学位論文

Prognostic Significance of Soluble PD-L1 on Cardiovascular Outcomes in Patients with Coronary Artery Disease

（冠動脈疾患患者における可溶性 PD-L1 の心血管転帰の予後的意義）

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Prognostic Significance of Soluble PD-L1 on Cardiovascular Outcomes in Patients with Coronary Artery Disease

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Aims: Programmed cell death-1 (PD-1) and its ligand (PD-L1) regulate T cells, leading to immunotolerance. We previously demonstrated that patients with coronary artery disease (CAD) had increased circulating levels of soluble PD-L1 (sPD-L1). However, the prognostic significance of sPD-L1 on cardiovascular outcomes is unknown. In the present study, we evaluated the association between sPD-L1 and cardiovascular events in patients with CAD.

Methods: We prospectively measured sPD-L1 in patients with CAD admitted to Kumamoto University Hospital between December 2017 and January 2020 and observed their cardiovascular event rate. The primary outcome was a composite of death from non-cardiovascular causes, death from cardiovascular causes, non-fatal myocardial infarction, unstable angina pectoris, revascularization, hospitalization for heart failure, and ischemic stroke.

Results: Finally, 627 patients were enrolled, and 35 patients were lost to follow-up. The median follow-up duration was 522 days. In total, 124 events were recorded. The Kaplan–Meier curve showed that the event rate was higher in the higher sPD-L1 group (median ≥ 136 pg/dL) than in the lower sPD-L1 group (25.0% vs. 16.9%; p=0.028, log-rank test). Univariate Cox proportional hazards analysis showed that high-sensitivity C-reactive protein, an estimated glomerular filtration rate of <60 mL/min/1.73m^2, B-type natriuretic peptide, left ventricular ejection fraction, and sPD-L1 were significantly associated with cardiovascular events. Multivariable Cox proportional hazards analysis of factors that were significant in univariate analysis identified that sPD-L1 was significantly and independently associated with cardiovascular events (hazard ratio: 1.364, 95% confidence interval: 1.018–1.828, p=0.038).

Conclusions: Higher sPD-L1 levels were significantly associated with future cardiovascular events in patients with CAD.

Key words: Soluble programmed cell death ligand-1, Inflammation, Immune checkpoint, Atherosclerotic cardiovascular disease, Prognostic predictor

1. Introduction

Inflammation plays an important role in coronary artery disease (CAD), as high-sensitivity C-reactive protein (hsCRP) has been shown to be a biomarker relating to the severity and prognosis of CAD[1]. The Canakinumab Anti-inflammatory Thrombosis Outcomes Study trial demonstrated that administration of an interleukin-1β inhibitor improved cardiovascular events in patients with

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CAD[2]. There has also been interest in the effect of colchicine on improving cardiovascular outcomes[3]. Therefore, an anti-inflammatory strategy is now highlighted in the management of CAD.

It has been reported that T cells are involved in the development of coronary plaque and its rupture[4]. A recent study showed that immune checkpoint proteins are associated with the pathogenesis of atherosclerosis. These proteins are expressed on the surface of T cells and regulate the T cell response[5]. Some molecules, such as CD28–CD80/86 and CD40–CD40L, work to activate an immune response as proatherogenic immune checkpoint proteins[6, 7]. However, some molecules, such as CD27–CD70 and programmed cell death-1 (PD-1)–programmed cell death ligand-1 (PD-L1), act as anti-atherogenic immune checkpoint proteins[8, 9]. PD-1 binds to PD-L1 and suppresses the proliferation of T cells and the secretion of cytokines, leading to immunotolerance[10]. While immune checkpoint proteins can suppress activation of T cells, disruption of the immune checkpoint pathway can lead to immune imbalances, which is a critical problem known as immune-related adverse events. We have previously reported a possible case of acute myocardial infarction caused by nivolumab, an immune checkpoint inhibitor[11]. When activated by immune checkpoint inhibitors, T cells produce proatherogenic cytokines, such as interferon (IFN)-γ and tumor necrosis factor-α, which can lead to the development and destabilization of coronary plaque. We have previously found that soluble PD-L1 (sPD-L1) levels are significantly higher in patients with CAD than in those without CAD and also significantly higher in patients with acute coronary syndrome (ACS) than in those with stable CAD[12]. We hypothesized that sPD-L1 could be associated with cardiovascular events based on our previous results.

2. Aim

This study aimed to assess the association between serum sPD-L1 levels and cardiovascular outcomes in patients with CAD.

3. Methods

3.1. Study Design and Patients

Consecutive patients who were admitted to Kumamoto University Hospital for the purpose of treatment or the examination of CAD were screened between December 2017 and January 2020. CAD was defined as ACS, a history of angina or myocardial ischemia confirmed by stress testing coupled with $>50\%$ stenosis of a coronary vessel diameter detected by coronary angiography, or a history of myocardial infarction, percutaneous coronary intervention, or coronary artery bypass grafting. ACS was defined as ST-elevation myocardial infarction (STEMI), non-STEMI, or unstable angina pectoris. Myocardial infarction was diagnosed by an increase or decrease in cardiac troponin above the 99th percentile of the upper limit of the normal range, together with evidence of myocardial ischemia and at least one of the following: electrocardiographic changes (novel ST-T changes, left bundle branch block, or pathological Q waves) and angiographically confirmed coronary obstruction or severe coronary stenosis[13]. Unstable angina pectoris was diagnosed by new or accelerating symptoms of myocardial ischemia accompanied by novel ischemic ST-T-wave changes. Patients with active malignant diseases, those on hemodialysis, and those with active systemic inflammatory diseases (autoimmune disease and rheumatoid disease requiring immunosuppressive therapy) were excluded. The study protocol applied the principles of the Declaration of Helsinki and was approved by the Human Ethics Review Committee of Kumamoto University. The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000036618). Written informed consent was obtained from all study participants at the time of admission.

