Serum soluble PD-1 plays a role in predicting infection complications in patients with acute pancreatitis

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Abstract

**Background:** Most of acute pancreatitis (AP) are mild and self-limiting, however, 15%-20% of patients develop severe AP (SAP) or moderately SAP (MSAP) with local or systemic complications. Infection complications (ICs) result in 40-70% morbidity and high mortality rates among SAP and MSAP patients. It’s require that early identification of SAP and MSAP patients at risk of developing ICs. Several studies have indicated that serum soluble programmed cell death protein (sPD-1) or programmed cell death 1 ligand (sPD-L1) levels were higher in patients with severe sepsis than in healthy volunteers, and have a predictive capacity for mortality. However, the role of serum soluble PD-1/PD-L1 in AP remains unclear. This study aimed to investigate whether the ICs of AP patients is associated with their sPD-1 and sPD-L1 levels, which were determined via enzyme-linked immunosorbent assay of peripheral blood samples from 63 MSAP and SAP patients and 30 healthy volunteers.

**Results:** The serum sPD-1 levels in AP patients on days 1, 3 and 10 after onset were significantly increased in a time-dependent manner compared with that in healthy volunteers. Moreover, the AP patients with ICs had significantly higher serum sPD-1 levels than the AP patients without ICs. While serum sPD-L1 levels in AP patients were similar to that in healthy volunteers. Besides, serum levels of sPD-1/sPD-L1 10 were negatively correlated with circulating lymphocytes. Univariate and Multivariate regression analyses showed that the up-regulated serum sPD-1 level was an independent risk factor for ICs in AP. The area under the receiver operating characteristics (ROCs) curve indicated that combination with Acute Physiology and Chronic Health Evaluation II (APACHE II) score and serum sPD-1 level had a high accuracy in predicting ICs in AP patients.

**Conclusion:** Serum sPD-1/sPD-L1 may be involved in the immunosuppressive process in AP. Moreover, the serum sPD-1 level may be an independent risk factor for predicting ICs in AP patients.

**Background**

Acute pancreatitis (AP) is a common acute abdomen in general surgery, and most of AP are mild and self-limiting, without complications and only needing a short hospitalisation [1]. However, 15%-20% of patients develop severe AP (SAP) or moderately SAP (MSAP) with local or systemic complications,
which has high mortality [2, 3]. The main reason for the high mortality among SAP and MSAP patients is the infection complications (ICs), morbidity for which can be approximately 40–70% [4–6]. It’s required that early identification of SAP and MSAP patients at risk of developing ICs.

Studies showed that early immunosuppression of SAP has led to the occurrence of systemic ICs and multiple organ failure [7–8]. Programmed cell death protein (PD-1) is a co-inhibitory molecule belonging to the CD28 family, mainly expressed in activated T lymphocytes, natural killer T cells, and bone marrow cells [9–10]. The programmed cell death 1 ligand (PD-L1) is a ligand for PD-1 expressed on antigen presenting and hematopoietic cells [10]. The PD-1/PD-L1 pathway has been shown to regulate lymphocyte proliferation and apoptosis and play an important role in immune regulation [11–13]. Previous studies had shown that PD-1 and PD-L1 exist in two forms: cell membrane-bound and soluble forms [14]. Soluble PD-1 and PD-L1 (sPD-1/sPD-L1) can be detected in human serum. sPD-1 may promote T cell responses by inhibiting the PD-1/PD-L1 signaling pathways, while excessive sPD-1 may lead to immunosuppression [14]; sPD-L1 was released into blood by the surface of PD-L1-expressing cells that may reflect PD-L1 levels [15]. Additionally, sPD-L1 may retain immunosuppression induction [15]. A recent study revealed that PD-1 expression in peripheral T cells and PD-L1 expression in monocytes increased significantly in sepsis patients than in healthy controls [16], and in AP patients with ICs than the patients without ICs [17]. Several studies have indicated that serum sPD-1/sPD-L1 levels were higher in patients with severe sepsis than in healthy volunteers and had a predictive capacity for mortality [18–19]. However, the relationship between serum sPD-1/sPD-L1 levels and ICs in AP has not been certified. Furthermore, serum sPD-1/sPD-L1 expression is easy to examine and has potential applications.

