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PAI-1 genetic polymorphisms influence septic patients' outcomes by regulating neutrophil activity

Shaowei Jiang[1], Yang Wang[1], Liang Chen[1], Honghua Mu[2], Connor Meaney[2], Yiwen Fan[3], Janesh Pillay[4], Hairong Wang[1], Jincheng Zhang[5], Shuming Pan[1], Chengjin Gao[1]

[1]Department of Emergency, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China;
[2]Division of Rheumatology, Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA;
[3]Department of Pathology Medicine Biology, University Medical Center Groningen, The Netherlands;
[4]Department of Critical Care, University Medical Center Groningen, The Netherlands;
[5]Department of Critical Care Medicine, Zhongshan Hospital, Fudan University, Shanghai 200032, China.

Abstract

Background: Plasminogen activator inhibitor-1 (PAI-1) plays an important role in the pathophysiology of sepsis, but the exact mechanism remains debatable. In this study, we investigated the associations among the serum levels of PAI-1, the incidence of 4G/5G promoter PAI-1 gene polymorphisms, immunological indicators, and clinical outcomes in septic patients.

Methods: A total of 181 patients aged 18–80 years with sepsis between November 2016 and August 2018 in the intensive care unit in the Xinhua Hospital were recruited in this retrospective study, with 28-day mortality as the primary outcome. The initial serum level of PAI-1 and the presence of rs1799768 single nucleotide polymorphisms (SNPs) were examined. Univariate logistic regression and multivariate analyses were performed to determine the factors associated with different genotypes of PAI-1, serum level of PAI-1, and 28-day mortality.

Results: The logistic analysis suggested that a high serum level of PAI-1 was associated with the rs1799768 SNP of PAI-1 (4G/4G and 4G/5G) (Odds ratio [OR]: 2.49; 95% confidence interval [CI]: 1.09, 5.68). Furthermore, a high serum level of PAI-1 strongly influenced 28-day mortality (OR 3.36; 95% CI 1.51, 7.49). The expression and activation of neutrophils (OR 0.96; 95% CI 0.93, 0.99), as well as the changes in the expression patterns of cytokines and chemokine-associated neutrophils (OR: 1.00; 95% CI: 1.00, 1.00), were both regulated by the genotype of PAI-1.

Conclusions: Genetic polymorphisms of PAI-1 can influence the serum levels of PAI-1, which might contribute to mortality by affecting neutrophil activity. Thus, patients with severe sepsis might clinically benefit from enhanced neutrophil clearance and the resolution of inflammation via the regulation of PAI-1 expression and activity.

Keywords: Gene polymorphism; Neutrophil; Plasminogen activator inhibitor-1; PAI-1; Sepsis; Single nucleotide polymorphism

Introduction

Sepsis is a life-threatening organ dysfunction caused by the host's defective response to infection and is defined as a complex clinical syndrome of physiological, pathological, and biochemical abnormalities caused by infection.[1,2] Sepsis was also one of the leading causes of the death associated with coronavirus disease 2019 (COVID-19).[3] Despite improved treatment options, sepsis remains a leading cause of death in intensive care units (ICUs).[2] Many biomarkers have been studied in patients with sepsis,[4-6] and the most significant features of sepsis are thrombocytopenia and disseminated intravascular coagulation (DIC).[7] In blood, the balance between coagulation and fibrinolysis is strictly regulated. Plasminogen activator inhibitor-1 (PAI-1) is a member of the serine protease inhibitor (serpin) family, which regulates fibrinolysis by inhibiting both tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). During sepsis, the coagulation system is quickly activated and amplified, and PAI-1 expression increases rapidly. Elevated PAI-1 levels have also been associated with severe consequences in numerous cases of sepsis.[8-10]

In addition to coagulation, PAI-1 plays an important role in the process of inflammation. PAI-1 is involved in the regulation of the host inflammatory response through

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Shaowei Jiang, Yang Wang, and Liang Chen contributed equally to this study.

