undergoing radical GC surgery (n=116) were divided into two groups according to the high and low sPD-L1 levels, and patient background, laboratory values, and prognosis (overall and relapse-free survival) were compared in each group. The pathological factors were tested in the non-preoperative treatment group (n=141).

Disease classification. The staging classification was confirmed by the International Union Against Cancer TNM staging system, 8th edition. Histological classification was performed according to the Japanese gastric cancer classification. As previously reported (16), the histological types were divided into intestinal predominant, diffuse predominant, and diffuse mixed intestinal types. The latter two were designated diffuse type.

Measurement of sPD-L1 levels. The serum concentrations of sPD-L1 were measured as previously reported (17), using a commercially available ELISA kit for human PD-L1 (R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's protocol. Briefly, 100 μl of serum samples and standards were added to each well and incubated for 2 h at room temperature on a horizontal orbital microplate shaker. After the liquid was removed and washed four times, 200 μl of Human/Cynomolgus Monkey B7-H1 conjugate was added and incubated for 2 h at room temperature on a shaker. After the liquid was removed and washed four times, 200 μl of the substrate solution was added to each well and incubated for 30 min at room temperature on the benchtop and protected from light. The stop solution was added to each well, and the optical density was measured using a microplate reader. All experiments were performed with technical duplicates for each sample, and the average values were calculated. The median (57 pg/ml) was used as the cut-off value, and the two groups were compared.

Factors governing cancer cachexia. To evaluate the association with host cachexia status, we analyzed (1) low body weight (BMI <18.5), (2) anemia (Hb <12 g/dl), (3) malnutrition (Alb <3.2 g/dl), and (4) chronic inflammation (CRP >0.5 mg/dl) as factors of cancer cachexia, and the relationship between the number of items satisfying these criteria and sPD-L1 was evaluated. Data from 148 cases with no missing items in the initial blood draw or clinical information were used for the analysis. The association between pathological Stage (pStage) and the number of cachexia items, excluding the preoperative treatment group, was examined.

Statistical analysis. Continuous variables were denoted by the median (min-max). The range of sPD-L1 levels in each item was indicated by the median and interquartile range. The Wilcoxon rank-sum tests and chi-square tests were used to compare the two groups, and the Kruskal-Wallis and Steel-Dwass tests were used to compare three or more groups. Univariate and multivariate Cox regression analyses were performed. Survival rates were evaluated using the Kaplan-Meier curve and log-rank test. The neutrophil-to-lymphocyte ratio (NLR) cut-off values were set using receiver operating characteristic (ROC) curves with five-year recurrence-free survival as the outcome. Statistical analysis was performed using JMP ver. 15(SAS Institute Inc., Cary, NC, USA), and statistical significance was set at $P<0.05$.

Results

Characteristics of included cases. The flowchart of the patients included in this analysis is displayed in Fig. 1. The clinicopathological features of all the patients (n=173) are listed in Table I, and values are expressed as medians (min-max). The success or failure of 14 patients with a history of Helicobacter pylori (H. pylori) eradication was unclear, and for those without a history of eradication, the results were determined by IgG antibody in blood: 61 patients were negative and 96 patients were positive. Surgical procedures included distal, total, and proximal gastrectomy in 88, 55, and 4 cases, respectively. Bypass and trial laparotomy were performed in 18 cases, and endoscopic treatment in eight cases.

The median value of sPD-L1 in GC patients was 57 pg/ml and was used as the cut-off value to compare the two groups.

sPD-L1 and pathological factors. The pathological features and sPD-L1 levels were compared between the 141 patients who did not receive preoperative treatment. The number of cases in

Table I. Clinicopathological features of all patients (n=173).

Variables	Value
Median age, years (min-max)	70 (28-93)
Sex, n	
Male	120
Female	53
Median BMI, kg/m^2 (min-max)	22.5 (15.5-33.1)
Median WBC, /μl (min-max)	6,400 (3,300-15,700)
Median NLR (min-max)	2.3 (0.2-18.6)
Median Hb, g/dl (min-max)	13.2 (6.6-17.4)
Median Plt, x10^3/μl (min-max)	233 (13-544)
Median T-Cho, mg/dl (min-max)	199 (106-325)
Median Alb, g/dl (min-max)	4.2 (2.0-5.2)
Median CRP, mg/dl (min-max)	0.1 (0.0-13.4)
Median CEA, ng/ml (min-max)	2.5 (0.3-776.0)
Median CA19-9, U/ml (min-max)	14.2 (0.1-2860.0)
Median sPD-L1, pg/ml (min-max)	57.1 (3.2-400.5)
H. pylori infection, n	
Negative	61
Positive	96
Eradication history	14
Clinical staging, n	
Tumor depth	
T1	41
T2	29
T3	42
T4	58
LN metastasis	
N0	97
N1-3	73
Distant metastasis	
M0	149
M1	21
Procedure, n	
DG	88
TG	55
PG	4
ESD	8
Bypass, other	18