3.2. Follow-Up and Outcomes

We measured serum levels of sPD-L1 in patients with CAD and recorded their cardiovascular events for up to 2 years. The primary outcome was a composite of death from non-cardiovascular causes, death from cardiovascular causes, non-fatal myocardial infarction, unstable angina pectoris, coronary revascularization, hospitalization for heart failure, and ischemic stroke.

3.3. Coronary Heart Disease Risk Models

Framingham risk models were used to evaluate if the addition of the sPD-L1 level to the Framingham risk score (FRS), which predicts the 10-year cardiovascular risk in individuals, could improve risk stratification for the primary outcome. The method used to calculate the FRS has been described in detail in a previous study[14].

3.4. Measurement of Various Biomarkers

Blood samples were obtained on emergency admission in the catheterization laboratory soon after insertion of a sheath and before administration of heparin in patients with ACS. In the elective cases of stable CAD, blood samples were obtained in the

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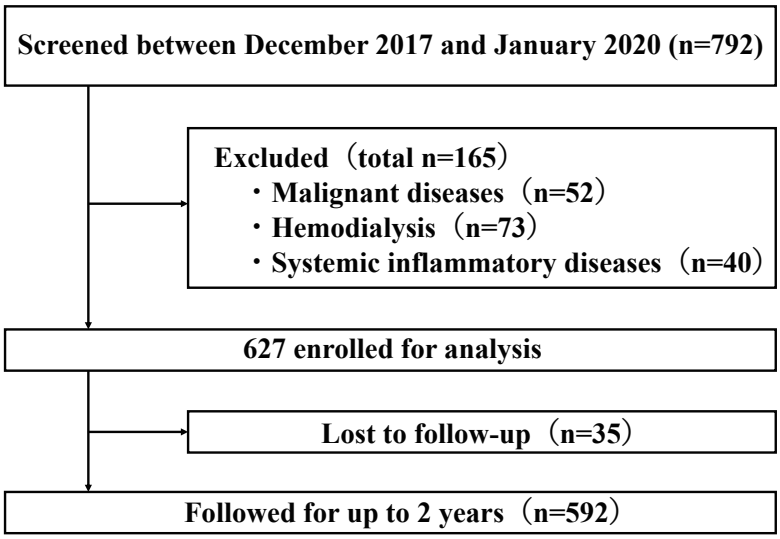

Fig. 1. Study flow chart showing the enrollment process for patients

catheterization laboratory soon after insertion of a sheath and before administration of heparin. Serum samples were kept frozen at −80℃ until measurement. Serum levels of sPD-L1 were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human PD-L1/B7-H1 Duo Set; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The intra- and inter-assay coefficients of variation were 1.5% and 4.2%–7.6%, respectively. The lower limit of detection (LOD) of this ELISA kit was 39.1 pg/mL. Values below the LOD were substituted to half of the LOD[15, 16]. The other laboratory data, including the high-sensitivity cardiac troponin T (hsTnT) and B-type natriuretic peptide (BNP), were measured at our institution soon after obtaining the samples. All biochemical analyses were performed by investigators blinded to the patients' clinical data.

3.5. Statistical Analysis

Data confirmed to be normally distributed by the Shapiro–Wilk test are expressed as the mean ± standard deviation, and those found to be non-normally distributed are shown as the median and interquartile range (IQR). Categorical data are presented as frequency and percentage. Differences between two groups were examined using Fisher's exact test for categorical variables and the unpaired t-test or Mann–Whitney *U* test for continuous variables, as appropriate.

The probability of cardiovascular events during the 2 years after enrollment was estimated using the Kaplan–Meier method. The study population was dichotomized into a lower sPD-L1 group and a higher sPD-L1 group based on a median sPD-L1 cut-off level of 136 pg/mL. The log-rank test was used to compare the distribution of event-free times between the higher sPD-L1 group and the lower sPD-L1 group. Cox proportional hazards models were used to estimate hazard ratios (HRs) for cardiovascular events in univariate and multivariable analyses with forced inclusion modeling including sPD-L1 as a continuous variable. The HRs are presented with their 95% confidence intervals (CIs). The estimates of the C-statistics in the Cox proportional hazards regression models were compared after the addition of the sPD-L1 level to the risk categories of FRS using R version 4.0.2 (R Development Core Team, 2014, http://www.R-project.org). Natural logarithmic scales were used for the Cox analyses and the C-statistics regarding non-normally distributed values such as hsCRP, BNP, hsTnT, and sPD-L1. The risk categories of FRS have been described elsewhere and widely used internationally (low risk, <10%; intermediate risk, 10%–19%; or high risk, ≥ 20%)[17, 18]. The statistical analyses were performed using SPSS version 27 for Macintosh (SPSS Inc., Tokyo, Japan) and R version 4.0.2. A *p*-value of <0.05 was considered statistically significant.

4. Results

4.1. Flow of Patients through the Study

Fig. 1 shows the patient enrollment process. A total of 792 consecutive patients with CAD were screened for eligibility between December 2017 and January 2020. After exclusion of patients with active malignant disease (*n*=52), those on hemodialysis

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(n=73), and those with active systemic inflammatory disease (autoimmune or rheumatoid disease requiring immunosuppressive therapy; n=40), 627 patients with CAD were enrolled for follow-up for up to 2 years. Thirty-five patients were lost to follow-up, leaving 592 patients who were eligible for analysis.

