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The prognostic value of platelet aggregation in patients with sepsis

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Abstract

Background: This study aims to investigate the relevance of platelet aggregation markers, specifically arachidonic acid (AA) and adenosine diphosphate (ADP), in relation to the prognosis of sepsis patients.

Methods: A cohort of 40 sepsis patients was included and stratified, based on their 28-day post-treatment prognosis, into two groups: a survival group (n = 31) and a severe sepsis group (n = 9). Then, their various clinical parameters, including patient demographics, platelet counts (PLT), inflammatory markers, and platelet aggregation rates (PAR) induced by AA and ADP between the two groups, were compared. Long-term health implications of sepsis were assessed using the Acute Physiologic Assessment and Chronic Health Evaluation II (APACHE II) score, and logistic regression analysis was conducted to evaluate the prognostic significance of PAR in sepsis patients.

Results: Patients with severe sepsis exhibited significantly elevated levels of procalcitonin (PCT), platelet adhesion rates, and PAR induced by ADP (P < 0.05), but having lower PLT (P < 0.05), compared to those in the survival group. Logistic regression analysis demonstrated that PAR induced by ADP was a protective factor in predicting prognosis in sepsis patients (P < 0.01).

Conclusions: Activation of platelets in sepsis intensifies inflammatory response. Patients with sepsis whose ADP-induced PAR was <60% displayed significant impairment in platelet aggregation function, and had higher mortality rate. Monitoring ADP-induced PAR is crucial for management of sepsis.

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Introduction

Sepsis is a common and severe complication typically arising from infections, septic shock, or traumatic injuries, often leading to the development of multiple organ dysfunction syndrome (MODS) or septic shock. Its key clinical manifestations include systemic inflammatory responses, immune dysregulation, and coagulation disturbances.¹⁻³ With a notably high incidence and mortality rate, sepsis is a primary contributor to fatality among critically ill patients. Notably, during the COVID-19 pandemic, a significant rise in infections was observed, with some individuals progressing to viral sepsis or secondary bacterial sepsis, frequently resulting in multiple organ failure and posing a substantial life-threatening risk.⁴

Platelets constitute an important class of immune cells, assuming a pivotal role in the immune response, particularly in the context of infection and sepsis. Numerous studies have demonstrated a strong correlation between reduced platelet counts (PLT) and mortality.⁵⁻⁷ In order to improve patient prognosis through early intervention, this study evaluated platelet aggregation rate (PAR), inflammatory markers, and the 28-day post-treatment survival outcomes of sepsis patients. Additionally, it aimed to analyze the correlation between these variables and patient prognostic outcomes.

Material and Methods

Objects

The basic clinical data of 40 eligible patients with sepsis, such as gender, age, diagnosis, and history of diseases, who received intensive care unit (ICU) treatment at our hospital, were collected. Among the 40 patients, 23 were male and 17 female patients, with an average age of 64 ± 19 years.

The inclusion criteria for this study were as follows: all the patients were diagnosed with sepsis 3.0 in accordance with the criteria proposed in 2016. Specifically, individuals with suspected or confirmed infections were classified as having sepsis if their sepsis-related organ failure assessment (SOFA) scores were ≥ 2 points. Additionally, all patients were required to be over 18 years of age and had been admitted to the hospital for more than 24 h.

The exclusion criteria for this study were as follows: patients who either died or were discharged within 24 h of admission to the hospital; patients with $PLT < 100 \times 10^9/L$;⁸ patients with other immune system disorders or liver and kidney dysfunction; and patients who had undergone treatment with antiplatelet drugs within the past week.

Based on their conditions 28 days after hospital admission, these patients were categorized into two groups: survival group ($n = 31$) and severe sepsis group ($n = 9$). Ethical approval for this study was obtained from the Ethics Committee of Shanghai Jing'an District Zhabei Central Hospital.

Within 2 h of admission, comprehensive patient data were collected, such as the Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, sequential organ failure assessment (SOFA) scores, disseminated

intravascular coagulation (DIC) scores, and the incidence of shock. Routine blood examination included PLT, red blood cell (RBC) count, and hemoglobin (Hb), and were analyzed using a BC-6900 automatic blood cell analyzer (Shenzhen Mindray, Shenzhen, China). Inflammatory markers, such as white blood cell (WBC) count, C-reactive protein (CRP), and procalcitonin (PCT) were measured utilizing a UPT-3A-1800 up-converting phosphor immunoassay analyzer (Beijing Hotgen, Beijing, China). Routine blood coagulation parameters included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB), thrombin time (TT), and fibrinogen degradation product (FDP). PAR induced by arachidonic acid (AA) and adenosine diphosphate (ADP) was determined with LBY-NJ4A automatic platelet aggregation detector (Beijing Precil, China). All tests were conducted in accordance with the instructions.