BMI, body mass index; WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; Hb, hemoglobin; Plt, platelet count; T-cho, total cholesterol; Alb, albumin; CRP, C-reactive protein; sPD-L1, soluble programmed death-ligand 1; *H. pylori*, *Helicobacter pylori*; LN, lymph node; DG, distal gastrectomy; TG, total gastrectomy; PG, proximal gastrectomy; ESD, endoscopic submucosal dissection.

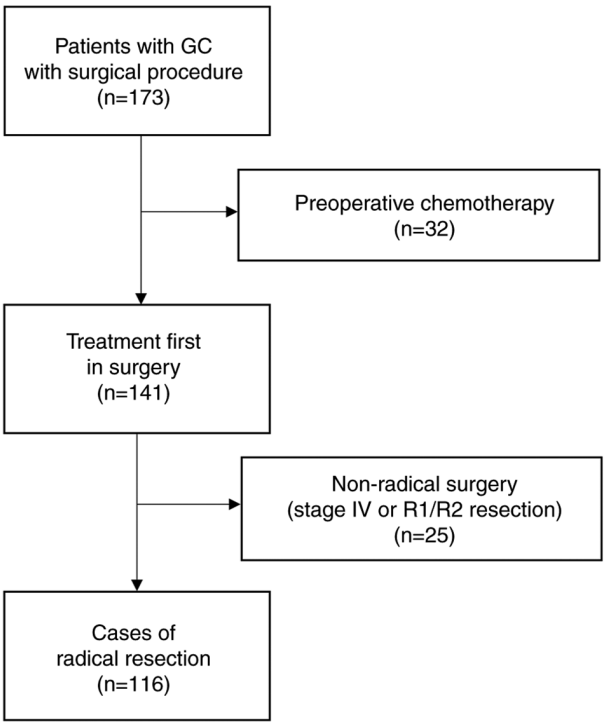

Figure 1. Patient flowchart. The initial serum and clinical information from 173 patients with gastric cancer were used. Preoperative treatment was performed in 32 cases, and histopathological analysis was performed in 141 cases after excluding these cases. Prognostic analysis was performed on 116 curatively treated cases, excluding stage IV and nonoperatively treated cases that did not undergo R0 resection (n=25). GC, gastric cancer.

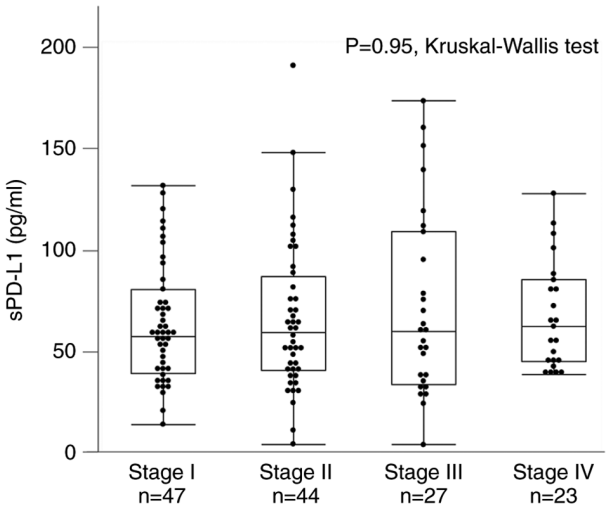

Figure 2. sPD-L1 levels and pathological stage in gastric cancer. sPD-L1 levels of 141 preoperatively untreated patients were analyzed according to the pathological stage. sPD-L1 levels did not significantly differ among the groups of patients based on pathological stage. P=0.95, Kruskal-Wallis test. sPD-L1, soluble programmed death-ligand 1.

the pStage I, II, III, and IV was 47, 44, 27, and 23, with sPD-L1 values of 57.1 [39.0-80.6] pg/ml, 59.2 [40.4-87.0] pg/ml, 59.6 [33.4-109.2] pg/ml, and 62.1 [44.7-85.3] pg/ml, respectively. There were no differences in sPD-L1 levels at each stage (P=0.95, Kruskal-Wallis test) (Fig. 2).