Correspondence to: Dr. Chengjin Gao, Department of Emergency, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, No. 1665 of Kongjiang Road, Shanghai 200092, China
Email: gaochenjing@xinhuamed.com.cn;
Dr. Shuming Pan, Department of Emergency, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, No. 1665 of Kongjiang Road, Shanghai 200092, China
Email: panshuming@xinhuamed.com.cn

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the Toll-like receptor-4 (TLR4) -mediated activation of macrophages.[11] Under ischemia-reperfusion conditions, PAI-1 rapidly accumulates on microvascular endothelial cells, allowing this inhibitor to exhibit previously unrecognized functional properties by inducing an increase in the affinity of Beta-2 integrin on intravascular rolling neutrophils.[12] PAI-1 enhances lipopolysaccharide (LPS)-induced neutrophil activation[13] and promotes the migration and infiltration of neutrophils into inflammatory sites,[14] independent of its participation in coagulation and microvascular thrombosis. Inflammation and coagulation are interconnected and interact during sepsis. Inflammatory mediators activate coagulation, while intravascular coagulation causes inflammation.[15] Neutrophil activity is also amplified during sepsis, with a series of related genes and signaling pathways activated or suppressed;[16] however, the exact relation between PAI-1 and neutrophils remains unclear.

Although patients infected with the same microorganisms experience similar general clinical symptoms and undergo similar therapies, they can have different clinical outcomes, indicating that genetic factors are major factors involved in these processes. Genetic variation may play a role in the development and clinical outcomes of sepsis.[17] One of the signature markers frequently observed in the serum of patients with sepsis is PAI-1, which in humans is located on chromosome 7q21.3-22. There exists a 4G/5G polymorphism within the promoter region 675 base pairs upstream of the transcription start site that plays a role in regulating PAI-1 expression levels.[18]

In this study, we investigated the associations among the serum levels of PAI-1, the incidence of 4G/5G promoter PAI-1 gene polymorphisms, immunological indicators, and clinical outcomes in septic patients.

Methods

Ethical approval

The study design was approved by the Ethics Committee of Xinhua Hospital affiliated with the Shanghai Jiaotong University School of Medicine, China (No. XHEC-D-2020-010), and strictly conformed to the principles of the *Declaration of Helsinki* (Seoul, 2008). Written informed consent was obtained from either the patients or their legal representatives.

Patients

One hundred and eighty-one patients (68 women and 113 men) aged 18–80 years (average age: 58.7 ± 10.7 years) with sepsis between November 2016 and August 2018 in the ICU of Xinhua Hospital affiliated with Shanghai Jiaotong University School of Medicine, Shanghai, China, were recruited for this retrospective study.

Inclusion criteria

The inclusion criteria were in accordance with the Assessment of Clinical Criteria for Sepsis (Sepsis-3).[2] Patients were enrolled within 24 h after confirming the sepsis with the Sepsis-3 criteria.

Exclusion criteria

The exclusion criteria were as follows: age <18 years; pregnancy; lactation; hematopoietic malignancy; liver cirrhosis (Child–Pugh class C); treatment with immunosuppressive therapy (e.g., cyclosporine or azathioprine), cancer-related chemotherapy, or radiotherapy; human immunodeficiency virus (HIV) infection or end-stage AIDS; terminal disease with expected mortality; participation in any other investigational study (drug or device); and unwillingness or inability to be fully evaluated during the study period.

Data collection

The patients' 28-day mortality was monitored as the primary outcome. The following parameters and patient information were recorded at the time of admission to the ICU: sex; age; comorbid diabetes mellitus, hypertension, chronic heart failure, and chronic obstructive pulmonary disease; history of cancer; history of stroke; responsible microorganisms; and site of infection. "D-dimer and fibrinogen degradation products were detected by immunoturbidimetry, prothrombin time, and activated partial thromboplastin time, while thrombin time and fibrinogen were detected by coagulase. The Japanese Association for Acute Medicine (JAAM) DIC criteria[19] and the International Society on Thrombosis and Haemostasis (ISTH) criteria for overt DIC[20] were used to assess septic patients. Initial DIC scores were defined as the highest DIC scores within the first 24 h after ICU admission; maximum DIC scores were defined as the highest DIC scores during the 28-day hospitalization period. Initial DIC onset was defined as DIC occurring within the first 24 h after ICU admission; DIC onset was defined as DIC occurring within the first 28 days of hospitalization. DIC was managed with a combination of anticoagulants and the transfusion of plasma and/or platelets. The Sequential Organ Failure Assessment (SOFA)[2] and Acute Physiology and Chronic Health Evaluation (APACHE) II[21] scores were evaluated at the onset of sepsis. Follow-up evaluations were performed for up to a maximum of 28 days. To determine the immunological status of septic patients, human leukocyte antigen (HLA-DR); the percentage of cluster of differentiation 14 (CD14)+ HLA-DR+ ; the CD3, CD4, and CD8 counts; the percentages of CD3 and CD8; and the ratio of CD4 to CD8 were also analyzed. Furthermore, we also examined the serum levels of interleukin (IL)-1β, interleukin 2 receptor (IL-2R), IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ at admission.

