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# Plasma cytokines can help to identify the development of severe acute pancreatitis on admission

Li-Hui Deng, MD, PhD[a], Cheng Hu, MD[a], Wen-Hao Cai, MD[a], Wei-Wei Chen, MD, PhD[a,b], Xiao-Xin Zhang, MD[a], Na Shi, MD[a], Wei Huang, MD, PhD[a,c], Yun Ma, MD[a], Tao Jin, MD, PhD[a], Zi-Qi Lin, MD, PhD[a], Kun Jiang, MD[a], Jia Guo, MD, PhD[a], Xiao-Nan Yang, MD[a,*], Qing Xia, MD[a]

**Abstract**

Severe acute pancreatitis (AP) is associated with high morbidity and mortality. Early severity stratification remains a challenging issue to overcome to improve outcomes. We aim to find novel plasma cytokines for the early identification of severe AP according to the revised Atlanta criteria.

In this prospective observational study, 30 cytokines, screened semiquantitatively with a human multicytokine array, were submitted to quantitative determination using either microparticle-based multiplex immunoassays analyzed on a Luminex 100 platform or enzyme-linked immunosorbent assay kits. The cytokine profiles of patients and the discriminative value of cytokines for severe AP were analyzed.

Plasma samples of 70 patients with AP (20 mild, 30 moderately severe, and 20 severe) were selected in this study if they were admitted within 48 hours of the onset of symptoms. Plasma from healthy volunteers was collected as the healthy control. Growth differentiation factor-15 (GDF-15) and pentraxin 3 (PTX3) on admission were independent prognostic markers for the development of severe AP and had higher discriminative powers than conventional markers (GDF-15 vs hematocrit, $P = .003$; GDF-15 vs C-reactive protein, $P = .037$; GDF-15 vs creatinine, $P = .048$; GDF-15 vs Acute Physiology and Chronic Health Evaluation II, $P = .007$; PTX3 vs hematocrit, $P = .006$; PTX3 vs C-reactive protein, $P = .047$; PTX3 vs Acute Physiology and Chronic Health Evaluation II, $P = .011$; PTX3 vs Bedside Index for Severity in Acute Pancreatitis, $P = .048$).

Plasma GDF-15 and PTX3 can help to identify the development of severe AP on admission. Future work should validate their accuracy in a larger, multicenter patient cohort.

**Abbreviations:** Ang = angiopoietin, AP = acute pancreatitis, APACHE-II = Acute Physiology and Chronic Health Evaluation II, AUC = area under the curve, BISAP = Bedside Index for Severity in Acute Pancreatitis, BMI = body mass index, BUN = blood urinary nitrogen, C5 = complement component 5, CHI3L1 = chitinase-3-like protein 1, CI = confidence interval, CRP = C-reactive protein, DPPIV = dipeptidyl peptidase-4, ELISA = enzyme-linked immunosorbent assay, FGF-2 = fibroblast growth factor-2, GDF-15 = growth differentiation factor-15, GH = growth hormone, GRO-α = growth regulated oncogene alpha, HGF = hepatocyte growth factor, ICU = intensive care unit, IFN-γ = interferon gamma, IL = interleukin, LDH = lactate dehydrogenase, LIF = leukocyte inhibition factor, MCP = monocyte chemotactic protein, M-CSF = macrophage colony-stimulating factor, MIF = macrophage migration inhibitory factor, MPO = myeloperoxidase, NIPPV = noninvasive positive pressure ventilation, NLR = negative likelihood ratio, NPV = negative predictive value, OF = organ failure, OR = odds ratio, PLR = positive likelihood ratio, PPV = positive predictive value, PTX3 = pentraxin 3, RAGE = receptor for advanced glycation endproducts, RANTES = Regulated on Activation Normal T-cell Expressed and

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*[a] Department of Integrated Traditional and Western Medicine, Sichuan Provincial Pancreatitis Center, West China Hospital, Sichuan University, Chengdu, Sichuan Province, [b] Department of Gastroenterology, Northern Jiangsu People's Hospital, Yangzhou, Jiangsu Province, China, [c] NIHR Liverpool Pancreas Biomedical Research Unit, Royal Liverpool University Hospital, University of Liverpool, Liverpool, UK.*

*[*] Correspondence: Dr Xiao-Nan Yang, Department of Integrated Traditional Chinese and Western Medicine, West China Hospital, Sichuan University, 37# Guoxue Street, Chengdu 610041, China (e-mail: yangxiaonan1@medmail.com.cnz).*

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Secreted, ROC curve = receiver-operating characteristic curve, SIRS = systemic inflammatory response syndrome, SOFA = Sequential Organ Failure Assessment, ST2 = suppression of tumorigenicity 2.

**Keywords:** acute pancreatitis, biomarker, cytokines, moderately severe acute pancreatitis, severe acute pancreatitis, severity, suspension liquid chip

## 1. Introduction

Acute pancreatitis (AP) is an inflammatory disease of the exocrine pancreas with highly variable severity, ranging from self-limited disease to severe progressive disease with organ dysfunction and death.[1–3] There is no intervention to modify the progression or severity of pancreatitis. However, it would be helpful to know whether the disease will progress or resolve when patients arrive with AP. Furthermore, extensive studies in the past 2 decades have demonstrated that the first 24 hours after the onset of symptoms are critical for identifying those patients who are at risk for developing complications or dying.[4] More accurate predictions of outcomes on admission would potentially permit stratification of patients during the initial stage of the disease, which is critical to improving clinical outcomes. Various conventional biochemical markers and scoring systems have been investigated.[4–8] Nevertheless, none of them fulfils a definitive role, has widespread applicable value, or is consistently accurate. Clinicians are still struggling to identify the severity of AP at the bedside.

Cytokines are a family of low-molecular-weight proteins that have been extensively investigated in inflammatory conditions. Overview of the inflammatory cascade in AP, premature activation of trypsin within pancreatic acinar cells leads to pancreatic autodigestion and a local inflammatory process, which is characterized by the activation of various leukocyte subsets and endothelium and the release of inflammatory cytokines, chemokines, and other inflammatory mediators into the pancreas.[9,10] A drastic and sustained activation of proinflammatory mediators leads to systemic inflammatory response syndrome (SIRS), which tends to trigger organ failure (OF), multiple OF, infected necrosis, and sepsis.[10,11] The persistence of OF over 48 hours is associated with a 35% to 50% mortality rate in AP. [4]

The link between cytokine levels and the development of severe AP has been studied by several investigators. [12–14] However, because of their limitations, no cytokines are useful enough to indicate disease progression of AP with simplicity and accuracy for routine clinical use. As a result, it is a matter of great urgency to screen new markers for AP progression.