In this study, we investigated the levels of serum sPD-1/sPD-L1 in SAP and MSAP patients and healthy volunteers to understand the association of these parameters with immune status and ICs in AP patients.

Results

**Characteristics of the patients**

According to the revised Atlanta classification [20], a total of 63 patients with AP (28 SAP and 35
MSAP patients) were included in this study, with an average age of 51.08 ± 13.56 years. For the classification of AP etiology, hypertriglyceridemia-induced pancreatitis was the main cause, accounting for 38.1%, followed by biliary 30.2%, alcoholicity 6.3%, and other factors 25.4%. All AP patients underwent three AP-related scoring system after admission, including the BISAP (assessment after 24-h admission), Ranson (48-h), and APACHE II (48-h) scores. The clinical characteristics of these patients are shown in Table 1.

**Serum sPD-1 and sPD-L1 levels in patients of AP**

Serum levels of sPD-1 and sPD-L1 were measured in patients of AP on day 1 (d1), day 3 (d3) and day 10 (d10) after admission. The serum sPD-1 levels in AP patients on d1, d3 and d10 were significantly elevated compared with that in healthy controls (P < 0.05, P < 0.01, P < 0.01; Figure 1A). Moreover, serum sPD-1 level in AP patients were up-regulated in a time-dependent manner, and were most elevated on day 10 compared to that on day 1 (P < 0.01; Figure 1A). However, serum sPD-L1 levels on d1, d3 and d10 in AP patients were similar to that in healthy controls (Figure 1B).

**Correlation between clinical indicators and serum sPD-1/sPD-L1 levels**

We further investigated the relationship between clinical indicators and serum sPD-1/sPD-L1 levels. We observed that the serum levels of sPD-1 and sPD-L1 on day 10 were both negative correlated with lymphocyte count (r = -0.335, P = 0.015; r = -0.294, P = 0.035; Table2), whereas the serum level of sPD-1 on day 1 was positive correlated with lymphocyte-monocyte ratio (LMR) (r = 0.269, P = 0.034; Table2). Moreover, the serum level of sPD-1 on days 3 and 10 were negative associated with the hematocrit (HCT) (r = -0.289, P = 0.021; r = -0.331, P = 0.016).

**Correlation between serum sPD-1/sPD-L1 levels and ICs of AP**

To investigate the relationship between ICs of AP and serum sPD-1/sPD-L1 levels, all patients were divided into two groups: AP with (n=36) and without (n=27) ICs. We found that APACHE II scores was significantly higher in the AP with ICs group than in the AP without ICs group (P< 0.001; Table 3). Whereas the HCT was significantly higher in the AP without ICs group than in the AP with ICs group (P = 0.003; Table 3). The AP with ICs group had significantly higher serum sPD-1 levels on days 3 and 10 than the AP without ICs group (P < 0.001, P < 0.001; Table 3). However, there were no significant
differences between AP with ICs group and AP without ICs group with regard to serum sPD-L1 levels.

**Serum sPD-1 may be an independent factor for predicting ICs in AP**

To determine the predictive effect of age, APACHE II scores, HCT, and lymphocyte, monocyte, and neutrophil counts on day 1, and serum sPD-1 and sPD-L1 levels on days 1 and 3 for ICs, we performed a logistic regression analysis. Univariate analysis demonstrated that HCT (OR 0.908, 95% CI 0.842–0.980, P = 0.013), APACHE II score (OR 1.420, 95% CI 1.134–1.776, P = 0.002), and serum sPD-1 level on day 3 (OR 1.013, 95% CI 1.005–1.021, P = 0.002) were significantly associated with ICs of AP (Table 4). Furthermore, we performed multivariate analysis to evaluate HCT, APACHE II score, and serum sPD-1 level on day 3 as independent predictors of ICs. The results suggested that serum sPD-1 levels on day 3 (OR 1.009, 95% CI 1.001–1.018, P = 0.029) and the APACHE II score (OR 1.281, 95% CI 1.008–1.629, P = 0.043) were independent risk predictors of ICs in AP (Table 4).