The initial leukocyte count, neutrophil granulocyte count, and neutrophil-lymphocyte ratio (NLR) were recorded. The maximum leukocyte count, neutrophil granulocyte count, NLR, initial platelet count, and minimum platelet count during hospitalization were assessed. The serum samples with the highest NLR during hospitalization were evaluated with a proinflam-

matory chemokine panel (13-plex) (cat# 740003, BioLegend, San Diego, CA, USA) to measure the levels of C-X-C motif ligand 1 (CXCL1) (GROα), CXCL5 (ENA-78), CXCL8 (IL-8), CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC), C-C motif chemokine 2 (CCL2) (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL11 (eotaxin), CCL17 (TARC), and CCL20 (MIP-3α) on a flow cytometer (CytoFLEX S, Beckman, Brea, CA, USA). The PAI-1 antigen was detected with a specific enzyme-linked immunosorbent assay (Human PAI-1 ELISA Kit, cat# 1818272A, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

Determination of haplotypes, DNA extraction, and polymerase chain reaction (PCR)

A 5-mL sample of venous blood was collected from each patient into ethylenediamine tetraacetic acid (EDTA) sterile tubes and stored at −80°C until DNA extraction. Data on the haplotypes of PAI-1 are available on the NCBI website (https://www.ncbi.nlm.nih.gov/nuccore/). DNA was extracted with a kit according to the manufacturer's instructions (Axygen Scientific Inc., CA, USA). The 1.2-kilobase region upstream of the transcription start site on the promoter of PAI-1 was amplified with HD Taq (TOYOBO, Shanghai, China) and sequenced on an ABI 3730XL (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Quantitative variables are described as the means and standard deviations, while qualitative data are described as numbers and percentages. Univariate (logistic regression) and multivariate (stepwise logistic regression, with entrance and removal limits set at 0.10 and the inclusion of factors significant at P <0.05) analyses were performed to determine the factors associated with different genotypes of PAI-1, serum levels of PAI-1, and 28-day mortality. A P-value <0.05 was considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (CIs) were also calculated. The statistical software used was SAS version 9.4 (SAS Inc., Cary, NC, USA).

Results

Baseline characteristics

A total of 181 adult Chinese patients with sepsis were enrolled in this study, 113 of whom were male (62.4%) and 68 of whom were female (37.6%; Table 1). The patient ages ranged from 18 to 80 (average age 58.7 ± 10.7) years. Based on severity, the sepsis was bifurcated into sepsis (n = 137) and septic shock (n = 42). At baseline, the SOFA and APACHE II scores were 5.33 ± 3.29 (2.00–15.00) and 14.44 ± 8.50 (0–42.00), respectively [Table 1]. The initial and maximum DIC scores according to the JAAM and ISTH criteria were 2.17 ± 2.05 and 2.44 ± 1.39 and 3.01 ± 2.38 and 3.01 ± 1.48, respectively. Of all the enrolled patients, 32 (17.7%) died within 28 days. We also found that hypertension, chronic heart failure, chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), a history of cancer, and a history of stroke were the most common in this sample [Table 1].

Table 1: Baseline clinical characteristics of the population (n = 181).