4.2. Baseline Characteristics of Enrolled Patients

The study population was divided into two groups based on the median cut-off sPD-L1 level of 136 pg/mL. The baseline characteristics of the enrolled patients are shown in **Table 1**. The mean patient age was significantly higher in the lower sPD-L1 group than in the higher sPD-L1 group (72.59 ± 9.37 vs. 69.30 ± 11.92 years, p<0.001), as was total cholesterol level (162 [139, 185] vs. 154 mg/dL [131, 185], p=0.030), high-density lipoprotein cholesterol (51 [41, 62] vs. 47 mg/dL [40, 58], p=0.001), and left ventricular ejection fraction (LVEF; 61.7% [53.9, 65.3] vs. 60.5% [53.8, 64.1], p=0.049). The ACS rate was higher in the higher sPD-L1 group than in the lower sPD-L1 group (24.3% vs. 32.4%, p=0.036).

4.3. Cardiovascular Events according to the sPD-L1 Level

The median follow-up duration was 522 days [IQR, 266,730], during which 124 cardiovascular events were observed (death from non-cardiovascular causes, n=15; cardiovascular death, n=9; non-fatal myocardial infarction, n=6; unstable angina pectoris, n=12; coronary revascularization, n=43; hospitalization for heart failure, n=32; and ischemic stroke, n=7; **Table 2**).

The Kaplan–Meier curves for the primary outcome **(Fig. 2)** show that the cardiovascular event rate was significantly higher in the higher sPD-L1 group than in the lower sPD-L1 group (25.0% vs. 16.9%; p=0.028, log-rank test). The Kaplan–Meier curves for each endpoint were compared between the study groups in **Fig. 3A–3G**. There were no significant between-group differences in the rates of death from non-cardiovascular causes (1.4% vs. 3.7%; p=0.087, log-rank test), death from cardiovascular causes (1.7% vs. 1.4%; p=0.612, log-rank test), non-fatal myocardial infarction (1.0% vs. 1.0%; p=0.996, log-rank test), unstable angina pectoris (2.4% vs. 1.7%; p=0.462, log-rank test), coronary revascularization (6.4% vs. 8.1%; p=0.424, log-rank test), and ischemic stroke (1.0% vs. 1.4%; p=0.747, log-rank test). The rate of hospitalization for heart failure was significantly higher in the sPD-L1 group than in the lower sPD-L1 group (7.8% vs. 3.0%; p=0.015, log-rank test).

4.4. Cox Proportional Hazards Analysis of Cardiovascular Events

The results of the univariate and multivariable Cox proportional hazards analyses for cardiovascular events are shown in **Table 3**. Univariate Cox proportional hazards analysis identified log hsCRP (HR: 1.164, 95% CI: 1.041–1.302, p=0.008), estimated glomerular filtration rate <60 mL/min/1.73 m^2 (HR: 1.543, 95% CI: 1.082–2.200, p=0.017), log BNP (HR: 1.462, 95% CI: 1.288–1.660, p<0.001), log hsTnT (HR: 1.152, 95% CI: 1.051–1.263, p=0.003), LVEF (HR: 0.975, 95% CI: 0.961–0.988, p<0.001), and log sPD-L1 (HR: 1.430, 95% CI: 1.085–1.884, p=0.011) to be factors that were significantly associated with cardiovascular events. Multivariable Cox proportional hazards analysis using these five significant factors from the univariate analysis identified log BNP (HR: 1.449, 95% CI: 1.207–1.741, p<0.001) and log sPD-L1 (HR: 1.364, 95% CI: 1.018–1.828, p=0.038) to be significant and independent predictors of cardiovascular events.

Moreover, we used univariate and multivariable analyses to compare the clinical outcomes between sPD-L1 <136 (the lower sPD-L1 group) and sPD-L1 ≥136 (the higher sPD-L1 group) **(Supplementary Table 1)**. Univariate Cox proportional hazards analysis identified that the higher sPD-L1 group (HR: 1.493, 95% CI: 1.042–2.138, p=0.029) and LVEF (HR: 0.975, 95% CI: 0.961–0.988, p<0.001) were significantly associated with cardiovascular events. ACS (HR: 1.327, 95% CI: 0.902–1.952, p=0.15) was not associated with cardiovascular events. Even after adjustment by LVEF and ACS, the higher sPD-L1 group was associated with cardiovascular events (the higher sPD-L1 group, HR: 1.527, 95% CI: 1.055–2.209, p=0.025; LVEF, HR: 0.974, 95% CI: 0.960–0.988, p<0.001, and ACS, HR: 1.271, 95% CI: 0.851–1.899, p=0.241).

4.5. C-statistics for Prediction of Cardiovascular Events in the Cox Proportional Hazards Model

We estimated the C-statistic for FRS alone (0.603, 95% CI: 0.557–0.649). After adding the log sPD-L1 to the FRS, the C-statistic for prediction of cardiovascular events significantly increased from 0.603 to 0.640 (p=0.048, **Table 4**).

