Increased soluble programmed cell death-ligand 1 is associated with acute coronary syndrome

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ARTICLE INFO
Keywords:
Soluble programmed cell death-ligand 1
Immune checkpoint pathways
Acute coronary syndrome
Chronic coronary syndrome

ABSTRACT

Background: Programmed cell death (PD)-1 and its ligand (PD-L1) plays crucial roles in T-cell tolerance as immune checkpoint. Previous studies reported that increased serum levels of soluble PD-L1 (sPD-L1) reflect myocardial and vascular inflammation. However, little is known about the clinical relationship between sPD-L1 and acute coronary syndrome (ACS). We investigated the relation of sPD-L1 and ACS.

Methods: We prospectively measured serum levels of sPD-L1 using a commercially available enzyme-linked immunosorbent assay kit in patients with coronary artery disease (CAD) and continuous non-CAD admitted to Kumamoto University Hospital between December 2017 and June 2019. All malignant diseases, patients who underwent hemodialysis, active collagen diseases, and severe infectious diseases were excluded.

Results: Totally, 446 CAD patients [ACS, n = 124; chronic coronary syndrome (CCS), n = 322] and 24 non-CAD patients were analyzed. The levels of sPD-L1 were significantly higher in patients with ACS than those both with non-CAD and CCS {ACS, 188.7 (111.0–260.8) pg/mL, p < 0.001 vs. non-CAD [83.5 (70.8–130.4) pg/mL]; and p = 0.009 vs. CCS [144.2 (94.8–215.5) pg/mL], respectively}. Univariate logistic regression analysis identified that sPD-L1 was significantly associated with ACS [odds ratio (OR): 1.459, 95% confidence interval (CI): 1.198–1.778, p < 0.001]. Multivariable logistic regression analysis with nine significant factors identified from the univariate analysis revealed that sPD-L1 was significantly and independently associated with ACS (OR: 1.561, 95% CI: 1.215–2.006, p < 0.001).

Conclusions: This is the first clinical study to demonstrate the increased level of sPD-L1 in patients with CAD, and the significant association with ACS.

1. Introduction

Various inflammatory cascades contribute to vascular injury and myocardial remodeling in the pathogenesis of acute coronary syndrome (ACS) [1]. Innate and adaptive immunities have been shown to promote plaque development and destabilization [2]. T cells are known to induce atheromatous plaque development through the interactions between immune cells such as macrophages and their products in the arterial walls [3]. Although anti-inflammatory strategy was re-focused after the recent positive results of clinical trials regarding canakinumab and colchicine in patients with coronary artery disease (CAD) [4,5], that has not been established. Hence, the development of novel inflammatory markers is required for the management of ACS.

Recently, immune checkpoint pathways are highlighted as a therapeutic target for various malignant diseases. Programmed cell death (PD)-1 is mainly expressed on activated T cells, and binding of PD-1 and its ligand (PD-L1) suppresses T cell proliferation and secretion of cytokines, leading to immunotolerance. A wide range of malignant cells express PD-L1 and avoid attack by T cells [6]. Moreover, PD-L1 is widely expressed on antigen-presenting cells (APCs), specifically dendritic cells,
macrophages, and B cells, and also on endothelial cells [7,8]. It was reported that the elevated levels of PD-L1 has a positive correlation with the levels of interferon (IFN)-γ in diabetes mellitus (DM) patients complicated with atherosclerotic disease [9]. Additionally, PD-L1 on the myocardial endothelium demonstrated a critical role in the control of immune-mediated cardiac injury in a murine myocarditis model [10]. While PD-L1 expression could reflect vascular and myocardial injuries in ACS patients, the association between the PD-L1 pathway and the pathogenesis of ACS has not been well studied. Recently, soluble form of PD-L1 (sPD-L1) has been reported to reflect the cancer status [11]. An experimental study using various cultured human cell lines reported that the concentrations of PD-L1 in supernatants were correlated with the expression of membrane PD-L1 [12]. Furthermore, a recent study reported that both circulating and tissue PD-L1 were associated with recurrence and prognosis in patients with gastric cancer [13]. Although both PD-1 and PD-L1 expressions on the cell surface are too complex to measure in clinical settings, the measurement of its soluble form released from the cell membrane might be a relatively simple and provides quick information of patients’ statuses. Thus, the present study was conducted to investigate the association between the serum levels of sPD-L1 and ACS.