Histological features, such as tumor depth (T1: 56.7 [38.9-80.6] pg/ml, T2: 59.2 [37.2-71.6] pg/ml, T3: 61.7 [41.1-108.5] pg/ml, T4: 61.1 [38.0-88.8] pg/ml, P=0.70, Kruskal-Wallis test), mode of invasion (infα: 68.6 [37.5-120.6] pg/ml, infβ: 60.7 [38.6-104.7] pg/ml, infγ: 50.5 [40.4-75.6] pg/ml, P=0.47, Kruskal-Wallis test),

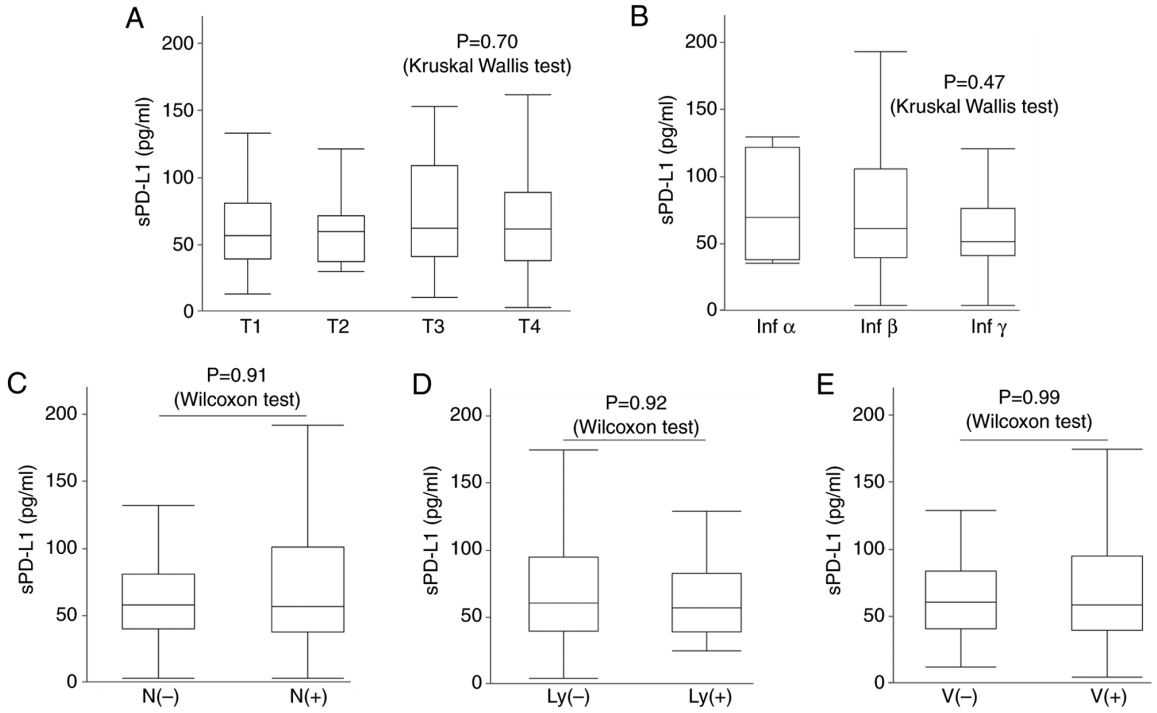

Figure 3. sPD-L1 and histological findings. sPD-L1 levels of a total of 141 preoperatively untreated patients divided into groups based on pathological findings were analyzed. (A) Tumor depth. (B) Mode of invasion. (C) Lymph node metastasis. (D) Lymph vessel invasion. (E) Vessel invasion. Inf, infiltrative growth pattern; Ly, lymph vessel invasion; N, lymph node metastasis; sPD-L1, soluble programmed death-ligand 1; V, vessel invasion.