4.6. Subgroup Analyses for the Association between sPD-L1 Levels and Cardiovascular Outcomes in Patients with and without ACS

We performed subgroup analyses for the association between sPD-L1 levels and cardiovascular outcomes in patients with and without ACS and also

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Table 1. Baseline characteristics of enrolled patients

	Total $n = 592$	sPD-L1 < 136 pg/mL $n = 296$	sPD-L1 ≥ 136 pg/mL $n = 296$	p-value
Demographics				
Age, years	70.85 ± 11.04	72.59 ± 9.37	69.30 ± 11.92	< 0.001
Male, *n* (%)	422 (71.3)	201 (67.9)	221 (74.7)	0.840
Body mass index, kg/m^2	24.07 ± 4.08	23.75 ± 3.57	24.39 ± 4.54	0.055
Comorbidities				
ACS, *n* (%)	168 (28.4)	72 (24.3)	96 (32.4)	0.036
Hypertension, *n* (%)	458 (77.2)	233 (78.7)	225 (76.0)	0.492
Diabetes mellitus, *n* (%)	293 (49.5)	139 (47.0)	154 (52.0)	0.250
Dyslipidemia, *n* (%)	448 (75.7)	226 (76.4)	222 (75.0)	0.774
COPD, *n* (%)	22 (3.7)	13 (4.1)	9 (3.0)	0.515
Atrial fibrillation, *n* (%)	74 (12.5)	38 (12.8)	36 (12.2)	0.901
Clinical history				
History of heart failure hospitalization, *n* (%)	33 (5.6)	12 (4.1)	21 (7.1)	0.151
History of myocardial infarction, *n* (%)	169 (28.5)	83 (28.0)	86 (29.1)	0.856
History of cerebral infarction, *n* (%)	66 (11.1)	33 (11.1)	33 (11.2)	1.000
Previous PCI, *n* (%)	209 (35.3)	101 (34.1)	108 (36.5)	0.606
Previous CABG, *n* (%)	9 (1.5)	3 (1.0)	6 (2.0)	0.504
Current smoker, *n* (%)	94 (16.0)	45 (15.2)	49 (16.6)	0.736
Family history of heart disease, *n* (%)	127 (21.5)	63 (21.3)	64 (21.6)	0.151
Laboratory results				
Hemoglobin, g/dL	13.3 [11.8, 14.7]	13.2 [11.8, 14.7]	13.4 [11.8, 14.7]	0.827
eGFR, mL/min/1.73m^2	59.0 [47.0, 73.0]	59.0 [48.0, 72.5]	60.0 [47.0, 74.0]	0.896
hsCRP, mg/dL	0.09 [0.04, 0.27]	0.09 [0.04, 0.28]	0.10 [0.04, 0.28]	0.655
Total cholesterol, mg/dL	157 [135, 185]	162 [139, 185]	154 [131, 185]	0.030
LDL-C, mg/dL	85 [69, 110]	86 [71, 108]	85 [66, 112]	0.296
HDL-C, mg/dL	49 [40, 60]	51 [41, 62]	47 [40, 58]	0.001
Triglycerides, mg/dL	110 [77, 148]	113 [81, 153]	103 [72, 147]	0.231
HbA1c, %	6.2 [5.8, 6.9]	6.1 [5.8, 6.8]	6.2 [5.7, 6.9]	0.656
BNP, pg/mL	46.5 [20.6, 120.8]	49.2 [21.0, 121.4]	45.1 [18.9, 118.2]	0.464
hsTnT, ng/mL	0.015 [0.009, 0.035]	0.017 [0.010, 0.032]	0.014 [0.010, 0.046]	0.762
LVEF, %	60.9 [53.7, 64.5]	61.7 [53.9, 65.3]	60.5 [53.8, 64.1]	0.049
Medications				
Loop diuretics, *n* (%)	80 (13.5)	38 (12.8)	42 (14.2)	0.718
Mineralocorticoid receptor antagonist, *n* (%)	46 (7.8)	19 (6.4)	27 (9.1)	0.282
β-blockers, *n* (%)	251(42.4)	123 (41.6)	128 (43.2)	0.739
Calcium channel blockers, *n* (%)	341 (57.6)	181 (61.1)	160 (54.1)	0.079
ACE-Is or ARBs, *n* (%)	323 (54.6)	161 (54.4)	162 (54.7)	1.000
Statins, *n* (%)	419 (70.8)	218 (73.6)	201 (67.9)	0.121
Anticoagulants, *n* (%)	77 (13.0)	40 (13.5)	37 (12.5)	0.807
Aspirin, *n* (%)	397 (67.1)	194 (65.5)	203 (68.6)	0.481
P2Y12 inhibitors, *n* (%)	168 (28.4)	73 (24.7)	95 (32.1)	0.055

Data are shown as the number (percentage), mean ± standard deviation, or median [interquartile range]. ACE-I, angiotensin-converting enzyme inhibitor; ACS, acute coronary syndrome; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; DOAC, direct oral anticoagulants; eGFR, estimated glomerular fraction rate; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity troponin T; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; sPD-L1, soluble programmed cell death ligand-1

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Table 2. Incidence of events according to sPD-L1 category

	Total $n=592$	sPD-L1 <136 pg/mL $n=296$	sPD-L1 ≥ 136 pg/mL $n=296$	p-value
All events	124 (20.9)	50 (16.9)	74 (25.0)	0.020
Death from non-cardiovascular causes	15 (2.5)	4 (1.4)	11 (3.7)	0.114
Death from cardiovascular causes	9 (1.5)	5 (1.7)	4 (1.4)	1.000
Non-fatal myocardial infarction	6 (1.0)	3 (1.0)	3 (1.0)	1.000
Unstable angina pectoris	12 (2.0)	7 (2.4)	5 (1.7)	0.772
Coronary revascularization	43 (7.3)	19 (6.4)	24 (8.1)	0.527
Hospitalization for heart failure	32 (5.4)	9 (3.0)	23 (7.8)	0.017
Ischemic stroke	7 (1.2)	3 (1.0)	4 (1.4)	1.000

Data are shown as the number (percentage). sPD-L1, soluble programmed cell death ligand-1