The development of new assay techniques has overcome the problems of conventional enzyme-linked immunosorbent assay (ELISA) measurements to make the determinations of cytokines fast and automatically. The magnetic bead suspension array, characterized by high throughput, large-scale, and multiplex screening, enables the users to perform proteomic research using small-volume samples. [15–17] A recent study by Nieminen et al[18] set an example of the performance of this method in cytokine screening. Unfortunately, the study did not propose a new cytokine whose prognostic value was superior to the conventional markers. Because the discriminative value of cytokines for severe AP is not firmly established, it is important to continue to explore this topic.

In this study, we sought to find the differentially expressed cytokines in AP and to validate their values as markers in identifying severe AP by using commercially available multiplex cytokine kits.

## 2. Materials and methods

### 2.1. Subject enrolment

Plasma samples and data from patients with AP and healthy volunteers were obtained from a prospective observational clinical study that undertook the measurement of various severity markers in a range of patients with mild to severe AP (Registration Number: chictr-DOD-15005864). The study protocol and informed consent were approved by the West China Hospital of Sichuan University Clinical Trials and Biomedical Ethics Committee. All procedures performed in studies of human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The informed consent of patient was given.

According to the Revised Atlanta Classification in 2012, the diagnosis of AP requires 2 of the following 3 features: abdominal pain characteristic of AP; serum amylase and/or lipase activity at least 3 times greater than the reference limit; and findings characteristic of AP on abdominal computerized tomography scan or transabdominal ultrasonography. [19] Mild AP was defined as the absence of both OF and local or systemic complications. Moderately severe AP was defined as the presence of transient OF or local or systemic complications in the absence of persistent OF. SAP was characterized by persistent OF. Using the Modified Marshall Scores (MMS), OF was defined as a score of 2 or more for 1 of 3 organ systems. [20] Persistent OF was defined as OF for more than 48 hours.

Acute pancreatitis patients between 18 and 70 years of age, who were admitted to West China Hospital of Sichuan University within 72 hours after the onset of disease, were enrolled. Patients were excluded if they were pregnant or lactating; had any malignant disease; had serious primary systemic diseases; or were not willing to provide written informed consent before enrolling. Healthy volunteers were included for study patients.

### 2.2. Collection of human plasma

Peripheral blood samples were collected from AP patients using purple-top tubes (BD Vacutainer, China) within 24 hours of admission. All blood samples were processed with the Standard Operation Procedures of the AP Biobank in West China Hospital: the tubes were placed upright for 20 to 25 minutes and centrifuged at room temperature at 600 *g* for 30 minutes, and the supernatant was further centrifuged at 24°C at 1500 *g* for 10 x0200A;minutes. Plasma was aliquoted and stored at −80°C until analyses.

### 2.3. Collection of clinical data

Electronic medical records and paper charts were reviewed by 2 independent physicians. Demographics and clinical data for all study participants were recorded. For AP patients, routine blood and biochemical tests, C-reactive protein (CRP), procalcitonin,

and interleukin (IL)-6 were measured on admission using an automated clinical chemical analyzer in the Department of Clinical Laboratory in West China Hospital. Scoring systems including SIRS, Acute Physiology and Chronic Health Evaluation II (APACHE-II), Bedside Index for Severity in Acute Pancreatitis (BISAP), Sequential Organ Failure Assessment (SOFA), and MMS were evaluated on 24 hours, 48 hours, 72 hours, 5 days, 7 days, and on the occasion of exacerbation of the disease. The treatments of the patients were based on the Revised Atlanta Classification and Management Guidelines of Acute Pancreatitis proposed by the American College of Gastroenterology.[4,19]

### 2.4. Semiquantitative determination with human multicytokine array

In the first portion of the experiment, we aimed to preliminarily screen the differentially expressed cytokines in plasma among patient with mild, moderately severe, or severe AP, and also a healthy volunteer (data are shown in Supplementary Materials, http://links.lww.com/MD/B767). To this end, cytokine levels were determined semiquantitatively with a Proteome Profiler Human XL Cytokine Array (ARY022, R&D systems, Minneapolis, MN),[21,22] which contains 4 membranes and each spotted in duplicate with 102 different cytokine antibodies. Consequently, a densitometric evaluation revealed that 30 cytokines were significantly differentially expressed among these 4 human plasma samples (Fig. 1).

### 2.5. Quantitative determination with human multicytokine suspension array

In the second portion of the experiment, 30 cytokines in the plasma of patients with mild, moderately severe, or severe AP were quantified. Each sample (50 μL) was processed using