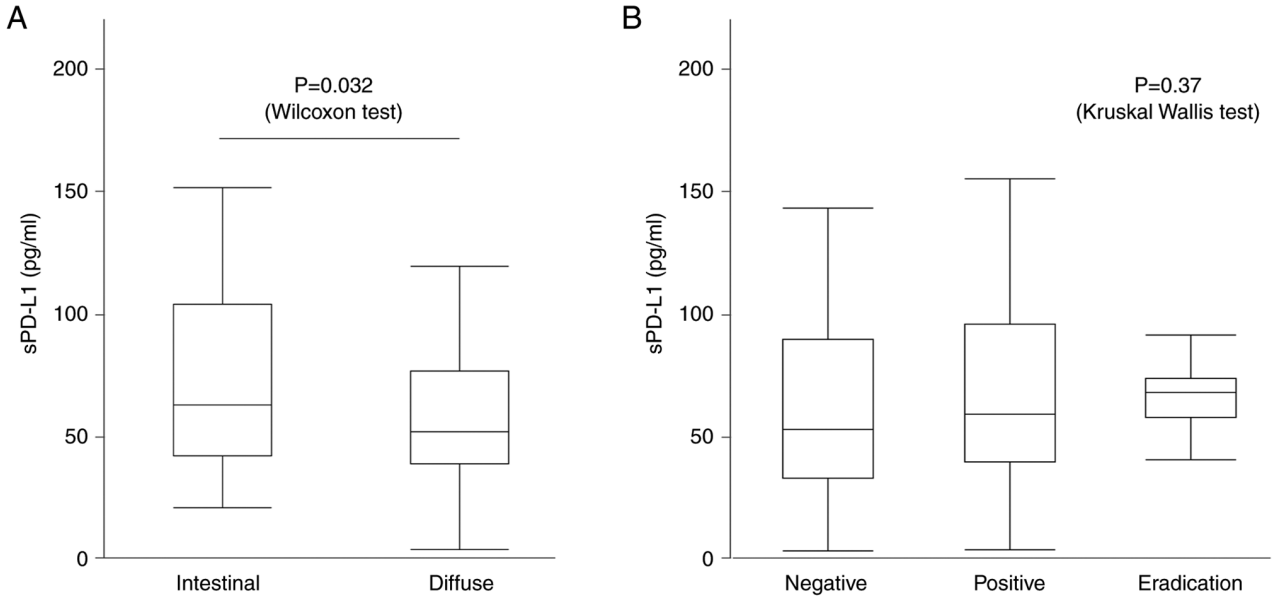

Figure 4. sPD-L1 and histological and clinical factors related to *Helicobacter pylori*. (A) Histological classification. (B) *Helicobacter pylori* infection and history of eradication. sPD-L1, soluble programmed death-ligand 1.

lymph node metastasis (N(-): 58.0 [40.4-81.4] pg/ml, N(+): 57.1 [38.0-101.1] pg/ml, P=0.91, Wilcoxon test), lymph vessel invasion (ly(-): 59.6 [38.9-94.0] pg/ml, ly(+): 56.3 [38.4-82.0] pg/ml, P=0.92, Wilcoxon test), and vessel invasion (v(-): 59.6 [39.4-82.6] pg/ml, v(+): 57.4 [38.0-94.0] pg/ml, P=0.99, Wilcoxon test) did not significantly differ (Fig. 3).

The intestinal type had high sPD-L1 levels compared with that of the diffuse type (68.3 [41.5-104.1] pg/ml vs. 56.4 [38.2-76.6] pg/ml, P=0.032, Wilcoxon test) (Fig. 4A). However, for H. pylori infection, the median sPD-L1 was 51.8 [37.4-95.3] pg/ml for H. pylori negative, 57.7 [38.9-80.7] pg/ml for positive, and 66.4 [59.3-75.0] pg/ml for those with history of eradication, which were not significant (P=0.37, Kruskal-Wallis test) (Fig. 4B).

sPD-L1 and patient characteristics. Patient background, laboratory values, and survival for groups with high and low median sPD-L1 values are listed in Table II.

Table II. Patient background, laboratory values and survival in the high and low sPD-L1 groups.