To evaluate the predictive accuracy of serum sPD-1 levels on day 3 and the APACHE II score for ICs in AP patients, the receiver operating characteristics (ROCs) curve analysis was performed. The areas under the curve (AUC) values for serum sPD-1 levels on day 3 and APACHE II score were 0.796 (95% CI 0.681–0.911, P < 0.001) and 0.769 (95% CI 0.649–0.889, P < 0.001; Table 5 and Figure 2). By combining these two variables, a high accuracy for AP IC prediction was achieved (AUC = 0.826, 95% CI 0.721–0.931, P < 0.001; Table 5 and Figure 2).

**Discussion**

MSAP and SAP can develop into immunosuppression, leading to secondary infection and pancreatic necrosis [21,22]. Our study showed that compared with healthy volunteers, serum sPD-1 levels in the MSAP and SAP patients increased continuously during the early course of the disease, especially the patients with ICs. Moreover, elevated sPD-1 level was associated with enhanced occurrence of ICs. Studies showed that serum sPD-1 may promote T cell responses by inhibiting the PD-1/PD-L1 signaling pathway, but continuously excessive level of serum sPD-1 may serve as an antibody to block the PD-1/PD-L1 pathway, which leads to the aberrant activation and proliferation of T cells [14,24]. The uncontrolled immune regulation resulted in hyperimmune behavior in the early stage of SAP, however, with the consumption of lymphocyte, the hyperimmune status transformed into
immunosuppression and increased the incidence of ICs. Finally, a marked increase in sPD-1 levels may represent more severe immune damage in patients [26]. In addition, sPD-L1 may retain immunosuppressive condition and continuously increased of sPD-L1 ultimately aggravates immunosuppression [15,27]. Hence, serum sPD-1/sPD-L1 levels may play an important role in monitoring the immune status of AP patients and predicting the ICs and prognosis. Furthermore, our data indicated that serum sPD-1/sPD-L1 levels of AP patients are associated with LMR, HCT, and lymphocyte counts. Immune dysfunction in AP patients may be caused by decreased peripheral blood lymphocytes [27]. The decreased expression of human leucocyte antigen-DR (HLA-DR) on monocytes may lead to early immunosuppression of AP [28]. Moreover, serum sPD-L1 was reported to be involved in lymphocyte apoptosis [15]. Our investigation of the correlation between serum sPD-1/sPD-L1 and clinical indicators revealed that dynamic monitoring of serum sPD-1/sPD-L1 levels in AP patients may reflect systemic immunologic functions in AP patients.

Previous studies indicated that BISAP, Ranson, and APACHE II scores predict the mortality of AP patients with high accuracy [29-31] and high Ranson, BISAP, and APACHE II scores were also associated with organ failure and complications in AP patients [32,33]. In this study, we showed that the elevated serum sPD-1 level was an independent risk factor for ICs in patients of AP. Combination of APACHE II score and serum sPD-1 level may better predict ICs of AP patients. However, there remain some limitations in this study, we investigated these variates in AP patients from a single center and the number of cases in this study was small. Further study involving a large cohort from multiple centers is needed to confirm these results.

Conclusions
Serum sPD-1/sPD-L1 levels may be involved in the immunosuppressive process of AP, and sPD-1, which increases continuously in the peripheral blood of AP patients, may be an independent risk factor for predicting ICs in AP patients, which is potentially applicable in determining or improving AP patient prognosis.

Methods
Peripheral blood was obtained from 63 patients with MSAP or SAP at Fujian Medical University Union
Hospital, Fuzhou, China, from October 2017 to April 2019. Patient inclusion criteria: (1) patients with MSAP or SAP, according to 2012 edition of the Atlanta Convention AP classification criteria [20]; (2) aged 18 years or older; (3) admitted to the hospital within 48 hours of onset. Exclusion criteria: Patients (1) with mild acute pancreatitis; (2) treated for <10 days; (2) with chronic pancreatitis, pregnancy, breastfeeding, acute and chronic hepatitis, end-stage liver and kidney disease, immunodeficiency disease, and malignant tumor; (3) who had received immunosuppressive therapy. All patients were followed until discharge or hospital mortality. Patient baseline characteristics, Bedside Index for Severity in Acute Pancreatitis (BISAP), Ranson, and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were collected and recorded. Patient characteristics were collected and are shown in Table 1. This study was approved by the Committee for the Ethical Review of Research, Fujian Medical University Union Hospital.