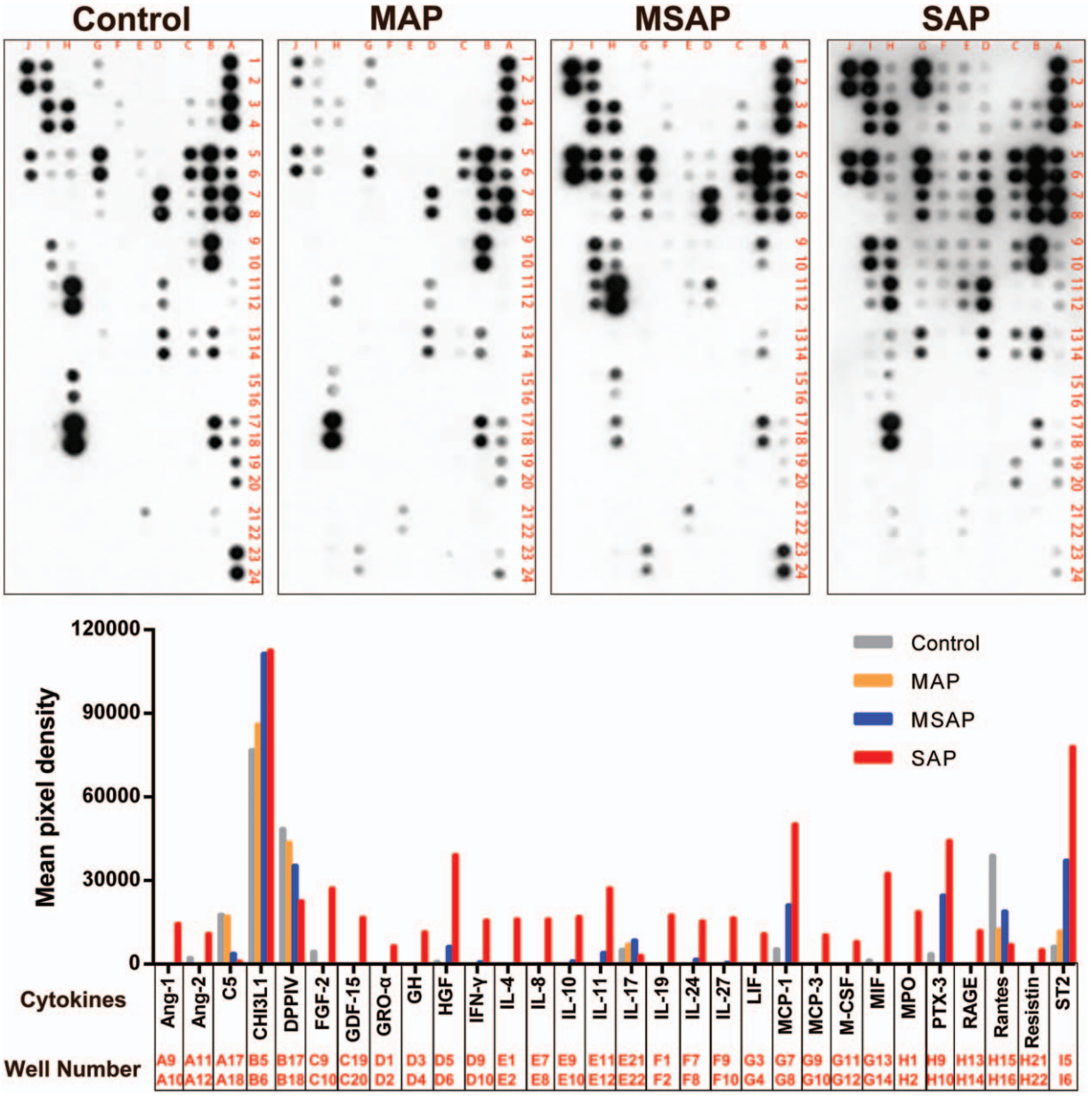


**Figure 1.** The differential expression of 30 cytokines associated with AP screened out by the Proteome Profiler Human XL Cytokine Array kit. AP = acute pancreatitis.

either microparticle-based multiplex immunoassays (R&D systems, Minneapolis, MN) analyzed on Luminex 100 platform[23,24] or using human PTX3 or IL-11 ELISA kits (R&D systems, Minneapolis, MN) according to the manufacturer's instructions. Except for 2 cytokines measured by ELISA kits, 28 cytokines were measured by flexible, customized bead-based multiplex panels for Luminex assays. Except for 2 cytokines measured by ELISA kits, 28 cytokines were measured by flexible customized bead-based multiplex panels for Luminex assays: Human Premixed Multi-Analyte Kit (LXSAH-14, 14 PLEX),Human Premixed Multi-Analyte Kit (LXSAH-01, 1 PLEX), Human Angiogenesis Premixed Kit A (FCST02-02, 2 PLEX), Human Obesity Premixed Kit (FCST08-03, 3 PLEX), Human Cytokine Premixed Kit A (FCST03-04, 4 PLEX), Human Cardiac Premixed Kit A (FCST11-02, 2 PLEX), Human Cardiac Base Kit B (FCST12-01, 1 PLEX), Human Cytokine Base Kit B (FCST04-01, 1 PLEX). The contains of each multiplex kit are shown in the Supplementary Materials, http://links.lww.com/MD/B767. The samples were analyzed using the Luminex 100 platform, and the results were calculated using Master Plex QT software.

No detectable values for suppression of tumorigenicity 2 (ST2) were obtained from any sample, and the rest of the 29 cytokines were included in the data analysis. The persons doing the cytokine measurements were blinded to the clinical data of the patients.

### 2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 22 (SPSS, Chicago, IL) statistical software. Descriptive data are presented as median and interquartile range for continuous variables. Categorical data are presented as proportions. For univariate analysis, comparisons between groups were made using the Mann–Whitney *U* test. Multiple group comparisons were performed using the chi-square test for categorical variables and the Kruskal–Wallis test for continuous data. A Bonferroni correction was used in multiple group comparisons, and a *P* value of $<0.017$ was considered statistically significant. Stepwise forward logistic regression analysis was performed to identify independent markers for SAP. Optimal cut-off values for each cytokine were determined by receiver-operating characteristic (ROC) curves with corresponding sensitivities and specificities. Pairwise area under the curve (AUC) comparisons were also performed using the nonparametric approach developed by DeLong et al[25] using MedCalc 15.0 software. A *P* value $<0.05$ (2-tailed) was considered statistically significant.

## 3. Results

### 3.1. Clinical characteristics of the patients included in this study

From September 1, 2014 to November 30, 2014, a total of 70 patients AP (20 mild, 30 moderately severe, and 20 severe AP patients) consecutively admitted to West China Hospital of Sichuan University within 48 hours of the onset of symptoms were prospectively included. The plasma samples of admitted within 48 hours of the onset of symptoms were selected. Seventy consecutive patients with admitted to West China Hospital of Sichuan University within 48 hours of the onset of symptoms were prospectively included, comprising 20 MAP, 30 MSAP, and 20 SAP. To discriminate the differential expression of the cytokines in AP, 10 healthy volunteers were also enrolled. There were no significant differences in age ($P=.430$), sex ($P=.748$), or body mass index (BMI) ($P=.458$) among the subjects.

The demographics and outcomes of the patients with AP are shown in Table 1. There was no significant difference in the etiology among any of the groups. The overall inhospital mortality was 5.7%. Four of 20 patients (20%) with severe AP died of refractory multiple OF, of which 2 patients died within 48 hours, and the others died within 7 to 14 days. All patients with mild and moderately severe AP survived.