Variables	sPD-L1 high (n=60)	sPD-L1 low (n=56)	P-value
Median age, years (min-max)	72.5 (47.4-93.0)	68.0 (46.0-83.7)	0.0289[a]
Sex, n			0.0678[b]
Male	43	31	
Female	17	25	
Median BMI (min-max)	22.6 (15.5-33.1)	22.8 (18.7-30.2)	0.4370[a]
Median WBC, /μl (min-max)	6,200 (3,900-13,000)	6,050 (3,300-15,700)	0.8160[a]
Median NLR (min-max)	2.7 (0.8-8.1)	1.8 (0.2-18.6)	0.0018[a]
Median Hb, g/dl (min-max)	12.5 (7.0-16.6)	13.6 (6.9-16.7)	0.0080[a]
Median Plt, x10^3/μl (min-max)	228 (45-419)	226 (13-515)	0.8186[a]
Median T-Cho, mg/dl (min-max)	187 (107-325)	210 (140-308)	0.0016[a]
Median Alb, g/dl (min-max)	4.1 (2.0-4.9)	4.3 (3.5-5.2)	0.0027[a]
Median CRP, mg/dl (min-max)	0.1 (0.0-13.4)	0.1 (0.0-5.0)	0.0307[a]
Median CEA, ng/ml (min-max)	2.7 (0.5-14.2)	2.4 (0.6-8.5)	0.3340[a]
Median CA19-9, U/ml (min-max)	12.2 (0.1-232.9)	12.2 (0.1-228.9)	0.6495[a]
H. pylori, n			0.3924[b]
Negative	19/	25	
Positive	30	28	
Procedure, n			0.2131[b]
TG	19	12	
No TG	41	44	
Histology, n			0.3359[b]
Intestinal	26	19	
Diffuse	32	34	
pStage, n			0.9929[b]
I	24	23	
II	23	21	
III	13	12	
5-year OS, %	69.6	81.5	
5-year RFS, %	58.7	78.4	

[a]Wilcoxon test. [b]χ^2 test. BMI, body mass index; WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; Hb, hemoglobin; Plt, platelet count; T-cho, total cholesterol; Alb, albumin; CRP, C-reactive protein; sPD-L1, soluble programmed death-ligand 1; *H. pylori*, *Helicobacter pylori*; TG, total gastrectomy; pStage, pathological stage; OS, overall survival; RFS, relapse-free survival.

No consistent trends in sex, BMI, H. pylori infection, procedure, or pStage were observed (P=0.0678, 0.4370, 0.3924, 0.2131, and 0.9929, respectively). However, the group with higher sPD-L1 included patients with advanced age (72.5 vs. 68, P=0.0289), higher NLR (2.74 vs. 1.95, P=0.0015), lower Hb (12.5 g/dl vs. 13.6 g/dl, P=0.012), lower Alb (4.0 g/dl vs. 4.3 g/dl, P=0.0006), lower total cholesterol (T-Cho) (186 mg/dl vs. 206 mg/dl, P=0.0005), and higher CRP (0.2 mg/dl vs. 0.1 mg/dl, P=0.008). Among the tumor markers, carcinoembryonic antigen levels tended to be higher (2.85 ng/ml vs. 2.25 ng/ml, P=0.037), whereas that of cancer antigen CA19-9 did not differ.

sPD-L1 can predict the survival of GC patients. The Kaplan-Meier curve and log-rank test were used to evaluate the overall and recurrence-free survival in high and low sPD-L1 groups (Fig. 5). The five-year overall survival rate was lower in the high sPD-L1 group (69.6%) than that in the low sPD-L1 group (81.5%); however, the difference was not statistically significant (P=0.13, log-rank test). The five-year relapse-free survival rate in the high sPD-L1 group was 58.7%, which was significantly lower than the 78.4% in the low sPD-L1 group (P=0.025, log-rank test).

In univariate analysis, older age (≥70), high CA19-9 (>35.4 U/ml), high sPD-L1 (≥57 pg/ml), diffuse type, pStage II/III were poor prognostic factors for poor relapse-free survival (P=0.0311, 0.0095, 0.0211, 0.0496, 0.0010, respectively), and multivariate Cox regression analyses revealed that high sPD-L1 was an independent prognostic factor for poor relapse-free survival after radical surgery (HR, 3.54; 95% CI 1.54-8.15, P=0.0029), independent of pStage II/III (HR 44.31, 95% CI, 5.60-350.50, P=0.0003), and high CA19-9 (HR 7.13, 95% CI 2.72-18.65, P<0.0001). Older age was associated with high sPD-L1 and was a significant poor prognostic factor in

Table III. Univariate and multivariate Cox regression analyses of relapse-free survival after radical surgery.