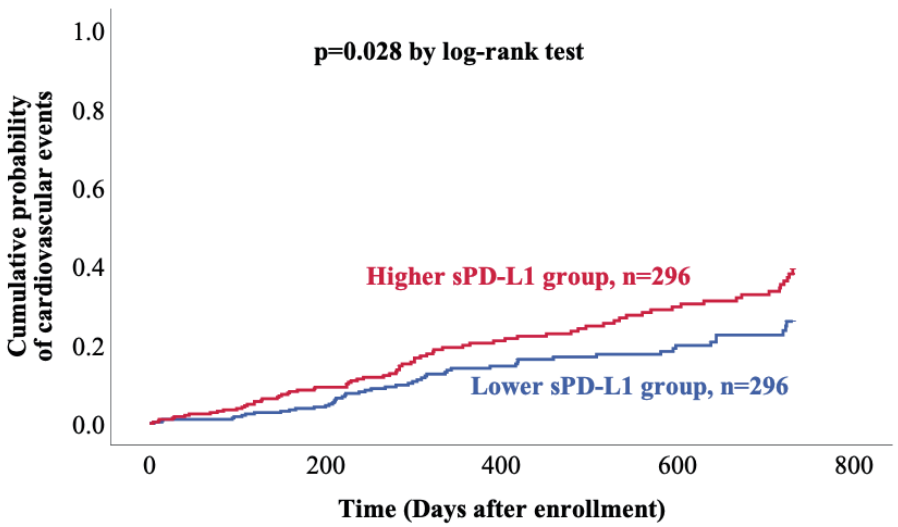

Fig. 2. Kaplan–Meier curve for the probability of cardiovascular events in the higher sPD-L1 group and the lower sPD-L1 group
Abbreviation: sPD-L1, soluble programmed cell death ligand-1.

evaluated the interaction between ACS and non-ACS for cardiovascular outcomes. The Kaplan–Meier curve showed that there was no significant difference in cardiovascular outcomes between the higher sPD-L1 group and the lower sPD-L1 group in patients with ACS (20.8% vs. 22.9%; $p=0.784$, log-rank test) **(Supplementary Fig. 1)**, whereas the cardiovascular event rate was significantly higher in the higher sPD-L1 group than in the lower sPD-L1 group in patients without ACS (15.6% vs. 26.0%; $p=0.021$, log-rank test) **(Supplementary Fig. 2)**. However, relative risk according to sPD-L1 between patients with and without ACS was similar in the interaction analysis (P for interaction =0.828) **(Supplementary Fig. 3)**.

5. Discussion

To the best of our knowledge, this is the first study to demonstrate the prognostic significance of serum levels of sPD-L1 on cardiovascular outcomes in patients with CAD. The cardiovascular event rate was significantly higher in patients with a higher sPD-L1 level than in those with a lower sPD-L1 level. A multivariable Cox proportional hazards analysis confirmed that the sPD-L1 level was significantly and independently associated with cardiovascular events. Moreover, we demonstrated that addition of the sPD-L1 level to the FRS improved estimation of future cardiovascular risk using the C-statistics.

We have previously found that sPD-L1 levels are significantly higher in patients with CAD than in those without CAD and also significantly higher in patients with ACS than in those with stable CAD[12]. Another study reported that upregulation of PD-1 and increased sPD-L1 levels were associated with the severity of diabetic atherosclerotic macrovascular diseases[19]. Therefore, the sPD-L1 level can reflect the severity of atherosclerotic disease.

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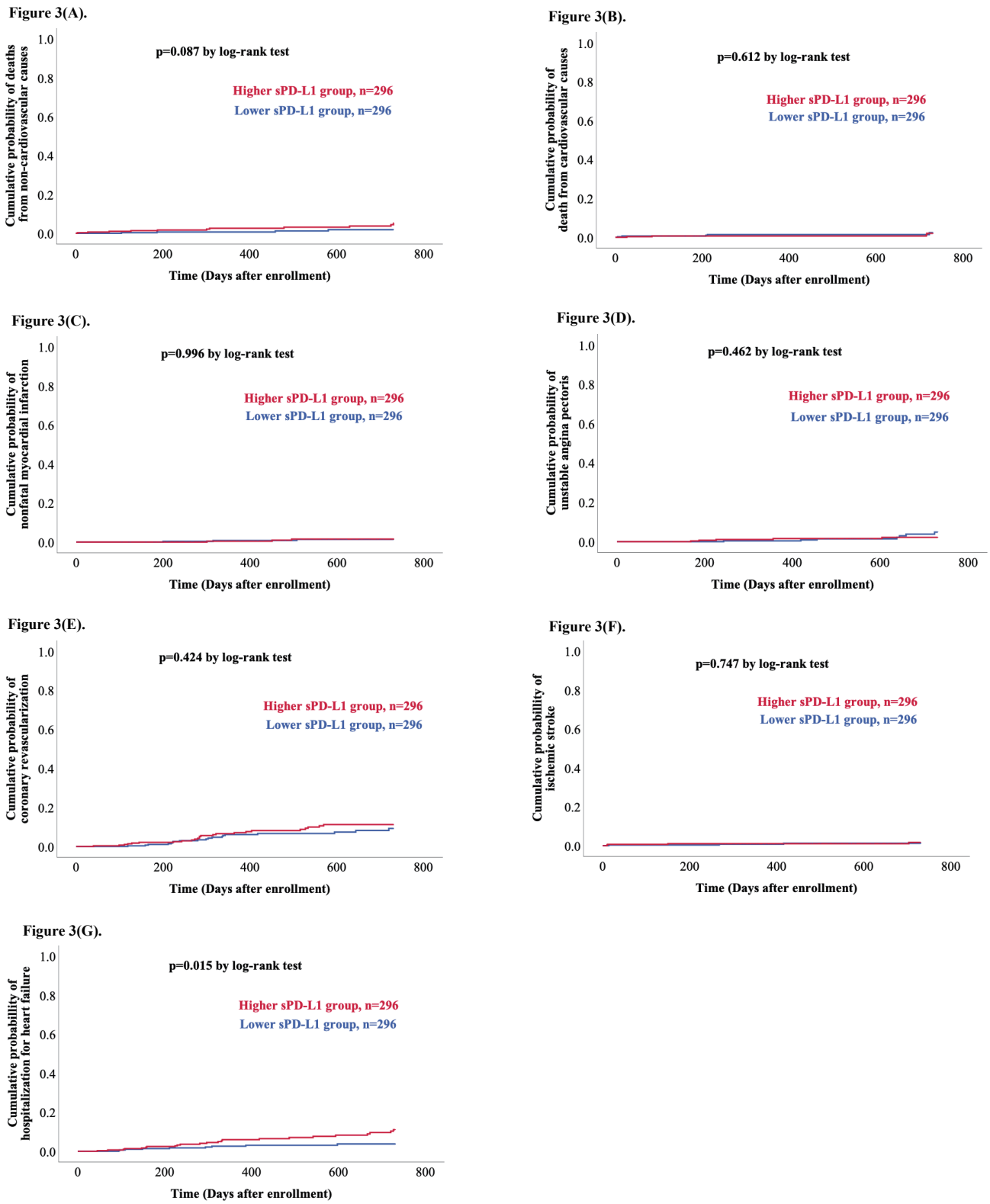