As shown in Table 2, there were no significant differences in the 8 routine markers (hematocrit, blood urine nitrogen [BUN], creatinine, lactate dehydrogenase [LDH], calcium, CRP, PCT, IL-6) and the 5 scoring systems (SIRS, APACHE-II, SOFA, BISAP, and MMS) when comparing severe AP with mild or moderately severe AP. No single marker or scoring system was able to differentiate all 3 groups of patients with AP.

As shown in Table 3, 29 (41.4%) of 70 patients with AP developed OF, of which 24 patients had OF within 24 hours and 5 patients developed OF within 2 to 7 days after admission.

**Table 1**

**Demographics and clinical outcomes of patients with acute pancreatitis (n = 70).**

| | Total (n = 70) | Mild (n = 20) | Moderately severe (n = 30) | Severe (n = 20) | *P* |
|---|---|---|---|---|---|
| Age, y | 44.1 (21–68) | 45 (21–65) | 42 (27–68) | 43.5 (33–66) | .359 |
| Sex, male | 54 (77.1) | 15 (75) | 22 (73.33) | 17 (85) | .648 |
| Body mass index | 25.9 (17.99–34.9) | 24.70 (21.3–34.9) | 25.74 (18–31.2) | 25.85 (21.8–32.9) | .701 |
| Etiology | | | | | .282 |
| Biliary | 47 (67.2) | 12 (60) | 20 (66.67) | 15 (75) | |
| Hypertriglyceridemia | 15 (21.4) | 5 (25) | 8 (26.67) | 2 (10) | |
| Alcohol | 3 (4.3) | 1 (5) | 2 (6.66) | 0 (0) | |
| Others | 5 (7.1) | 2 (10) | 0 (0) | 3 (15) | |
| Length of hospital stay | 9.5 (1–94) | 7 (3–32) | 9.5 (5–26) | 14.5 (1–94) | .002 |
| Inhospital mortality | 4 (5.7) | 0 | 0 | 4 (20) | <.001 |
| The need for ICU or ICS | 21 (30) | 0 | 6 (20) | 15 (75) | <.001 |
| Duration of ICU or (and) ICS | 0 (0–32) | 0 | 0 (0–8) | 7 (0–32) | <.001 |
| Mechanical ventilation | 7 (10) | 0 | 0 | 7 (35) | <.001 |
| Noninvasive ventilation | 18 (25.7) | 0 | 5 (16.7) | 13 (65) | <.001 |
| Infected pancreatic necrosis | 5 (7.1) | 0 | 1 (3.3) | 4 (20) | .079 |

Data are presented as median (IQR) or n (%).

ICS = intermediary care setting, ICU = intensive care unit.

**Table 2**

**Routine clinical parameters of the patients upon admission.**

| | Mild (n = 20) | Moderately severe (n = 30) | Severe (n = 20) | P* | P† | P‡ |
|---|---|---|---|---|---|---|
| Biochemical parameters | | | | | | |
| Hematocrit, % | 43.5 (39.75–46) | 44 (41–47.25) | 46.5 (44–51) | .974 | .013 | .024 |
| Blood urea nitrogen, mmol/L | 5.16 (3.94–6.38) | 4.96 (3.79–6.56) | 7.85 (5.58–11.36) | .913 | .001 | .001 |
| Creatinine, μmol/L | 73.5 (64–77.75) | 74.5 (65–88.25) | 96.5 (76.25–215.25) | .559 | .008 | .003 |
| Lactic dehydrogenase, U/L | 186.5 (152.5–274.75) | 221 (153–316.75) | 414 (294–843.75) | .289 | .001 | <.001 |
| Calcium, mmol/L | 2.25 (2.16–2.31) | 2.21 (1.97–2.31) | 1.74 (1.54–2.23) | .276 | .005 | .003 |
| C-reactive protein, mg/L | 45.8 (13.73–103.28) | 84.2 (84.2–222.75) | 301 (11.75–449.75) | .198 | .006 | <.001 |
| Procalcitonin, μg/L | 0.11 (0.02–0.62) | 0.3 (0.08–1.85) | 4.56 (0.71–8.32) | .234 | .001 | <.001 |
| Interleukin-6, μg/L | 50.05 (23.44–112.17) | 93.97 (53.43–225.8) | 355.1 (238.53–840.7) | .081 | <.001 | <.001 |
| Scoring systems | | | | | | |
| SIRS score | 1 (1–2) | 2 (1–3) | 3 (2–3) | .029§ | .007 | <.001 |
| APACHE-II score | 4 (2–6) | 4 (2–5) | 6 (4–12) | .724 | <.001 | <.001 |
| BISAP score | 0 (0–1) | 1 (0–2) | 2 (1–2) | .115 | .002 | <.001 |
| Modified Marshall score | 0 (0–1) | 0 (0–3) | 3 (2–5) | .547 | <.001 | <.001 |

Data are presented as median (IQR).

APACHE-II = Acute Physiology and Chronic Health Evaluation II, BISAP = Bedside Index for Severity in Acute Pancreatitis, SIRS = systemic inflammatory response syndrome.

* Mild AP vs moderately severe AP.

† Moderately severe AP vs severe AP.

‡ Mild AP vs severe AP.

§ Level of significance was defined as $P < .017$ (Bonferroni-adjusted).

Among the 24 patients who had OF within 24 hours after admission, 6 patients responded promptly to treatment and resolved within 48 hours, 16 patients developed persistent OF, and 2 died within 48 hours of admission. Of the 4 patients who developed OF within 2 to 7 days after admission, 2 developed transient OF, and 2 developed persistent OF. There was 1 case of new-onset transient OF after the first week of treatment. In total, 9 patients had transient OF, 20 had persistent OF, and these patients were classified as moderately severe or severe AP accordingly.

### 3.2. Cytokine profiles of patients with severe acute pancreatitis

The levels of 13 of 29 cytokines were significantly different among the mild, moderately severe, and severe AP patients, and also healthy volunteers (Table 4). Of these cytokines, 12 were significantly higher in the severe AP patients than in the mild and moderately severe AP patients. IL-11 was significantly lower in patients with severe AP and did not differentiate severe AP from the healthy controls. Table 5 shows the levels of 16 cytokines with insignificant differences among all categories of AP and the healthy volunteers.