Demographics	Values
Age (years)	58.7 ± 10.7 (18.0–80.0)
Sex (male)	113 (62.4)
Septic shock	42 (23.2)
Use of vasopressors	33 (18.2)
Initial SOFA score	5.33 ± 3.29 (2.00–15.00)
Initial APACHE II score	14.44 ± 8.50 (0–42.00)
Comorbidities	
Hypertension	74 (40.9)
COPD	6 (3.3)
Chronic heart failure	33 (18.2)
Diabetes mellitus	53 (29.3)
Cancer history	11 (6.1)
Stroke history	30 (16.6)
Site of infection	
Respiratory	84 (46.4)
Abdominal	66 (36.6)
Urinary	26 (14.4)
Bloodstream	3 (1.7)
Neurological	1 (0.6)
Soft tissue	1 (0.6)
Initial DIC score according to the JAAM criteria	2.17 ± 2.05 (0–8.00)
Initial DIC onset according to the JAAM criteria	40 (22.1)
Maximum DIC score according to the JAAM criteria	3.01 ± 2.38 (0–8.00)
DIC onset according to the JAAM criteria	70 (38.7)
Initial DIC score according to the ISTH criteria	2.44 ± 1.39 (0–8.00)
Initial DIC onset according to the ISTH criteria	9 (5)
Maximum DIC score according to the ISTH criteria	3.01 ± 1.48 (0–8.00)
DIC onset according to the ISTH criteria	20 (11)
28-day mortality	32 (17.7)

Data are presented as mean ± standard deviation with range or n (%) . Initial SOFA score: Highest Sequential Organ Failure Assessment scores within the first 24 h after intensive care unit (ICU) admission; Initial APACHE II score: Highest Acute Physiology and Chronic Health Evaluation II score within the first 24 h after ICU admission; COPD: Chronic obstructive pulmonary disease; DIC: Disseminated intravascular coagulation; ISTH: International Society on Thrombosis and Hemostasis; JAAM: Japanese Association for Acute Medicine.

Prognostic factors for PAI-1 (rs1799768)

All of the PAI-1 polymorphisms in patients were analyzed by genotyping. Four patients could not have their PAI-1 genotypes tested. The distributions of the 4G/5G genotypes were as follows: 64 (4G/4G), 77 (4G/5G), and 36 (5G/5G); the power of sample size according to the ratio of PAI-1 SNP was 0.56209. The PAI-1 rs1799768 SNPs (4G/4G and 4G/5G) were accompanied by higher serum levels of PAI-1 (OR: 0.40; 95% CI: 0.18, 0.91; chi-

squared value 4.72, *P*-value 0.03) [Supplementary Table 1, http://links.lww.com/CM9/B278]. Differential analyses among patients with the 4G/4G, 4G/5G, and 5G/5G genotypes were performed with univariate and multivariate logistic regression analyses, the results of which revealed a higher frequency of DM (OR: 3.98; 95% CI: 1.66, 9.49), lower maximum NLR (OR: 0.96; 95% CI: 0.94, 0.99), and lower serum levels of PAI-1 (OR: 0.40; 95% CI: 0.18, 0.91), and CCL5 (OR: 1.00; 95% CI: 1.00, 1.00) in 5G/5G patients than in either 4G/4G or 4G/5G patients [Table 2]. Higher serum levels of glycosylated hemoglobin (HbA1c) and higher APACHE II scores were observed in 5G/5G patients than in 4G/4G and 4G/5G patients using univariate logistic regression analysis; these data are shown in Table 2.

Prognostic factors for serum PAI-1 levels

As Table 2 suggests, there are multiple associations between PAI-1 SNP and diabetes, HbA1c, NLR, and higher serum PAI-1 levels. Increased initial serum levels of IL-1β (OR: 6.02; 95% CI: 1.33, 27.23) and decreased serum levels of CCL11 (OR: 0.99; 95% CI: 0.98, 1.00) were significantly associated with the serum levels of PAI-1 in the multivariate analysis. Higher initial serum levels of IL-2R and IL-6 and higher serum levels of CXCL8, CCL2, CCL3, CCL4, and CCL20 remained strongly associated with elevated PAI-1 serum levels in the univariate analysis, implying that elevated serum PAI-1 levels lead to excessive chemokine and cytokine reactivity. Thus, these tested biomarkers are closely consistent with clinical assessments, such as higher DIC scores based on the JAAM and ISTH criteria and a higher frequency of DIC onset based on the JAAM and ISTH criteria. Furthermore, the presence of the PAI-1 SNP (rs1799768) (4G/4G and 4G/5G) was associated with higher serum levels of PAI-1 [Table 3]. Furthermore, we examined 28-day survival for patients with different serum levels of PAI-1. The overall 28-day survival rate in patients with serum levels of PAI-1 >4363 ng/mL was much lower than that in patients with serum levels of PAI-1 ≤4363 ng/mL ($P = 0.0017$). The survival plot for patients with different serum levels of PAI-1 is shown in Figure 1.