2. Methods

2.1. Study design and patients

We prospectively enrolled consecutive patients with atherosclerotic coronary artery disease (CAD) who were aged ≥20 years who were admitted to Kumamoto University Hospital between December 2017 and June 2019. Exclusion criteria included active malignant diseases, undergoing hemodialysis, and active systemic inflammatory diseases (autoimmune diseases, rheumatoid diseases required immunosuppression therapies, and severe infectious diseases). During the same study period, we enrolled consecutive non-CAD patients for control.

The study protocol conformed to the principles of the Declaration of Helsinki and was approved by the Human Ethics Review Committee of Kumamoto University. Written informed consent was obtained from all participating patients upon admission. This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000036618). This study was undertaken without patient involvement. Patients were not invited to comment on the study design and were not consulted to interpret the results, contribute to writing, or editing of this manuscript for readability and accuracy.

2.2. Definition of ACS, CCS, and non-CAD

ACS was defined as ST-elevation myocardial infarction (STEMI), non-STEMI, or unstable angina pectoris. Chronic coronary syndrome (CCS) was defined as history of angina or myocardial ischemia by stress tests coupled with coronary stenosis of >50% of the vessel diameter detected by coronary angiography, and/or history of myocardial infarction, percutaneous coronary intervention (PCI), or coronary artery bypass grafting. This study also excluded patients with ACS or CCS patients caused by coronary vasospasm. An expanded method section is available in the Supplementary Material.

2.3. Measurements of various biomarkers

Blood samples were obtained in the catheter-laboratory room soon after sheath insertion and before administration of heparin. Serum samples were kept frozen at −80 °C until measurement. Serum levels of sPD-L1 were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human PD-L1/B7-H1 DuoSet; R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. The intra- and inter-assay coefficient 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. The other laboratory data, including high-sensitive C-reactive protein (hsCRP), high-sensitive cardiac troponin T (hsTnT), and B-type natriuretic peptide (BNP), were measured soon after obtaining samples at our institute. All biochemical analyses were performed by investigators blinded to the clinical data of the patients.

2.4. Statistical analysis

Missing data were excluded from the analyses. Categorical variables are presented as frequencies and percentages. Continuous variables are expressed as mean ± standard deviation for normally distributed variables according to the Shapiro–Wilks test. Continuous variables with a non-normal distribution are expressed as the median value with interquartile range (IQR). ANOVA was used for approximately normally distributed variables in three groups. Kruskal-Wallis was used in three groups. Chi-square test was used for categorical variables. Bonferroni method was used to adjust for comparisons among three groups. Receiver operating characteristic (ROC) analysis was used to evaluate the diagnostic accuracy of sPD-L1 for ACS. In addition, we attempted to validate the risk score by 10-fold cross-validation (internal validation).

3. Results

3.1. Baseline clinical characteristics

A study flowchart is shown in Fig. 1. In total, 612 patients were screened coronary artery disease by coronary angiography between December 2017 and June 2019. Finally, this study enrolled 579 patients with CAD. One hundred and thirty-three patients with CAD met the exclusion criteria: active malignant diseases (n = 3), undergoing hemodialysis (n = 67), and active systemic inflammatory diseases (autoimmune diseases and rheumatoid diseases that required immunosuppressive therapies) (n = 31). During the same study period, 33 continuous non-CAD patients were enrolled. Nine patients with non-CAD met the exclusion criteria: active malignant diseases (n = 3), active systemic inflammatory diseases (n = 6). Finally, 446 patients with CAD (ACS, n = 124; CCS, n = 322) and 24 patients with non-CAD as control were analyzed in this study. Twelve cases showed below levels of LOD in the measurement of sPD-L1. Baseline characteristics of the enrolled patients are shown in Table 1. Age, male, and body mass index were similar among three groups. Compared with patients with CCS, patients with ACS showed a significantly higher rate of current smoking (22.6% vs 13.0%, p = 0.019), and higher levels of low-density lipoprotein cholesterol (LDL-C) (91.0 (75.5–124.5) vs. 82.0 (68.0–105.0) mg/dL, p = 0.001), triglycerides [163.5 (137.5–194.0) vs. 115.0 (77.0–158.0) mg/dL, p = 0.010], hsCRP [0.17 (0.04–0.55) vs. 0.08 (0.04–0.21), p = 0.000], hsTnT [0.0846 (0.0169–0.6005) vs. 0.0125 (0.0081–0.0202), p < 0.001], and BNP [75.3 (31.8–149.2) vs. 38.6 (16.9–91.5), p = 0.001], and showed significantly lower prevalence of hypertension (71% vs. 81.7%, p = 0.005). Compared with non-CAD patients, patients with CCS and ACS showed significantly higher levels of sPD-L1. Furthermore, the levels of sPD-L1 were
significantly higher in patients with ACS than those with CCS (ACS, 188.7 (111.0–260.8) pg/mL, p < 0.001 vs. non-CAD [83.5 (70.8–130.4) pg/mL] and p = 0.009 vs. CCS [144.2 (94.8–215.5) pg/mL], respectively; CCS, p = 0.010 vs. non-CAD, Table 1 and Fig. 2).