	Univariate			Multivariate		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Age, years						
≥70	2.22	1.08-4.58	0.0311	1.72	0.79-3.75	0.1751
<70	Ref.					
Sex						
Female	1.21	0.61-2.41	0.5916	2.21	0.97-5.03	0.0582
Male	Ref.					
BMI						
<22	1.98	0.95-4.10	0.0673	-	-	-
≥22	Ref.					
CEA, ng/ml						
>4.8	0.79	0.60-1.92	0.6002	-	-	-
≤4.8	Ref.					
CA19-9, U/ml						
>35.4	2.63	1.27-5.47	0.0095	7.13	2.72-18.65	<0.0001
≤35.4	Ref.					
NLR						
>1.9	2.06	0.92-4.60	0.0795	-	-	-
≤1.9	Ref.					
sPD-L1, pg/ml						
≥57	2.35	1.14-4.84	0.0211	3.54	1.54-8.15	0.0029
<57	Ref.					
Procedure						
TG	1.30	0.63-2.68	0.4777	-	-	-
DG, PG, other	Ref.					
Histology						
Diffuse type	2.22	1.00-4.92	0.0496	1.97	0.83-4.69	0.1261
Intestinal type	Ref.					
pStage						
II/III	28.57	3.90-209.28	0.0010	44.31	5.60-350.50	0.0003
I	Ref.					

Multivariate Cox regression analysis included CA19-9, sPD-L1, histological type and pStage, which were significant in univariate analysis, in addition to age and sex. HR, hazard ratio; BMI, body mass index; NLR, neutrophil-to-lymphocyte ratio; sPD-L1, soluble programmed death-ligand 1; DG, distal gastrectomy; TG, total gastrectomy; PG, proximal gastrectomy; pStage, pathological stage.

univariate analysis for relapse free survival, but not in multivariate analysis. (Table III).

sPD-L1 reflects cachexia status in GC patients. Our findings suggested that the sPD-L1 levels were 52.0 [38.0-68.3] pg/ml for zero items (n=83), 59.1 [38.5-97.0] pg/ml for one item (n=42), 75.7 [55.6-121.4] pg/ml for two items (n=17), 104.3 [84.4-126.0] pg/ml for three items (n=4), and 124.8 [101.1-148.6] pg/ml for four items (n=2). sPD-L1 values also tended to increase with the increasing number of matched items, with significantly higher results for two, three, and four items compared with that of zero items (two items vs. zero item, P=0.0025; three and four items vs. zero item, P=0.0053; Steel-Dwass test) (Fig. 6A). Thus, sPD-L1 likely reflects elements of cachexia in GC patients.

On the other hand, with respect to the association between the number of cachexia items and tumor progression, the percentage of patients with 0 items tends to decrease in pStage I, II, III, and IV to 65.8, 58.3, 53.9, and 50.0%, respectively, but there is no statistically significant difference in the distribution (P=0.793, Chi-square test) (Fig. 6B).

Discussion

In this study, we observed that sPD-L1 was higher in patients with a higher number of factors, such as inflammation, anemia, malnutrition, and cachexia-related weight loss, and the effect on relapse-free survival in radical resection cases of GC was clarified. Additionally, sPD-L1 was an independent prognostic