Prognostic factors for 28-day mortality

Both univariate and multivariate logistic regression analyses were performed to identify prognostic factors for 28-day mortality. Shock (OR: 41.60; 95% CI: 6.17, 280.61) and increased serum levels of PAI-1 (OR: 7.29; 95% CI: 1.12, 47.60) were related to adverse clinical outcomes as determined by multivariate logistic regression analysis [Table 4]. There were multiple other important factors predictive of mortality in septic patients, including (but not limited to) demographics, chronic illness, surface marker expression by T-cells, cytokine and chemokine levels, sepsis disease severity scores, and DIC onset. Demographic characteristics that contributed to increased mortality were female and aged >80 years. Chronic illnesses, such as heart failure and COPD, were found to be important factors that were predictive of mortality. Decreased surface marker expression by T-cells (CD3 and CD4) and increased serum chemokine and cytokine levels (IL-6, CXCL1, CXCL8, CCL2, CCL5, and CCL20) were also significantly predictive of mortality. We confirmed that patients had higher initial APACHE II scores and higher initial SOFA scores, indicating increased sepsis severity. High DIC scores were clearly associated with mortality [Table 4].

Discussion

In this study, we found that a high serum level of PAI-1 was associated with the rs1799768 SNP of PAI-1 (4G/4G and 4G/5G). Furthermore, a high serum level of PAI-1 strongly influenced 28-day mortality. In addition, we examined the immunological profiles of septic patients with differential PAI-1 polymorphisms. Of interest, the expression and activation of neutrophils (NLR), as well as the changes in the expression patterns of cytokines and chemokine-associated neutrophils (CCL5), were affected by the genotype of PAI-1.

Higher plasma PAI-1 levels have been reported in septic patients, and PAI-1 plays an important role in the fibrinolytic response associated with sepsis,[8-10] which is closely associated with the genetic control of PAI-1 genotypes. In a recent population-based study investigating 1054 DNA specimens from healthy control subjects, the proportions

Table 2: Analysis of potential factors associated with different genotypes of PAI-1 (rs1799768).

Factors	4G/4G and 4G/5G (*n* = 141)	5G/5G (*n* = 36)	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)*
Diabetes mellitus				
No	106 (75.2)	17 (47.2)	1.00	1.00
Yes	35 (24.8)	19 (52.8)	3.38 (1.59, 7.22)	3.98 (1.66, 9.49)
HbA1c	6.64 ± 2.09	8.04 ± 2.94	1.25 (1.04, 1.50)	
NLR max	29.00 ± 23.87	17.28 ± 14.15	0.96 (0.94, 0.99)	0.96 (0.93, 0.99)
PAI-1 (ng/mL)				
≤4363	77 (54.6)	27 (75.0)	1.00	1.00
>4363	64 (45.4)	9 (25.0)	0.40 (0.18, 0.91)	0.26 (0.10, 0.69)
CCL5 (pg/mL)	2167.6 ± 1417.3	1604.6 ± 862.87	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Initial APACHE II score	13.75 ± 8.27	17.47 ± 8.70	1.05 (1.00, 1.10)	–

Data are presented as mean ± standard deviation or *n* (%). APACHE II: Acute Physiology and Chronic Health Evaluation; CCL5: C-C motif chemokine 5; CI: Confidence interval; HbA1C: Glycosylated haemoglobin; NLR: Neutrophil-lymphocyte ratio; OR: Odds ratio; PAI-1: Plasminogen activat inhibitor-1; –: Not applicable. *Univariate logistic regression and multivariate analyses were performed to determine the factors associated with different genotypes of PAI-1. *P*-value <0.05 was considered statistically significant.

Table 3: Analysis of potential factors predicting the serum level of PAI-1.