3.2. ROC analysis was used to evaluate the diagnostic accuracy of sPD-L1 for ACS

The optimum sPD-L1 cut-off for the diagnosis of ACS was 203.55 pg/
were shown in Supplementary Table 1 and Table 2, respectively. Univariate and by transformation of biochemical covariates to fold cross-validation as internal validation. The risk score also showed good results by the 10-fold cross-validation (AUC: 0.602, 95% CI: 0.543–0.663, p = 0.001, Supplementary Fig. 1). In addition, we attempted to validate the risk score by 10-curve (AUC) was 0.603 (95% CI: 0.543–0.663, p = 0.001). In the present study, we firstly demonstrated that CAD patients showed significantly higher levels of sPD-L1 than non-CAD patients. Among CAD patients, in addition, the patients with ACS showed higher levels of sPD-L1 than those with CCS. Multivariable logistic regression analysis revealed that sPD-L1 was significantly associated with ACS, independent of conventional coronary risk factors and other biomarkers such as hsCRP, BNP, and hsTnT. Moreover, the linear regression analysis showed that sPD-L1 did not have an internal correlation with hsCRP, BNP, and hsTnT, which are globally established inflammatory and cardiac biomarkers in ACS. These results suggest that the severity of ACS reflected by infarct size or ACS-related heart failure was not associated with the levels of sPD-L1.

4. Discussion

In the present study, we firstly demonstrated that CAD patients showed significantly higher levels of sPD-L1 than non-CAD patients. Among CAD patients, in addition, the patients with ACS showed higher levels of sPD-L1 than those with CCS. Multivariable logistic regression analysis revealed that sPD-L1 was significantly associated with ACS, independent of conventional coronary risk factors and other biomarkers such as hsCRP, BNP, and hsTnT. Moreover, the linear regression analysis showed that sPD-L1 did not have an internal correlation with hsCRP, BNP, and hsTnT, which are globally established inflammatory and cardiac biomarkers in ACS. These results suggest that the severity of ACS reflected by infarct size or ACS-related heart failure was not associated with the levels of sPD-L1.
through the secretion of IFN-γ [15]. PD-1 is a CD28 family member expressed on the membrane of T cells, and PD-1 signaling affects T cell expression of other pro-atherogenic factors such as tumor necrosis factor (TNF-α) [16]. PD-L1 has been shown to decrease the production of proinflammatory cytokines, and to attenuate the activation of T cells [8,17]. Furthermore, it is well established that binding of PD-1/PD-L1 mediates a potent co-inhibitory signal to T cells, resulting in T cell exhaustion and dysfunction to prevent excessive immune injury and maintain the self-balance of the immune system [18].

It has been reported that sPD-L1 is associated with advanced cancer, myeloma and lymphoma [11,19,20], with crucial roles in tumor growth and metastasis processes. Matrix metalloproteinases (MMPs) also have vital function in these processes. It has been reported that the membrane-bound form of PD-L1 can be cleaved by protease such as MMPs [12,21]. MMPs also have a critical role in inflammatory processes underlying plaque rupture and erosion, indicating a crucial role in the pathogenesis of ACS. Furthermore, MMPs have become prime suspects for inducing plaque vulnerability because of their biological activity in the degradation of extracellular matrix components, leading to loss of mechanical properties in tissues displaying active MMPs. Yasuda et al. previously demonstrated that the cardiac production of MMPs, measured by their concentration gradients between at the coronary sinus and at aortic root, is increased in the patients with AMI compared with those with stable coronary artery disease [22]. Therefore, we speculated that elevated MMPs could increase the levels of sPD-L1 in ACS compared with CCS. It has also been reported that sPD-L1 is released through proteolytic cleavage of membrane-bound proteins by pro-inflammatory cytokines such as the TNF-α [12,23,24] and that PD-L1 expression on APC and endothelial cells is upregulated through cytokines such as IFN-γ [25]. In this study, however, there was no correlation between sPD-L1 and hsCRP, which is a major inflammatory biomarker. Although the association of sPD-L1 and CRP was reported in advanced pancreatic cancer [7], to the best of our knowledge, there are no reports regarding the relationship between sPD-L1 and CRP in atherosclerosis. It has been revealed that sPD-L1 has several forms, and some of them have bioactivity that can negatively regulate T cell activation through the PD-1/PD-L1 pathway [26]. Therefore, bioactive sPD-L1 may cancel the correlation between CRP and sPD-L1. However, we could not clearly demonstrate the mechanism that the elevation of sPD-L1 evaluation independent from CRP in patients with ACS, because of the absence of serum levels of MMPs and cytokines measurements in this study. Further detailed experimental studies are required to validate our results.