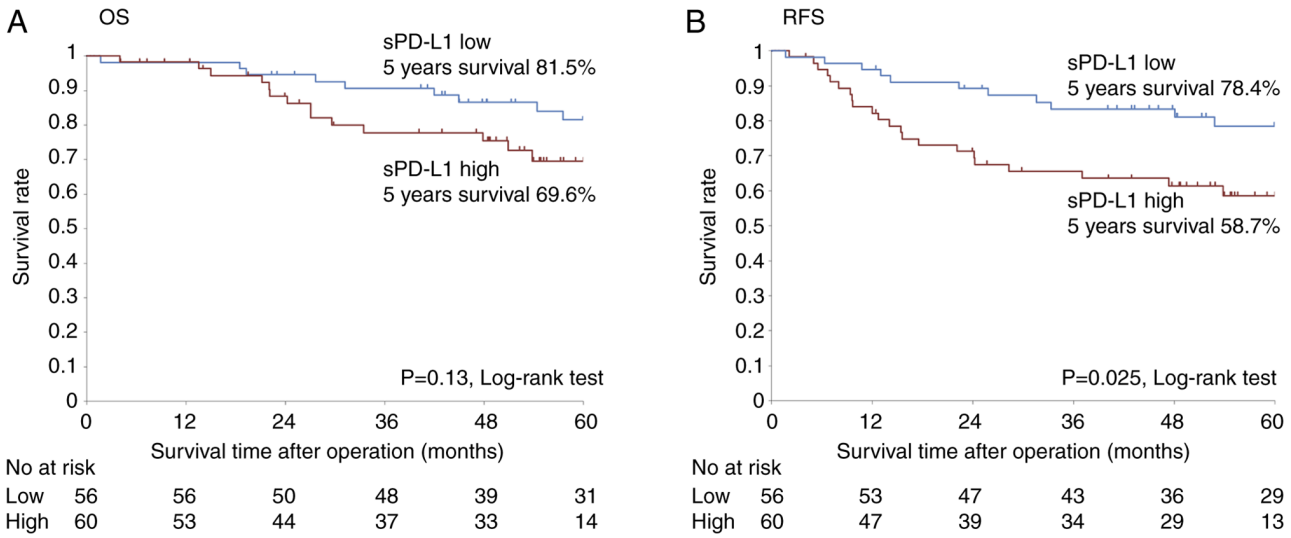

Figure 5. sPD-L1 levels and survival curve. Patients undergoing radical gastric cancer surgery (n=116) were divided into two groups (high and low) according to the median sPD-L1 levels, and (A) OS and (B) RFS were compared. OS, overall survival; RFS, relapse-free survival; sPD-L1, soluble programmed death-ligand 1.

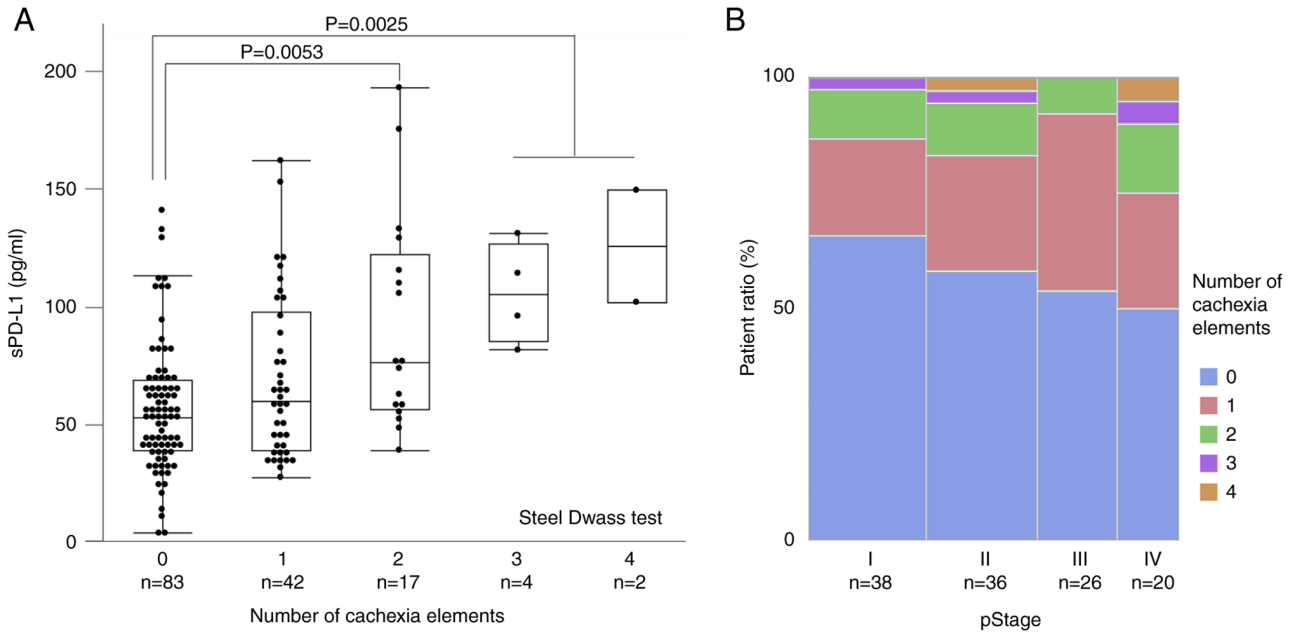

Figure 6. Association between the number of cachexia elements and sPD-L1 level or pStage. (A) sPD-L1 levels and the number of cachexia elements. The relationship between the sPD-L1 values and the number of cancer cachexia elements satisfying the following criteria: (1) low body weight (body mass index <18.5), (2) anemia (Hb <12 g/dl), (3) malnutrition (Alb <3.2 g/dl) and (4) chronic inflammation (CRP >0.5 mg/dl). sPD-L1 values tended to increase with the increasing number of elements, with significantly higher results for two, three and four items compared with that of zero items (two items vs. zero item, P=0.0025; three and four items vs. zero item, P=0.0053; Steel-Dwass test). (B) Number of cachexia elements and pStage in gastric cancer. The number of cachexia elements according to pStage was analyzed for 120 patients for whom complete medical information was available and who were excluded from preoperative treatment or who did not undergo radical resection. pStage, pathological stage; sPD-L1, soluble programmed death-ligand 1.

factor for postoperative recurrence-free survival in patients with GC, independent of tumor markers CA19-9 and pStage. Although the possibility that age, which is considered a prognostic factor for gastric cancer in multiple literatures (18-20), may be a confounding factor for sPD-L1 being a prognostic factor could be considered, older age was not significant in the multivariate analysis of this study.