Fig. 3. Kaplan–Meier curve for the probability of each individual endpoint

Kaplan–Meier curve for the probability of (A) death from non-cardiovascular causes, (B) death from cardiovascular causes, (C) non-fatal myocardial infarction, (D) unstable angina pectoris, (E) coronary revascularization, (F) hospitalization for heart failure, and (G) ischemic stroke in the higher sPD-L1 group and the lower sPD-L1 group.

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Table 3. Cox proportional hazards analysis of future cardiovascular events in patients with CAD

Variable	Univariate		Multivariable	
	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Age (per year)	1.013 (0.996–1.030)	0.127	–	–
BMI (per kg/m^2)	0.988 (0.945–1.033)	0.608	–	–
Male (yes)	0.861 (0.576–1.287)	0.465	–	–
Current smoker (yes)	0.829 (0.497–1.382)	0.471	–	–
ACS (yes)	1.327 (0.902–1.952)	0.150	–	–
Hypertension (yes)	0.864 (0.578–1.292)	0.476	–	–
Diabetes mellitus (yes)	1.171 (0.823–1.666)	0.381	–	–
Dyslipidemia (yes)	0.725 (0.492–1.069)	0.104	–	–
Atrial fibrillation (yes)	1.432 (0.910–2.253)	0.121	–	–
History of myocardial infarction (yes)	1.317 (0.908–1.910)	0.147	–	–
History of cerebral infarction (yes)	1.402 (0.850–2.311)	0.185	–	–
Previous PCI (yes)	0.893 (0.616–1.296)	0.552	–	–
β-blockers (yes)	1.094 (0.769–1.557)	0.618	–	–
ACE-Is/ARBs (yes)	1.223 (0.861–1.738)	0.261	–	–
Calcium blockers (yes)	1.034 (0.723–1.477)	0.856	–	–
Aspirin (yes)	1.134 (0.886–1.453)	0.317	–	–
P2Y12 inhibitors (yes)	0.836 (0.557–1.256)	0.388	–	–
Anticoagulants (yes)	1.216 (0.767–1.930)	0.406	–	–
HbA1c (per %)	1.009 (0.933–1.092)	0.815	–	–
log hsCRP (per 0.01)	1.164 (1.041-1.302)	0.008	1.059 (0.919-1.221)	0.429
Total cholesterol (per mg/dL)	1.000 (0.995–1.004)	0.903	–	–
LDL cholesterol (per mg/dL)	1.000 (0.955–1.006)	0.878	–	–
HDL cholesterol (per mg/dL)	0.993 (0.981–1.005)	0.247	–	–
Triglycerides (per mg/dL)	1.001 (0.99–1.003)	0.496	–	–
eGFR $<$60 mL/min/1.73 m^2 (yes)	1.543 (1.082–2.200)	0.017	1.143 (0.753-1.733)	0.531
log BNP (per 0.1)	1.462 (1.288-1.660)	$<$0.001	1.449 (1.207-1.741)	$<$0.001
log hsTnT (per 0.01)	1.152 (1.051-1.263)	0.003	1.001 (0.885-1.134)	0.981
LVEF (per %)	0.975 (0.961–0.988)	$<$0.001	0.995 (0.978-1.013)	0.581
log sPD-L1 (per 0.01)	1.430 (1.085-1.884)	0.011	1.364 (1.018-1.828)	0.038

ACS, acute coronary syndrome; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CI, confidence interval; COPD, chronic obstructive pulmonary disease; DOAC, direct oral anticoagulants; eGFR, estimated glomerular fraction rate; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity troponin T; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; sPD-L1, soluble programmed cell death ligand-1

Table 4. C-statistics for the Cox proportional hazards model in the prediction of cardiovascular events

	Harrell's C-statistic		
	Value	95% CI	*p*-value vs base model
FRS category	0.603	0.557–0.649	
FRS category + log sPD-L1	0.640	0.589–0.691	0.048

CI, confidence interval; FRS, Framingham risk score; sPD-L1, soluble programmed cell death ligand-1

Similar trends have been shown for several other acute and chronic inflammatory diseases. A recent study reported that serum levels of sPD-L1 were significantly higher in patients with systemic lupus erythematosus than in healthy controls, and that an increased serum sPD-L1 level was associated with increased activity and severe clinical manifestations of this disease[20]. Another study found that the sPD-L1 levels were significantly elevated in patients with sepsis and associated with mortality[21]. Overall, the evidence

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suggests that an increased sPD-L1 level is associated with the severity of immune-related diseases, including collagen disease, infection, and atherosclerosis.