Factors	PAI-1 ≤4363 ng/mL (*n* = 106)	PAI-1 > 4363 ng/mL (*n* = 75)	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)*
Diabetes mellitus				
No	80 (75.5)	46 (61.3)	1.00	
Yes	26 (24.5)	29 (38.7)	1.94 (1.02, 3.68)	
Death				
No	95 (89.6)	54 (72.0)	1.00	
Yes	11 (10.4)	21 (28.0)	3.36 (1.51, 7.49)	
IL-1β on admission (pg/mL)				
<5	54 (94.7)	29 (76.3)	1.00	1.00
≥5	3 (5.3)	9 (23.7)	5.58 (1.40, 22.24)	6.02 (1.33, 27.23)
IL-2R on admission (U/mL)	1148.40 ± 889.48	1694.80 ± 1105.40	1.00 (1.00, 1.00)	
IL-6 on admission (pg/mL)	88.87 ± 172.10	196.86 ± 280.73	1.00 (1.00, 1.00)	
CXCL8 (pg/mL)	149.80 ± 187.27	310.34 ± 444.34	1.00 (1.00, 1.00)	
CCL2 (pg/mL)	793.55 ± 1059.80	1451.80 ± 1577.80	1.00 (1.00, 1.00)	
CCL3 (pg/mL)	16.79 ± 20.68	33.38 ± 44.24	1.02 (1.01, 1.03)	
CCL4 (pg/mL)	18.25 ± 24.51	35.31 ± 55.08	1.01 (1.00, 1.02)	
CCL11 (pg/mL)	191.68 ± 96.67	139.89 ± 104.05	1.00 (1.00, 1.00)	0.99 (0.98, 1.00)
CCL20 (pg/mL)	113.51 ± 173.40	175.87 ± 209.04	1.00 (1.00, 1.00)	
Maximum DIC scores according to the JAAM criteria	2.54 ± 2.39	3.67 ± 2.23	1.22 (1.08, 1.40)	
DIC onset according to the JAAM criteria				
No	75 (70.75)	36 (48.00)	1.00	
Yes	31 (29.25)	39 (52.00)	2.62 (1.41, 4.86)	
Maximum DIC scores according to the ISTH criteria	2.75 ± 1.45	3.36 ± 1.47	1.33 (1.08, 1.65)	
DIC onset according to the ISTH criteria				
No	99 (93.40)	62 (82.67)	1.00	
Yes	7 (6.60)	13 (17.33)	2.97 (1.12, 7.84)	
PAI-1 (rs1799768)				
5G/5G	27 (25.96)	9 (12.33)	1.00	
4G/4G and 4G/5G	77 (74.04)	64 (87.67)	2.49 (1.09, 5.68)	

Data are presented as mean ± standard deviation or *n* (%). CCL2: C-C motif chemokine 2; CI: Confidence interval; CXCL1: C-X-C motif ligand 1; DIC: Disseminated intravascular coagulation; IL: Interleukin; ISTH: International Society on Thrombosis and Haemostasis; JAAM: Japanese Association for Acute Medicine; OR: Odds ratio; PAI-1: Plasminogen activator inhibitor-1. *Univariate logistic regression and multivariate analyses were performed to determine the factors associated with the serum level of PAI-1.

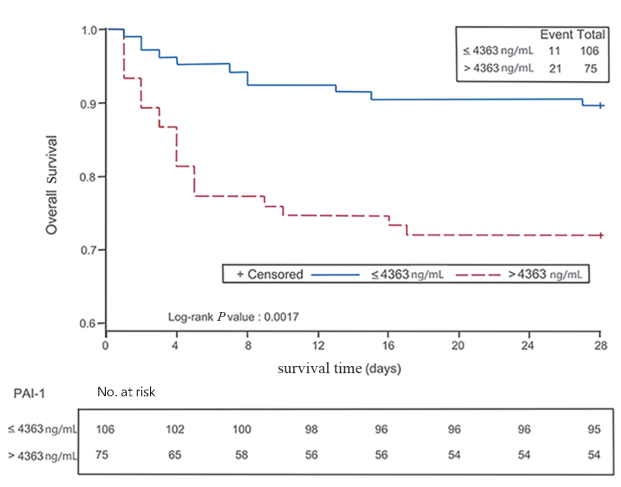

Figure 1: Survival plot for patients with different serum levels of PAI-1. The 28-day survival plot for patients with different serum levels of PAI-1. The overall 28-day survival rate in patients with serum levels of PAI-1 >4363 ng/mL was much lower than that in patients with serum levels of PAI-1 ≤4363 ng/mL (P = 0.0017). PAI-1: Plasminogen activator inhibitor-1.