**Table 3**

**Organ failure of patients with mild, moderately severe, and severe acute pancreatitis.**

| Outcomes | Total (n = 70) | Mild or moderately severe (n = 50) | Severe (n = 20) | P |
|---|---|---|---|---|
| Respiratory failure | 29 (41.4) | 9 (18) | 20 (100) | <.001 |
| Transient | 9 (12.9) | 9 (18) | 0 | |
| Persistent | 20 (28.5) | 0 | 20 (100) | |
| Cardiovascular failure | 6 (8.6) | 0 | 6 (30) | .003 |
| Transient | 2 (2.9) | 0 | 2 (10) | |
| Persistent | 4 (5.7) | 0 | 4 (20) | |
| Renal failure | 8 (11.4) | 0 | 8 (40) | .001 |
| Transient | 4 (5.7) | 0 | 4 (20) | |
| Persistent | 4 (5.7) | 0 | 4 (20) | |
| Single organ failure | 20 (28.6) | 10 (20) | 10 (50) | .003 |
| Multiple organ failure | 10 (14.3) | 0 | 10 (50) | <.001 |

Data are presented as median (range) or n (%).

### 3.3. The discriminative power of plasma cytokines for severe acute pancreatitis

By stepwise forward logistic regression analysis of the 13 cytokines, we identified growth differentiation factor-15 (GDF-15) (odds ratio [OR] 1.0, 95% confidence interval [CI] 1.000–1.001, $P = .019$) and pentraxin 3 (PTX3) (OR 4.101, 95% CI 1.559–10.788, $P = .006$) as independent prognostic markers of severe AP (Table 6). There was no significant difference between GDF-15 and PTX3 in the identification of severe AP ($P = .932$).

We also compared the pairwise AUCs of GDF-15 and PTX3 to those of conventional markers and scoring systems. The results showed that GDF-15 and PTX3 had higher AUCs than hematocrit, CRP, creatinine, APACHE-II, and BISAP (GDF-15 vs hematocrit, $P = .003$; GDF-15 vs CRP, $P = .037$; GDF-15 vs creatinine, $P = .048$; GDF-15 vs APACHE-II, $P = .007$; PTX3 vs hematocrit, $P = .006$; PTX3 vs CRP, $P = .047$; PTX3 vs APACHE-II, $P = .011$; PTX3 vs BISAP, $P = .048$) (Table 6). The ROC curves for the comparisons of these variables for the identification of severe AP are presented in Figure 2A and B.

When hematocrit, creatinine, CRP, APACHE-II, and BISAP were added into the logistic regression analysis with GDF-15 and PTX3, the results only showed that GDF-15 (OR 1, 95% CI 1.000–1.001, $P = .024$) and PTX3 (OR 3.886, 95% CI 1.508–10.011, $P = .005$) were able to identify severe AP independently.

### 3.4. Identification of severe acute pancreatitis in patients with the presence of systemic inflammatory response syndrome on admission

Of 70 patients with AP, 45 patients had SIRS (SIRS ≥2 scores) on admission, of which 28 developed OF. Among these 45 patients,

**Table 4**

**The 13 cytokines that differed among 3 categories of patients with AP and healthy volunteers.**

| Cytokines | | | Acute pancreatitis (n = 70) | | | |
|---|---|---|---|---|---|---|
| (pg/mL) | Control (n = 10) | $P^*$ | Mild (n = 20) | Moderately severe (n = 30) | Severe (n = 20) | $P^\dagger$ |
| Ang-2 | 606.99 (354.76–2190.70) | <.001 | 1014.84 (397.05–3709.33) | 1307.97 (108.77–10884.59) | 5119.93 (655.28–19829.66) | <.001 |
| GRO-α | 51.85 (35.82–95.93) | .028 | 55.10 (31.75–152.94) | 61.03 (38.49–387.09) | 78.66 (43.35–1462.84) | .001 |
| IL-8 | 1.62 (0.79–16.06) | .001 | 3.68 (0–27.73) | 9.29 (0–185.45) | 24.32 (2.85–1318.82) | <.001 |
| M-CSF | 0 (0–254.29) | .016 | 0 (0–267.68) | 8.88 (0–400.99) | 200.15 (0–1207.69) | .001 |
| MIF | 296.20 (57.31–556.79) | .001 | 438.94 (143.36–1452.97) | 716.51 (201.61–2631.12) | 2983.82 (73.92–44785.59) | <.001 |
| CHI3L1 | 13220.90 (6332.98–31451.50) | <.001 | 56920.68 (4738.32–116684.09) | 96907.49 (1892.68–141245.04) | 119598.04 (16277.23–138388.66) | <.001 |
| MPO | 13338.29 (11355.47–18532.60) | <.001 | 18482.17 (9847.99–32661.46) | 27300.88 (10487.13–54227.34) | 37677.32 (7649.51–77879.00) | <.001 |
| HGF | 122.40 (71.55–162.50) | <.001 | 338.00 (107.70–1347.55) | 428.23 (106.30–3274.00) | 1784.33 (289.85–9024.55) | <.001 |
| GDF-15 | 619.08 (227.64–1193.43) | <.001 | 1593.44 (664.35–4671.90) | 2314.65 (709.95–18548.34) | 10135.28 (1063.62–33922.62) | <.001 |
| MCP-1 | 115.93 (64.80–191.95) | .006 | 130.80 (81.80–1391.25) | 160.58 (58.65–9132.05) | 273.15 (82.30–5074.45) | .001 |
| Resistin | 5834.58 (3877.70–7620.55) | <.001 | 10144.23 (3882.45–51700.50) | 12714.23 (3571.55–100053.25) | 53264.28 (6414.65–175371.50) | <.001 |
| PTX3 | 0.10 (0.06–0.15) | <.001 | 0.25 (0.10–2.99) | 0.60 (0.17–2.75) | 2.15 (0.32–3.31) | <.001 |
| IL-11 | 0.13 (0.04–0.34) | .130 | 0.34 (0.08–1.45) | 0.21 (0.07–0.65) | 0.15 (0.09–0.38) | .003 |

Ang = angiopoietin, CHI3L = chitinase-3-like protein, GDF-15 = growth differentiation factor-15, GRO-α = growth regulated oncogene alpha, HGF = hepatocyte growth factor, IL = interleukin, MCP = monocyte chemotactic protein, M-CSF = macrophage colony-stimulating factor, MIF = macrophage migration inhibitory factor, MPO = myeloperoxidase, PTX3 = pentraxin 3.