It is considered that sPD-L1 originates from the cleaved form of membrane PD-L1 (mPD-L1) on the surface of T cells, endothelium, and myocardium. There is some evidence suggesting that mPD-L1 is cleaved and released in a soluble form by matrix metalloproteinase[22]. An experimental study in cultured human cell lines reported that sPD-L1 was detectable in the supernatant of mPD-L1-positive cells but not in the supernatant of mPD-L1-negative cells, and that the PD-L1 concentrations in the supernatants were correlated with the expression of mPD-L1 [22]. Another experimental study using ischemia reperfusion in a murine model of cryoinjury found increased expression of PD-1 and PD-L1 in damaged cardiomyocytes[23]. Another study in mice reported that IFN-γ increased the expression of PD-L1 on microvascular endothelial cells[24]. A further study reported that mPD-L1 was upregulated on monocytes in patients with type 2 diabetes mellitus, with progression of the severity of atherosclerotic lesions via upregulation of IFN-γ[19]. Therefore, sPD-L1 might reflect injured vascular endothelium and myocardium in patients with CAD and be associated with future cardiovascular events.

It has also been suggested that sPD-L1 blocks the interaction between PD-1 and PD-L1 by binding to the PD-1 receptor and promoting the proliferation of T cells[19]. It is considered that an amount of circulating sPD-L1 disturbs the PD-1/PD-L1 pathway to improve the survival of $CD4^+CD28^-$ T cells. Increased levels of IFN-γ secreted by $CD4^+CD28^-$ T cells have been shown to activate major histocompatibility complex class II molecules, enhancing the chronic inflammatory process in atherosclerosis. IFN-γ potentially also inhibits the production of collagen by fibroblasts and the proliferation of smooth muscle cells. Increased inflammation and decreased synthesis of collagen contribute to the destabilization of atherosclerotic plaques[25]. Therefore, an excessive level of sPD-L1 might inhibit the PD-1/PD-L1 pathway and participate in the chronic immune response and pathological progression of inflammation. Patients with higher sPD-L1 levels might be exposed to chronic inflammation and be at increased risk of cardiovascular events. Therefore, we hypothesized that sPD-L1 could be associated with atherosclerotic events. In the current study, there was a difference in the rate of hospitalization for heart failure between the higher sPD-L1 group and the lower sPD-L1 group, although there were comparable previous histories of hospitalization for heart failure, myocardial infarction, and BNP levels. In the sub-analysis, a higher sPD-L1 level appeared to be more associated with future hospitalization for heart failure than atherosclerotic events. One of the possible reasons is that LVEF was lower in the higher sPD-L1 group than in the lower sPD-L1 group, resulting in an increase in future heart failure-related events. More severe ischemic cardiomyopathy might be included in the population with lower LVEF in the higher sPD-L1 group. We also evaluated the effects of ACS on the result (Supplementary Materials). Our previous study showed that sPD-L1 was higher in the ACS group than in the non-ACS group[12]. However, relative risk according to sPD-L1 between patients with and without ACS was similar in the interaction analysis in this study. We did not have enough data regarding the difference in disease severity, complexity of coronary lesions, and degree of myocardial impairment to prove these associations, especially in ACS. Further investigation is required. A previous study reported that lower LVEF was associated with an increased risk of all-cause mortality and hospitalization for heart failure[26]. The other study demonstrated that lower LVEF was an independent predictor of hospitalization for heart failure in patients with CAD[27]. Therefore, it is possible that LVEF had some effects on heart failure events in this study.

This study has several limitations. First, it was performed at a single center and included a relatively small sample size. Therefore, multicenter clinical studies with larger cohorts are needed to confirm our results. Second, we could not elucidate the relationship between sPD-1 and sPD-L1. We were unable to measure the levels of sPD-1 using ELIZA in any of the study participants, and only sPD-L1 was evaluated. Third, the expression of mPD-1 on the cell surface was not assessed, and the relationship between sPD-L1 and other inflammatory markers, including cytokines, was not elucidated. Therefore, we could not demonstrate the mechanism via which the PD-1/PD-L1 pathway affects the prognosis in patients with CAD. Fourth, although we obtained blood samples on emergency admission in patients with ACS, the sampling timing after onset might be different. The sPD-L1 levels might change according to the time after the onset of ACS. We did not evaluate the changes in sPD-L1 levels over time after onset. These changes in sPD-L1 levels might affect the results. Fifth, over 5% of the study candidates were lost to follow-up. There might be a potential bias that affects the results. Further studies are required to confirm that this inflammatory response is associated with atherosclerosis.

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6. Conclusion

Higher sPD-L1 levels were significantly associated with future cardiovascular events in patients with CAD. This finding suggests that sPD-L1 could be a novel prognostic marker in patients with CAD.