Another possible mechanism of the elevated levels of sPD-L1 in ACS in this study could be the release of sPD-L1 from injured endothelium and myocardium. A previous experimental study using ischemia-reperfusion and cryoinjured murine models reported increased expression of PD-1 and PD-L1 in injured cardiomyocytes, and upregulation of PD-1 and PD-L1 was associated with DNA damage-inducible protein 153 and interleukin-17 [27]. Another experimental study also reported that PD-L1 was upregulated in the heart through IFN-γ produced by infiltrating T cells in a murine myocarditis model [10]. Because ACS also induces acute intense inflammation locally and systemically, we speculated that a similar phenomenon regarding PD-L1 may occur in patients with ACS.

On the other hand, some experimental studies reported a cardioprotective property of PD-L1. PD-L1 was more abundantly expressed in the heart, and studies regarding myocardial infarction reported that the PD-1/PD-L1 pathway has a dominant role in protecting the heart from T cells attack [25,27–29]. The present study could not demonstrate the origin, mechanism, and roles of increased sPD-L1 in ACS, but we believe our results provide novel insights into the pathogenesis of ACS.

5. Limitations

There are some limitations of this study. First, we evaluated only sPD-L1 in this study. Although we attempted to measure the levels of sPD-1 using ELISA, sPD-1 were not detectable in any of the patients. In addition, the expression of sPD-1 and sPD-L1 on the cell surface was not measured. Therefore, it is difficult to reveal the detailed role of the PD-1/PD-L1 pathway in the pathogenesis of ACS. Second, we did not evaluate the changes in sPD-L1 following treatment for ACS. Hence, this study could not provide the therapeutic implications in patients with ACS. Third, this study was the exploratory study to assess sPD-L1 levels in patients with CAD and without CAD. In addition, the small number of non-CAD patients might provide limited information on the levels of sPD-L1 in non-CAD patients. Further study is needed to confirm the usefulness of sPD-L1 for the diagnosis or future risk stratification in a different population. Finally, this was a single center study with a relatively small sample size study, and further multicenter clinical studies with larger cohorts are required to validate our results.

6. Conclusions

Despite these limitations, this is the first study to demonstrate the increased level of sPD-L1 in patients with CAD, and the significant association with ACS.

Source of funding

This study was supported in part by a Grant-in-Aid for Young Scientists (19 K1750 to K.F.) from the Ministry of Education, Science, and Culture, Japan.

Disclosures

KK has received significant research grant support from Bayer, Yakuhin, Ltd., Daiichi Sankyo Co., Ltd., Novartis Pharma AG., and SBI, Pharma Co., Ltd., and has received honoraria from Bayer Yakuhin, Ltd. and Daiichi Sankyo Co., Ltd., outside the submitted work. KT has received significant research grant support from Astra Zeneca Kabushiki Kaisha, Sugi Yohoen Kabushiki Kaisha, and Nihon Iryo Kiki Giken Company, Ltd., and has received honoraria from Kowa Company, Ltd., Sanofi Kabushiki Kaisha, Daiichi Sankyo Company, Ltd., Takeda Pharmaceutical Company, Ltd., Bayer Yakuhin, Ltd., and MSD Kabushiki Kaisha, outside the submitted work. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Author statement:


Clinical Trial Registration: The University Hospital Medical Information Network Clinical Trials Registry (https://upload.umin.ac.jp/cgi-bin/ctrctr_view_reg.cgi?receptno=R000041719) (UMIN000036618).

Acknowledgements

We thank Ms. Megumi Nagahiro, Ms. Saeko Tokunaga, and Ms. Michiyo Saito for their technical assistance with sample preparations, measurement of sPD-L1, and the other laboratory data. We also thank H. Nikki March, PhD, from Edanz Group (https://en-author-services.edanzgroup.com/ac) for editing a draft of this manuscript.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2021.11.060.

References