* Control group versus all AP patients.

† Comparison among patients with mild, moderately severe, and severe acute patients.

7 (15.6%) had mild, 19 (42.2%) had moderately severe, and 19 (42.2%) had severe AP ($P < .001$). The levels of GDF-15 and PTX3 were significantly higher in patients with severe AP than patients with mild and moderately severe AP ($P = .013$ and $P = .014$, respectively). The rest of the cytokines and conventional parameters had $P$ values >.1.

The discriminative powers of GDF-15, PTX3, and conventional markers for severe AP in patients with the presence of SIRS on admission are shown in Table 7. Pairwise AUC comparisons showed that GDF-15 and PTX3 were significantly different from APACHE-II and BISAP (GDF-15 vs APACHE-II, $P = .038$; GDF-15 vs BISAP, $P = .007$; PTX3 vs BISAP, $P = .011$). GDF-15 had better discriminating power than HCT ($P = .035$).

When using SIRS as the variable and cytokines as the covariables in a logistic regression analysis, the results showed GDF-15 (OR 1, 95% CI 1.000–1.001, $P = .035$) and PTX3 (OR 3.195, 95% CI 1.177–8.675, $P = .023$) were significant variables in the equation, indicating they were independent markers for the identification of severe AP in patients with the presence of SIRS on admission.

## 4. Discussion

The present study was designed for the potential of plasma cytokines for early identification of the development of severe AP, which is based on the detrimental roles of various cytokines in promoting both local tissue destruction and distal organ complications.

This study was a prospective observational study that included 70 patients with AP. Severe AP, defined as persistent OF and/or

**Table 5**

**Levels of 16 cytokines that did not differ among mild, moderately severe, and severe acute pancreatitis and healthy volunteers.**

| Cytokines | | | Acute pancreatitis (n = 70) | | | |
|---|---|---|---|---|---|---|
| (pg/mL) | Control (n = 10) | $P^*$ | Mild (n = 20) | Moderately severe (n = 30) | Severe (n = 20) | $P^\dagger$ |
| IL-19 | 252.76 (229.16–439.14) | .476 | 250.53 (213.18–294.53) | 260.54 (220.06–322.57) | 269.38 (230.30–297.78) | .010 |
| IL-23 | 156.15 (92.54–629.25) | .616 | 127.31 (71.78–274.01) | 237.63 (55.38–704.05) | 249.72 (134.75–444.88) | <.001 |
| MCP-3 | 25.82 (22.94–29.78) | .298 | 24.04 (21.03–28.14) | 27.46 (20.07–120.96) | 31.90 (22.67–48.00) | <.001 |
| IL-10 | 3.43 (0–9.80) | .227 | 2.80 (0–10.10) | 4.60 (0–330.60) | 12.35 (1.20–199.75) | .002 |
| C5 | 10.71 (2.50–22.74) | <.001 | 29.06 (1.68–58.34) | 29.75 (4.06–93.18) | 43.27 (10.77–123.28) | .120 |
| RANTES | 5147.42 (2503.68–7911.12) | .009 | 3253.39 (930.48–5898.60) | 2655.37 (168.14–7569.60) | 3467.78 (595.38–5893.22) | .767 |
| IFN-γ | 13.42 (9.70–21.02) | .018 | 11.20 (6.05–17.53) | 11.75 (3.32–36.13) | 10.53 (5.84–25.69) | .444 |
| GH | 157.38 (42.56–1000.00) | .116 | 286.37 (0–2990.87) | 519.06 (0–3067.64) | 260.03 (0–13864.71) | .260 |
| LIF | 1.24 (0–50.75) | .252 | 2.33 (0–19.59) | 5.29 (0–52.71) | 6.07 (0–51.07) | .408 |
| RAGE | 1094.33 (446.95–1709.38) | .907 | 1071.29 (585.70–1784.22) | 1015.08 (341.78–2866.02) | 1332.54 (562.03–10037.74) | .143 |
| IL-27 | 61.23 (53.01–74.48) | .238 | 55.52 (40.81–89.33) | 59.32 (41.61–153.18) | 59.95 (41.61–77.59) | .122 |
| Ang-1 | 986.00 (181.02–2696.04) | .556 | 753.70 (93.34–5763.04) | 577.22 (53.58–8588.54) | 628.69 (149.98–8663.92) | .975 |
| FGF-2 | 15.85 (0–35.74) | .778 | 16.92 (0–114.96) | 18.66 (0–86.22) | 4.00 (0–40.64) | .157 |
| DPPIV | 56220.75 (33456.00–136647.75) | .227 | 75059.00 (0–183106.25) | 70997.25 (26235.75–129307.25) | 75687.38 (34932.75–177753.75) | .471 |
| IL-17 | 2.71 (0.77–7.43) | .282 | 3.98 (0–14.47) | 4.78 (0–27.18) | 3.85 (0.21–13.25) | .808 |
| IL-4 | 10.57 (6.24–12.33) | .154 | 9.21 (6.24–13.11) | 8.19 (0–24.50) | 9.15 (1.56–12.44) | .520 |

Data are presented as median (range).

Ang = angiopoietin, DPPIV = dipeptidyl peptidase-4, FGF-2 = fibroblast growth factor-2, GH = growth hormone, IFN-γ = interferon gamma, IL = interleukin, LIF = leukocyte inhibition factor, MCP = monocyte chemotactic protein, RAGE = receptor for advanced glycation endproducts, RANTES = Regulated on Activation Normal T-cell Expressed and Secreted.

* Control versus AP.

† Comparison among 3 AP groups.