Definition of ICs: infected pancreatic necrosis, bacteremia, pneumonia, infectious ascites or urinary tract infections during admission. The diagnostic criteria for infected pancreatic necrosis were ‘positive for peripancreatic effusion or pancreatic necrosis tissue culture’ obtained at the first pancreatic perivascular drainage or the first surgical treatment. The diagnostic criterion for bacteremia was ‘positive for blood culture’. Diagnostic criteria for pneumonia: (1) newly developed cough, or symptoms of the original respiratory disease, with purulent sputum, with or without chest pain; (2) fever ≥ 38 °C; (3) lung consolidation signs and/or wet rales; (4) white blood cell (WBC) > 10×10^9/L or < 4×10^9/L with or without nuclear left shift; (5) lung imaging suggests patchy infiltrating shadow or interstitial changes with or without pleural effusion. Any of the above 1 to 4 plus the fifth item can lead to a diagnosis, except for tuberculosis, lung cancer, non-infectious pulmonary interstitial disease, pulmonary edema, atelectasis, pulmonary embolism, pulmonary eosinophilic infiltration, and pulmonary vasculitis. The diagnostic criterion for infectious ascites is the positive ascites specimen obtained during the first abdominal puncture drainage or the first surgery. Diagnostic criteria (and confirmation) for urinary tract infections: bacterial colony count ≥ 10^5/mL and white blood cell count > 10/HP following centrifugation of urine collected midstream. Multiple infections in the same patient were considered one endpoint [17].
**Blood samples**

Peripheral blood samples were obtained from 30 healthy volunteers (control) and AP patients on days 1, 3, and 10 after admission. Serum samples were collected immediately after centrifugation at 3000 rpm for 15 min at 4 ºC, and stored at -80 ºC for subsequent analysis.

**Serum sPD-1 and sPD-L1 analysis**

Serum sPD-1/sPD-L1 was quantified using the human sPD-1/sPD-L1 enzyme-linked immunosorbent assay (ELISA) kit (RayBio®, GA, USA). Serum sPD-1/sPD-L1 levels were measured in duplicates and analyzed according to manufacturers' recommendations. A 1:50 dilution was used for all the samples. The nonlinear standard curve was constructed based on polynomial regression (degree = 2).

**Statistical analysis**

SPSS 22.0 software (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis. Results were presented as medians and interquartile ranges (IQR) or means ± standard deviation (SD), and categorical variables were shown as frequency and percentage. The normal distribution of all variables was tested using the Shapiro-Wilk test. Chi-square or Fishers tests for two-category variables. The independent sample t test was used to compare variables that conform to the normal distribution, and the Mann-Whitney U test to compare variables that are not normally distributed. The correlation was assessed by a Spearman rank test. The concentrations at different times (days 1, 3, and 10) in each group were compared using One-way Repeated Measures Analysis of Variance. A two-category univariate logistic regression analysis was performed to assess the correlation between the variables (Tables 4) and AP infectious complications. Then only the significant differences in univariate analysis were using multivariate stepwise regression analysis of variables. The area under the receiver operating characteristics (ROCs) curve (AUC) was used to estimate the accuracy of the predicted model, and the area under the curve was bilaterally P < 0.05. Figures were prepared using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

**Abbreviations**

AP, acute pancreatitis; SAP, severe acute pancreatitis; MSAP, moderately severe acute pancreatitis; sPD-1, serum soluble programmed cell death; sPD-L1, serum soluble programmed cell death protein
ligand 1; IQR, interquartile range; APACHEII, Acute Physiology and Chronic Health Evaluation II; BISAP, The Bedside Index for Severity in Acute Pancreatitis; WBC, white blood cell; HCT, hematocrit; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PLR, platelet-lymphocyte ratio;

Declarations

Ethics approval and consent to participate:

All procedures performed in studies involving human participants were in accordance with the Helsinki declaration. All patients whose blood samples were used in this research provided written informed consent, and the study was approved by the Committee for the Ethical Review of Research, Fujian Medical University Union Hospital.