PD-L1 is permanently expressed in normal peripheral tissues, antigen-presenting cells, and vascular endothelial cells in various organs, and its expression is upregulated by inflammation. PD-L1 is upregulated by multiple inflammatory cytokines (IL-6, TNF-α, interferon-γ, and others) (2). In GC, IFN-γ treatment reportedly increases the expression of intracellular and membrane-bound PD-L1 *in vitro* (21). During an immune response, most immunocompetent cells, including activated lymphocytes, express PD-L1, which binds to PD-1 on T cells and inhibits T cell function, induces immune tolerance, suppresses excessive immune responses, and serves as a

negative feedback mechanism to protect the body from tissue injury. This may lead to immune escape in tumor immunity. Therefore, sPD-L1 is thought to be a marker of immune exhaustion (22-24).

We previously reported that sPD-L1 concentration is proportional to the expression of PD-L1 in tissues (17) and described the efficacy of sPD-L1 in peripheral blood as a valuable biomarker in esophageal squamous cell carcinoma (25). However, another report on GC found no significant correlation (11). Although there are reports of PD-L1 being highly expressed in H. pylori-infected gastric cancer tissues (26), there are no reports showing an association with sPD-L1. sPD-L1 is more highly expressed in the intestinal type than in the diffuse type, and its involvement in chronic inflammation caused by H. pylori infection was suspected, but no significant difference was found between sPD-L1 and H. pylori infection.

Various factors associated with sPD-L1 have been reported (27,28); however, discussion on host factors is limited. Our analysis revealed that sPD-L1 was not significantly different in stage progression but was lower in the diffuse type, which is consistent with previous reports (28). Wei *et al* (29) reported that the value of sPD-L1 did not change before and after surgery, suggesting that host-derived sPD-L1 accounts for some of the value. In our study, high sPD-L1 levels were associated with older age, high NLR, low Hb, low T-Cho, low Alb, and high CRP levels, which may be related to nutritional and inflammatory indicators and is consistent with a PD-L1 activation mechanism. Cachexia is often associated with chronic inflammatory diseases, various cancers such as gastrointestinal and lung cancer, and chronic infections, and inflammatory cytokines have been shown to play a major role in these diseases (2). Therefore, it is logical that sPD-L1 is associated with cachexia.

However, this study had the following limitations: We focused mainly on relapse-free survival and did not examine the regimen and duration of postoperative adjuvant therapy and the effect of post-relapse therapy, especially ICI. Many patients treated before ICI were covered by public insurance; we would like to analyze the relationship between sPD-L1 and ICI efficacy in the future. Various researchers have reported the usefulness of sPD-L1 or circulating exosomal PD-L1 biomarkers. The correlation between PD-L1 expression in tissues and sPD-L1 expression in the blood is controversial. Additionally, we did not compare PD-L1 expression between tissues and blood. Tumor proportion and combined positive scores are related to some extent according to previous immunostaining studies; however, such analysis is complicated and requires a pathologist. Therefore, such associations were not performed in this study. The analysis of cachexia was based primarily on changes (loss) in body weight and skeletal muscle mass, whereas low body weight (BMI <18.5) was a surrogate factor in this study.

Recently, ghrelin-like drugs have been launched as therapeutic interventions for cancer cachexia (30); however, multiple laboratory tests are required to confirm their indications. Our findings suggest that sPD-L1 may be a driving force and contribute to the early detection of pre-cachexia. sPD-L1 is associated with indices related to cachexia in patients with GC and may be a predictive marker for recurrence-free survival rate after radical surgery.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YM, MK, HS, KM, TT, RO, KH and YK were involved in the study design and surgical treatment. HH and HMa checked and revised the study design. YM, TSa, TSh, KM, KK, SI and HMo performed the clinical data extraction and analysis. TSa and TSh performed sPD-L1 measurements. Statistical analyses were performed by YM and YN, with validation by HH and HMa. YM and TSa confirm the authenticity of all the raw data. The manuscript was written by YM under the supervision of HH and HMa. All authors revised the manuscript critically for important intellectual content. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Graduate School of Medicine, Chiba University (Chiba, Japan), and written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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