**Table 6**

**Performances of GDF-15, PTX3, and conventional markers in predicting severe acute pancreatitis.**

| Predictive marker (optimum cut-off point) | AUC | Sensitivity (%) | Specificity (%) | PLR (%) | NLR (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|
| GDF-15 (>3288.87 pg/mL) | 0.900 (0.805–0.959) | 90 (68.3–98.8) | 86 (73.3–94.2) | 6.43 (3.2–13.0) | 0.12 (0.03–0.4) | 72.0 (50.6–87.9) | 95.6 (84.9–99.5) |
| PTX3 (>1.05 pg/mL) | 0.897 (0.801–0.957) | 85 (62.1–96.8) | 88 (75.7–95.5) | 7.08 (3.3–15.3) | 0.17 (0.06–0.5) | 73.9 (51.6–89.8) | 93.6 (82.5–98.7) |
| Hematocrit (>0.5) | 0.682 (0.559–0.788) | 30 (11.9–54.3) | 92 (80.8–97.8) | 3.75 (1.2–11.9) | 0.76 (0.6–1.0) | 60.0 (26.2–87.8) | 76.7 (64.0–86.6) |
| Creatinine (>137 μmol/L) | 0.744 (0.626–0.841) | 40 (19.1 – 63.9) | 98 (89.4–99.9) | 20 (2.7–149.7) | 0.61 (0.4–0.9) | 88.9 (51.8–99.7) | 80.3 (68.2–89.4) |
| CRP (>353 mg/L) | 0.781 (0.666–0.871) | 40 (19.1–63.9) | 96 (86.3–99.5) | 10.00 (2.3–43.1) | 0.63 (0.4–0.9) | 80.0 (44.4–97.5) | 80.0 (67.7–89.2) |
| BISAP (>1 score) | 0.798 (0.685–0.885) | 60 (36.1–80.9) | 80 (66.3–90.0) | 3.00 (1.6–5.8) | 0.50 (0.3–0.9) | 54.5 (32.2–75.6) | 83.3 (69.8–92.5) |
| APACHE-II (>10 scores) | 0.744 (0.626–0.841) | 40 (19.1–63.9) | 100 (92.9–100.0) | +∞ | 0.60 (0.4–0.9) | 100 (63.1–100.0) | 80.6 (68.6–89.6) |

APACHE-II = Acute Physiology and Chronic Health Evaluation II, AUC = area under curve, BISAP = Bedside Index for Severity in Acute Pancreatitis, BUN = blood urinary nitrogen, CRP = C-reactive protein, GDF-15 = growth differentiation factor-15, NLR = negative likelihood ratio, NPV = negative predictive value, PLR = positive likelihood ratio, PPV = positive predictive value, PTX3 = pentraxin 3.

death, occurred in 28.5% of patients, which was higher than the morbidity reported,[4,19] but was consistent with the reported data from our hospital.[26] Mild and moderately severe AP comprised 28.6% and 42.9% of patients, respectively. Our data were inconsistent with a higher rate of moderately severe AP and a lower rate of severe AP in another large center for AP in China.[27] As the largest center for severe AP, representing the specific severity spectrum in South West China, the patients who present with a severe course tend to be transferred to our center rapidly, and those who have mild disease are treated in other municipal hospitals. Because this group of patients was admitted to our center within 48 hours of symptom onset, these patients suffered from more severe symptoms and signs in the initial stage of the disease and endured a more complicated disease course in the late stage. This process might explain the high morbidity of severe AP and the low proportion of mild disease in this cohort. The value of the cytokines will be helpful for clinical use in our center.

The patients with severe AP suffered from significantly more severe outcomes compared with those with mild or moderately severe AP. The management of AP, which influences the development and outcomes of the disease, followed the Revised Atlanta Classification [19] and American College of Gastroenterology guidelines.[4] Typically, patients with OF were given closer observation in a monitored setting. When patients present with acute lung injury, noninvasive positive-pressure ventilation (NIPPV) might be considered, and invasive ventilation was performed when the patients developed severe acute respiratory distress syndrome or if a satisfactory oxygen saturation index could not be maintained by NIPPV.[28] When the patients presented with severe renal failure or a lethal imbalance of the internal environment, renal replacement therapy was considered. When organic supports or the development of disease were needed, the patients were managed in an intermediary care setting in our department. When there was need for mechanical ventilation or perioperation, the patients were transferred to the intensive care unit in our hospital.

The major findings of this study were that 13 out of 29 cytokines were significantly different on admission in patients who developed severe AP compared with those who developed mild or moderately severe AP or with healthy controls, and that GDF-15 and PTX3 were independent prognostic markers of severe AP. To select optimal cut-off values, we considered the prevalence of a target case and the cost of optimal value by setting the false positive cost (FPc) to 1, the false cost (FNc) to 1, the true positive cost (TPc) to 0, and the true negative cost (TNc) to 0. The positive likelihood ratio (PLR) was in the 5 to 10 range for GDF-15, PTX3, and BUN, which was consistent with a medium likelihood of disease. A PLR in excess of 10 in CRP and APACHE-II could be interpreted as being consistent with a high likelihood. However, both GDF-15 and PTX3 exhibited low NLR, indicative of a lower possibility for negative results. In the interesting group of patients who exhibited SIRS and further progressed to severe pancreatitis, GDF-15 and PTX3 were

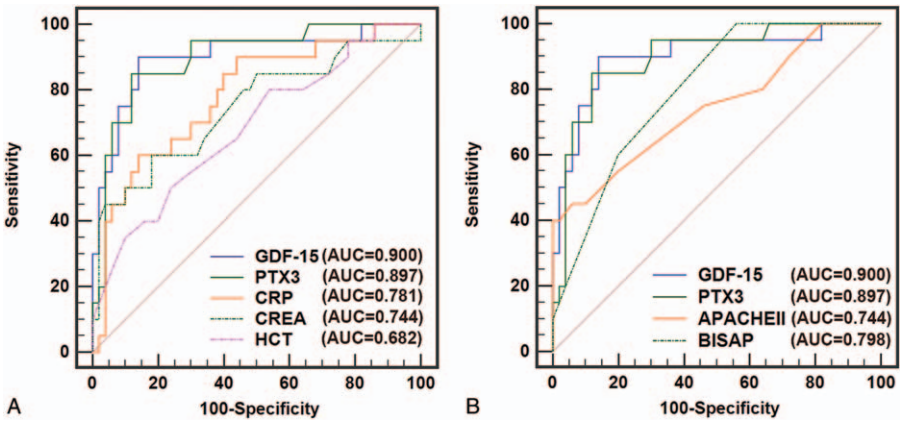


**Figure 2.** The ROC curves for the comparisons of growth differentiation factor-15 (GDF-15), pentraxin 3 (PTX3), and conventional markers on admission for the identification of severe acute pancreatitis. (A) GDF-15 and PTX3 versus single parameters. (B) GDF-15 and PTX3 versus scoring systems. ROC curve = receiver-operating characteristic curve.