Consent for publication:

Not applicable in this section.

Availability of data and materials:

Please contact with the authors.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

YP, XL, and HH conceived the concept. HH and MP supervised the study. YP, QF, XY, FL and PX designed and performed the experiments. YP, XL, and MP wrote the manuscript. All authors approve the manuscript.

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Tables
Table 1 Characteristics of AP patients
### Characteristics

| Characteristic                      | Data (n=63) |
|------------------------------------|-------------|
| Age (years, mean ± SD)             | 51.08±13.56 |
| Sex (n, female/male)               | 29/34       |
| Severity of AP, n (%)              |             |
| Moderately severe                  | 35 (55.6%)  |
| Severe                             | 28 (44.4%)  |
| Etiology of AP, n (%)              |             |
| Biliary                            | 19 (30.2%)  |
| Hypertriglyceridemia               | 24 (38.1%)  |
| Alcoholicity                       | 4 (6.3%)    |
| Other                              | 16 (25.4%)  |
| Ranson score, median (IQR)         | 2.0 (1–3)   |
| BISAP score, median (IQR)          | 2.0 (1–2)   |
| APACHE II score, median (IQR)      | 10.0 (8–15) |
| Infection complications, n         |             |
| Pneumonia                          | 36          |
| Infected necrosis                  | 20          |
| Bacteremia                         | 3           |
| Organ dysfunction, n               |             |
| Respiratory                        | 29          |
| Cardiovascular                     | 10          |
| Renal                              | 11          |
| Interventions, n                   |             |
| Surgical                           | 29          |
| Mechanical ventilation             | 7           |
| Renal replacement therapy          | 3           |
| Hospital mortality, n (%)          | 2 (3.2%)    |

Abbreviations: IQR, interquartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II; BISAP, The Bedside Index for Severity in Acute Pancreatitis

### Table 2 Correlation between traditional clinical indicators and serum sPD-1/sPD-L1

| Variable     | Day 1  | Day 3  | Day 10 | Day 1  | Day 3  | Day 1  | Day 3  | Day 10 |
|--------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Lymphocyte   | r      | 0.168  | 0.156  | -0.092 | -0.023 | -0.335 | -0.294 |
| count, ×10⁹/L| P      | 0.188  | 0.244  | 0.474  | 0.861  | 0.015  | 0.035  |
| WBC, ×10⁹/L  | r      | 0.048  | -0.029 | -0.088 | -0.032 | 0.133  | 0.060  |
| Monocyte     | r      | -0.068 | -0.144 | -0.049 | -0.040 | -0.211 | -0.080 |
| count, ×10⁹/L| P      | 0.595  | 0.259  | 0.703  | 0.756  | 0.134  | 0.574  |
| Neutrophil   | r      | -0.162 | -0.052 | -0.051 | -0.085 | -0.038 | 0.143  |
| count, ×10⁹/L| P      | 0.205  | 0.686  | 0.694  | 0.507  | 0.790  | 0.312  |
| HCT, %       | r      | -0.145 | 0.133  | -0.289 | 0.125  | -0.331 | 0.116  |
| NLR          | r      | -0.248 | -0.138 | 0.038  | -0.024 | 0.177  | 0.213  |
| LMR          | r      | 0.061  | 0.280  | 0.766  | 0.850  | 0.210  | 0.129  |
| PLR          | r      | 0.169  | 0.268  | -0.015 | 0.019  | -0.049 | -0.092 |

Abbreviations: WBC, white blood cell; HCT, hematocrit; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte-monocyte ratio; PLR, platelet-lymphocyte ratio