of patients with the various genotypes were determined to be 30.9% (4G/4G), 49.4% (4G/5G), and 19.8% (5G/5G).[22] It has been shown that the 5G allele has an additional binding site for a repressor, therefore leading to lower transcription rates and less PAI-1 activity.[23] The 4G/4G genotype of the PAI-1 polymorphism was associated with a higher plasma PAI-1 concentration and higher mortality rate in patients with sepsis.[24-26] It is apparent that the elevation in PAI-1 levels after stressful events is much more pronounced in patients with the 4G allele than in patients with the 5G allele. Consequently, the higher PAI levels resulted in impaired fibrinolysis and impaired microcirculation that lead to dysregulated immune function.

In the present study, we provided novel findings showing that the 4G/4G and 4G/5G genotypes were much more closely associated with higher serum PAI-1 levels than the 5G/5G genotype (OR: 2.49; 95% CI: 1.09, 5.68), which is inconsistent with the findings of previous studies.[24-26] Furthermore, a lower serum level of PAI-1 (OR: 0.40; 95% CI: 0.18, 0.91) and a lower maximum NLR (OR: 0.96; 95% CI: 0.93, 0.99) were found in our 5G/5G allele patients than in the other patients, according to the results of the multivariate logistic regression analysis. As described in our previous study, increased NLR levels were independently associated with an unfavorable clinical prognosis in patients with sepsis.[27]

Table 4: Analysis of potential factors predictive of 28-day mortality.

Factors	Survivors (*n* = 149)	Non-survivors (*n* = 32)	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)*
Sex				
Male	98 (65.77)	15 (46.88)	1.00	
Female	51 (34.23)	17 (53.13)	2.18 (1.01, 4.71)	
Age				
<60 (years)	40 (26.85)	3 (9.38)	1.00	
60–80 (years)	72 (48.32)	10 (31.25)	1.85 (0.48, 7.12)	
>80 (years)	37 (24.83)	19 (59.38)	6.85 (1.87, 25.05)	
Heart failure				
No	134 (89.93)	22 (68.75)	1.00	
Chronic	15 (10.07)	10 (31.25)	4.06 (1.62, 10.17)	
Shock				
No	124 (83.22)	15 (46.88)	1.00	1.00
Yes	25 (16.78)	17 (53.13)	5.62 (2.48, 12.72)	41.60 (6.17, 280.61)
CD3 (1141–1880/μL)	615.31 ± 341.44	356.66 ± 255.93	1.00 (0.99, 1.00)	
CD4 (478–1072/μL)	374.84 ± 232.54	192.83 ± 119.33	1.00 (0.99, 1.00)	
IL-6 on admission (pg/mL)	113.02 ± 194.87	277.48 ± 377.55	1.00 (1.00, 1.00)	
PAI-1				
≤4363 (ng/mL)	95 (63.76)	11 (34.38)	1.00	1.00
>4363 (ng/mL)	54 (36.24)	21 (65.63)	3.36 (1.51, 7.49)	7.29 (1.12, 47.60)
CXCL1 (pg/mL)	160.36 ± 189.72	366.24 ± 676.96	1.00 (1.00, 1.00)	
CXCL8 (pg/mL)	172.16 ± 273.17	425.55 ± 466.43	1.00 (1.00, 1.00)	
CCL2 (pg/mL)	972.18 ± 121 9.1	1506.2 ± 1744.2	1.00 (1.00, 1.00)	
CCL5 (pg/mL)	2208.0 ± 1414.5	1540.1 ± 1249.7	1.00 (1.00, 1.00)	
CCL20 (pg/mL)	113.73 ± 172.28	261.29 ± 228.41	1.00 (1.00, 1.01)	
Initial APACHE II score	12.82 ± 7.49	22.38 ± 8.32	1.15 (1.09, 1.21)	
Initial SOFA score	4.68 ± 2.77	8.34 ± 3.85	1.38 (1.21, 1.56)	
Initial DIC score according to the JAAM criteria	1.97 ± 1.89	3.09 ± 2.51	1.27 (1.07, 1.51)	
Initial DIC onset according to the JAAM criteria				
No	122 (81.88)	19 (59.38)	1.00	
Yes	27 (18.12)	13 (40.63)	3.09 (1.36, 7.02)	
Maximum DIC score according to the JAAM criteria	2.77 ± 2.26	4.13 ± 2.66	1.26 (1.07, 1.47)	
DIC onset according to the JAAM criteria				
No	98 (65.77)	13 (40.63)	1.00	
Yes	51 (34.23)	19 (59.38)	2.81 (1.28, 6.14)	
Maximum DIC scores according to the ISTH criteria	2.80 ± 1.35	3.97 ± 1.71	1.70 (1.30, 2.24)	
DIC onset according to the ISTH criteria				
No	138 (92.62)	23 (71.88)	1.00	
Yes	11 (7.38)	9 (28.13)	4.91 (1.83, 13.15)	