**Table 7**

**Performances of GDF-15 and PTX3 and conventional markers in predicting severe acute pancreatitis in patients with SIRS ≥2 on admission.**

| Predictive marker (optimum cut-off point) | AUC | Sensitivity (%) | Specificity (%) | PLR (%) | NLR (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|
| GDF-15 (>3183.36 pg/mL) | 0.862 (0.727–0.947) | 89.47 (66.9–98.7) | 76.92 (56.4–91.0) | 3.88 (1.9–8.0) | 0.14 (0.04–0.5) | 73.9 (51.6–89.8) | 90.9 (70.8–98.9) |
| PTX3 (>1.05 pg/mL) | 0.856 (0.720–0.943) | 89.47 (66.9–98.7) | 80.77 (60.6–93.4) | 4.65 (2.1–10.4) | 0.13 (0.03–0.5) | 77.3 (54.6–92.2) | 91.3 (72.0–98.9) |
| Hematocrit (>0.5) | 0.685 (0.530–0.815) | 31.58 (12.6–56.6) | 92.31 (74.9–99.1) | 4.11 (0.9–18.2) | 0.74 (0.5–1.0) | 75.0 (34.9–96.8) | 64.9 (47.5–79.8) |
| Creatinine (>111 μmol/L) | 0.764 (0.614–0.878) | 47.37 (24.4–71.1) | 96.15 (80.4–99.9) | 12.32 (1.7–89.2) | 0.55 (0.4–0.8) | 90.0 (55.5–99.7) | 71.4 (53.7–85.4) |
| CRP (>219 mg/L) | 0.696 (0.541–0.824) | 63.16 (38.4–83.7) | 76.92 (56.4–91.0) | 2.74 (1.3–6.0) | 0.48 (0.3–0.9) | 66.7 (41.0–86.7) | 74.1 (53.7–88.9) |
| BISAP (>2 scores) | 0.651 (0.494–0.787) | 21.05 (6.1–45.6) | 92.31 (74.9–99.1) | 2.74 (0.6–13.4) | 0.86 (0.7–1.1) | 66.7 (22.3–95.7) | 61.5 (44.6–76.6) |
| APACHE-II (>10 scores) | 0.726 (0.572–0.848) | 42.11 (20.3–66.5) | 100.00 (86.8–100.0) | +∞ | 0.58 (0.4–0.8) | 100 (63.1–100.0) | 70.3 (53.0–84.1) |

APACHE-II = Acute Physiology and Chronic Health Evaluation II, AUC = area under the curve, BISAP = Bedside Index for Severity in Acute Pancreatitis, BUN = blood urinary nitrogen, CRP = C-reactive protein, GDF-15 = growth differentiation factor-15, NLR = negative likelihood ratio, NPV = negative predictive value, PLR = positive likelihood ratio, PPV = positive predictive value, PTX-3 = pentraxin 3, SIRS = systemic inflammatory response syndrome.

significantly higher than in those who did not. Overall, GDF-15 and PTX3 presented higher discriminative power compared to current conventional markers.

Pentraxin 3, an acute-phase protein, is a multifunctional soluble pattern recognition receptor associated with innate immunity, inflammation, matrix deposition, and female fertility.[29] Another acute-phase protein, CRP, was the first pattern recognition receptor identified. Previous studies have found that the levels of PTX3 correlated with the severity of endotoxic shock, sepsis, acute myocardial infarction, and other inflammatory and infectious diseases.[30–33] In AP, PTX3 achieved maximum levels earlier than CRP, and the levels of PTX3 in the early phase of AP were similar to IL-6.[34] Our study confirms the association of PTX3 with the development of severe AP.

Growth differentiation factor-15, a cytokine in the transforming growth factor β superfamily, might play a role in regulating inflammation in injured tissues and the progress of the diseases.[35–40] It was considered a good biomarker in discriminating pancreatic and other periampullary cancers.[41,42] However, the relationship between GDF-15 and AP has not been fully illustrated. Our study showed that GDF-15 was an independent marker in identifying severe AP, but the mechanism of GDF-15 in the progression of AP remains unclear and should be elucidated further.

The early presence of SIRS was associated with the severity of AP. We found that 64.3% (45/70) of patients presented with SIRS upon admission, of which 42.2% (19/45) developed persistent OF or died and were classified as having severe AP accordingly. If the levels of GDF-15 and PTX3 could identify in advance those who presented with SIRS and would develop persistent OF or dying, comprehensive treatment and referral to superior hospitals would occur sooner, and clinical outcomes might be improved. The results of this study revealed that GDF-15 and PTX3 are independent markers for severe AP in patients with SIRS on admission, which has clinical significance for decision-making at bedside.

There are interesting issues in our study, which differ from a study on a similar topic and the methods reported by Nieminen et al. First, comparing the sample and data collected across 6 years before 2012 in the other study, our study was performed according to the latest classification and management guidelines, and the results in the present study would be more likely to be applicable to current practice. Second, a short period of study conducted by the same group of clinical and laboratory researchers might reduce the heterogeneity of the clinical data and aid in successful laboratory measurements. Third, different commercially available multiplex panels and suspension array systems were used in the 2 studies, and it is noteworthy that we semiquantitatively screened the discriminable cytokines before quantitative measurement of target cytokines. Fourth, healthy controls were included to screen the cytokines specifically expressed in the disease conditions. Lastly, a shorter admission interval was adopted in this study (admitted within 48 hours of the onset of symptoms in this study compared with 48-hour intervals in Nieminen et al's study), and the discovery of the markers in our study might help to identify of severe pancreatitis in the initial stage.

Despite the potential interest in our findings, our study still had limitations. First, a small sample size in short-term studying period would likely preclude multivariate analysis, which attenuates the predictive value. Second, as a single-center study, the results only represent 1 cohort of patients in our center. Third, pancreatitis is a complex and heterogeneous disease that evolves over time to time, and the discriminative ability of the optimal cut-off levels of GDF-15 and PTX3 on admission, at only 1 time-point, need to be determined in larger studies.

## 5. Conclusions

Growth differentiation factor-15 and PTX3 were independent markers to identify the development of severe AP at an early stage. The discrimination power of GDF-15 and PTX3 for severe AP was higher than conventional markers, such as hematocrit, CRP, and APACHE-II. A multicenter, prospective, consecutive cohort study with a large sample should be carried out to validate the present findings.

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