### Table 3 Clinical indicators of patients with AP with or without ICs
| Variable                          | AP with IC (n=36) | AP without IC (n=27) |
|----------------------------------|-------------------|----------------------|
| Age, years                       | 52.33 ± 13.76     | 49.41 ± 13.37        |
| Male/Female, n                   | 24/12             | 10/17                |
| APACHE II score                  | 13.56 ± 2.91      | 8.93 ± 2.43          |
| WBC count, ×10^9/L               | 12.42 ± 5.41      | 12.97 ± 5.67         |
| Neutrophil count on day 1, ×10^9/L | 9.98 ± 5.23    | 10.35 ± 5.54         |
| Monocyte count on day 1, ×10^9/L  | 0.84 ± 0.47       | 0.62 ± 0.30          |
| Lymphocyte count on day 1, ×10^9/L | 1.20 ±0.58     | 1.26 ± 0.66          |
| PLT, ×10^9/L                     | 282.06 ± 116.47   | 239.44 ± 105.32      |
| HCT, %                           | 31.66 ± 7.79      | 36.79 ± 6.75         |
| sPD-1 levels on day 1, pg/ml     | 186.29 ± 124.51   | 165.57 ± 62.25       |
| sPD-1 levels on day 3, pg/ml     | 266.03 ± 130.37   | 185.17 ± 78.79       |
| sPD-1 levels on day 10, pg/ml    | 323.76 ± 167.25   | 210.97 ± 102.33      |
| sPD-L1 levels on day 1, pg/ml    | 25.83 ± 16.01     | 30.78 ± 21.51        |
| sPD-L1 levels on day 3, pg/ml    | 31.02 ± 17.61     | 29.49 ± 16.63        |
| sPD-L1 levels on day 10, pg/ml   | 27.58 ± 15.02     | 33.75 ± 14.81        |

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; WBC, white blood cell; PLT, Platelet; HCT, hematocrit

Table 4 Univariate and Multivariate regression analysis of variables for ICs of AP

| Variable                          | Univariate analysis | Multivariate analysis |
|-----------------------------------|---------------------|-----------------------|
|                                   | OR (95% CI)         | P value               |
| Age                               | 1.016 (0.979–1.055) | 0.395                 |
| HCT, %                            | 0.908 (0.842–0.980) | 0.013                 |
| APACHE II score                   | 1.420 (1.134–1.776) | 0.002                 |
| Lymphocyte count on day 1         | 0.863 (0.380–1.957) | 0.724                 |
| Monocyte count on day 1           | 3.178 (0.757–13.344)| 0.114                 |
| Neutrophil count on day 1         | 0.987 (0.898–1.084) | 0.783                 |
| Serum sPD-1 levels on day 1       | 1.002 (0.997–1.007) | 0.428                 |
| Serum sPD-1 levels on day 3       | 1.013 (1.005–1.021) | 0.002                 |
| Serum sPD-L1 levels on day 1      | 0.985 (0.959–1.013) | 0.300                 |
| Serum sPD-L1 levels on day 3      | 1.005 (0.976–1.033) | 0.722                 |

Abbreviations: HCT, hematocrit; APACHE II, Acute Physiology and Chronic Health Evaluation II

Table 5 AUCs of various parameters for predicting ICs in AP patients

| Variable                                   | AUC    | P value | 95% CI       |
|--------------------------------------------|--------|---------|--------------|
| APACHE II score                            | 0.769  | < 0.001 | 0.649–0.889  |
| Serum sPD-1 levels on day 3                | 0.796  | < 0.001 | 0.681–0.911  |
| Combination of above two various           | 0.826  | < 0.001 | 0.721–0.931  |

Figures
Figure 1

The serum sPD-1 and sPD-L1 levels in patients with AP. (A) sPD-1 and (B) sPD-L1 were measured in peripheral blood from healthy volunteers (control, n=30) and patients with AP (n = 63) on day 1 (d1), day 3 (d3) and day 10 (d10) after onset. *P < 0.05, **P < 0.01.
The area under the ROC curve (AUC) was used to estimate the accuracy of the predicted model. AUC of serum sPD-1 level on day 3: 0.796; AUC of APACHE II score: 0.769; AUC of combined: 0.826.