Data are presented as mean ± standard deviation or *n* (%) except otherwise specified. APACHE II: Acute Physiology and Chronic Health Evaluation; CCL2: C-C motif chemokine 2; CI: Confidence interval; CXCL1: C-X-C motif ligand 1; DIC: Disseminated intravascular coagulation; IL: Interleukin; ISTH: International Society on Thrombosis and Haemostasis; JAAM: Japanese Association for Acute Medicine; OR: Odds ratio; PAI-1: Plasminogen activator inhibitor-1; SOFA: Sequential Organ Failure Assessment. *Univariate logistic regression and multivariate analyses were performed to determine the factors associated with 28-day mortality.

Diabetes mellitus patients are highly susceptible to sepsis.[28] Interestingly, a higher frequency of DM (OR: 3.98; 95% CI: 1.66, 9.49) was found in 5G/5G patients in the present study. As a further evidence, the higher serum levels of HbA1c (OR: 1.25; 95% CI: 1.04, 1.50) and higher APACHE II scores (OR: 1.05; 95% CI: 1.00, 1.10) were observed in 5G/5G septic patients. The patients with the 5G/5G genotype should have had a much higher 28-day mortality rate than those with the 4G/4G and 4G/5G genotypes, but there were no significant differences (OR: 1.47; 95% CI: 0.59, 3.62) [Supplementary Table 1, http://links.lww.com/CM9/B278]. We suppose that the lower transcription rates and lower PAI-1 activity due to the 5G/5G genotype counteracted the adverse effects of DM and the substantially higher HbA1c levels and APACHE II scores to a certain extent.

PAI-1 inactivates plasmin in the impaired fibrinolysis pathway[29] and is a key element involved in fibrinolysis inhibition in sepsis-induced DIC.[8] By conducting a single measurement of PAI-1 activity, persistent severe clotting disorders can be detected early in the course of

sepsis.[10] In the present study, an increased serum level of PAI-1 was clearly associated with the onset of DIC according to the JAAM criteria[19] (OR 2.62; 95% CI 1.41, 4.86). PAI-1 levels and shock were closely related to 28-day mortality.

Moreover, it has been well documented that serum PAI-1 can directly affect neutrophil activity, specifically the secretion of TNF-α and the chemokines CCL3, CCL4, CCL20, and CXCL8, upon the stimulation of neutrophils by LPS.[30] It is not surprising that the increased initial serum levels of IL-1β and decreased serum levels of CCL11 were significantly associated with the serum level of PAI-1 in the present study. Furthermore, increased serum levels of IL-2R, IL-6, CXCL8, CCL2, CCL3, CCL4, and CCL20 were also associated with higher serum levels of PAI-1. Interestingly, different serum levels of PAI-1 were not related to the maximum leukocyte count, neutrophil granulocyte count, percentage of neutrophils, or NLR in either univariate or multivariate logistic regression analyses [Supplementary Table 2, http://links.lww.com/CM9/B278]. There are two possible explanations. First, although the serum level of PAI-1 is controlled by genotypes of PAI-1, the patients were grouped together during the statistical analysis according to a cutoff value of the serum level of PAI-1. Second, the patients' baseline statuses were different; for example, the proportion of patients with DM was not the same across the different groups.

In conclusion, genetic polymorphisms of PAI-1 can influence the serum levels of PAI-1, which might cause mortality by affecting neutrophil activity. Thus, it would be clinically beneficial to control PAI-1 activity and modulate PAI-1 expression by enhancing neutrophil clearance and resolving inflammation in patients with severe sepsis. PAI-1 is likely to be a biomarker and a potential target for novel treatment of sepsis.

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Conflicts of interest

None.

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