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Conflicts of Interest

KT has received remuneration for lectures from Abbott Medical Co., Ltd., Amgen K.K., AstraZeneca K.K., Bayer Yakuhin, Ltd., Daiichi Sankyo Co., Ltd., Medtronic Japan Co., Ltd., Kowa Pharmaceutical Co., Ltd., Novartis Pharma K.K., Otsuka Pharmaceutical Co., Ltd., Pfizer Japan Inc., and Janssen Pharmaceutical K.K., has received trust research/joint research funds from PPD-Shin Nippon Biomedical Laboratories K.K. and Alexion Pharmaceuticals, Inc., has received scholarship funds from Abbott Medical Co., Ltd., Bayer Yakuhin, Ltd., Boehringer Ingelheim Japan, Daiichi Sankyo Co., Ltd., ITI Co., Ltd., Ono Pharmaceutical Co., Ltd., Otsuka Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd., and belongs to the endowed division sponsored by Abbott Japan Co., Ltd., Boston Scientific Japan K.K., Fides-one, Inc., GM Medical Co., Ltd., ITI Co., Ltd., Kaneka Medix Co., Ltd., Nipro Corporation, Terumo Co., Ltd., Abbott Medical Co., Ltd., Cardinal Health Japan, Fukuda Denshi Co., Ltd., Japan Lifeline Co., Ltd., Medical Appliance Co., Ltd., and Medtronic Japan Co., Ltd. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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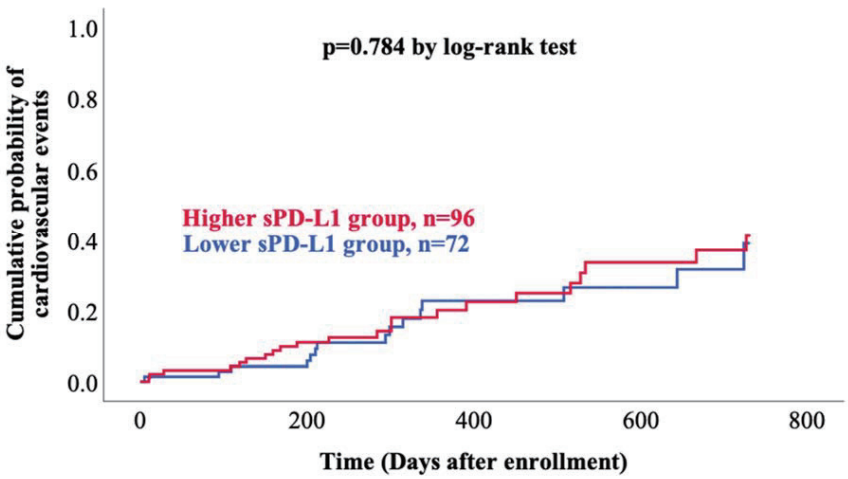
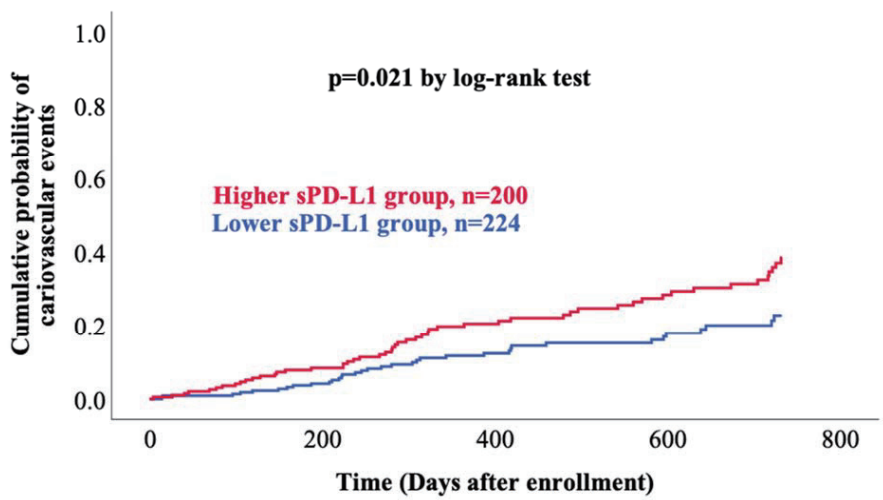
Supplementary Table 1. Cox proportional hazards analysis of future cardiovascular events in between sPD-L1 <136 vs. sPD-L1 ≥ 136

variable	Univariate		Multivariable	
	Hazard Ratio (95%CI)	*P* value	Hazard Ratio (95%CI)	*P* value
sPD-L1 ≥ 136 pg/mL	1.493 (1.042-2.138)	0.029	1.527 (1.055-2.209)	0.025
ACS (yes)	1.327 (0.902-1.952)	0.15	1.271 (0.851-1.899)	0.241
EF (per %)	0.975 (0.961-0.988)	<0.001	0.974 (0.960-0.988)	<0.001

ACS, acute coronary syndrome; CI, confidence interval; LVEF, left ventricular ejection fraction; sPD-L1, soluble programmed cell death ligand-1



Supplementary Fig. 1. Kaplan-Meier curve for the probability of cardiovascular events in patients with ACS



Supplementary Fig. 2. Kaplan-Meier curve for the probability of cardiovascular events in patients without ACS

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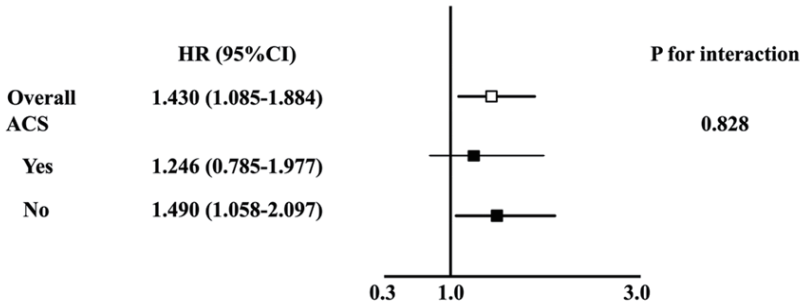

Supplementary Fig. 3. Risk of cardiovascular outcomes according to sPD-L1 with or without acute coronary syndrome

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