Indicators

Routine blood examination

The routine blood examination was conducted using the BC-6900 automatic hematology analyzer (Shenzhen Mindray), and parameters, such as RBC, WBC, and PLT, were recorded.

Procalcitonin

The expression level of PCT in the samples was detected using enzyme-linked immunosorbent serologic assay (ELISA; R&D Systems, Minneapolis, MN, US).⁹

Adhesion of blood platelets

Blood platelet adhesion was assessed using glass balloon flask method, in which 1.5-mL blood sample containing anticoagulant was introduced in a glass balloon flask containing citric acid. Subsequently, the flask was placed on a turntable and rotated at a speed of $0.5 \times g$ for 15 min. Following this, 1 mL of blood sample was analyzed in a tube that was treated with dimethyldichlorosilane. Hematology analyzer was used to enumerate PLT, and platelet adhesion was computed using the provided formula.

Platelet aggregation rate

Turbidimetry was used to measure PAR. Whole blood samples, treated with citric acid for anticoagulation, were centrifuged at $150 \times g$ for 10 min to obtain a supernatant containing platelet-rich plasma (PRP). Subsequently, the PRP was further centrifuged at $680 \times g$ for an additional 10 min to obtain platelet-poor plasma (PPP), which was stored under refrigeration for the future use. Using PPP as a reference, 300 μL of PRP was placed into a reaction cup of an automatic platelet aggregation analyzer (Beijing Prislabs, China) and preheated for 5 min. ATP was added to both PRP and PPP samples to measure PAR.

Statistics

Data analysis was conducted using SPSS version 25.0. Normally distributed measurement data were presented as mean \pm standard deviation ($\bar{x} \pm SD$) and included variables such as age, APACHE II score, WBC, PCT, PLT, adhesion rate of PLT, and PAR. Data with abnormal distribution were described as median and interquartile range (M [P25~P75]). Multi-factor logistic regression analysis was conducted to analyze independent risk factors associated with the early prognosis of sepsis patients; $P < 0.05$ was considered as statistically significant.

Results

Basic information

Our analysis showed no significant differences in terms of gender, age, or the source of infection among the sepsis patients ($P > 0.05$). However, SOFA scores of patients in the severe sepsis group were significantly higher than those in the survival group ($P < 0.05$). Conversely, there was no significant disparity in APACHE II scores between the two patient groups ($P > 0.05$). Additional information is provided in Table 1.

Comparison of inflammatory indexes

No significant difference was observed in inflammatory indexes (WBC, CRP, and PCT) in the patients of both groups ($P > 0.05$; Table 2).

Comparison of coagulation function

Our results showed that the thrombin time in the patients of severe sepsis group was significantly higher than that in the patients of survival group ($P < 0.05$). However, no significant differences were observed in other coagulation indexes, such as PT, APTT, FIB, FDP, and PLT ($P > 0.05$).

Table 1 Comparison of basic information between the two groups.

Variables	Survival group (n = 31)	Group with severe sepsis (n = 9)	P
Male (n [%])	23 (74.2)	4 (44.4)	0.086
Age (years)	68 (52~79)	82 (64~89)	0.047
Infection site			
Lungs	20 (64.5)	6 (66.7)	0.000
Extra-pulmonary organs	11 (35.5)	3 (33.3)	
Severity			
APACHE II scores	20 \pm 5	25 \pm 3	0.143
SOFA scores	6 (4~7)	9 (7~11)	0.012
RBC($\times 10^{12}/L$)	4.12 \pm 1.05	3.47 \pm 1.01	0.135
Hb (g/L)	118.62 \pm 28.73	103.69 \pm 31.08	0.472

Table 2 Comparison of inflammatory indexes between the two groups.

Variables	Survival group (n = 31)	Group with severe sepsis (n = 9)	P
Inflammatory indexes			
WBC ($10^9/L$)	10.27 (9.2~22.8)	15.45 (5.9~30.8)	0.932
CRP (mg/L)	87.3 (10.8~160.2)	164.5 (63.2~274.1)	0.062
PCT (ng/mL)	1.38 (0.19~10.72)	3.26 (1.24~17.81)	0.076

Regarding platelet aggregation function, it was observed that the PAR induced by AA and ADP in the patients of severe sepsis group was lower than those in the patients of survival group ($P < 0.05$). Further details are shown in Table 3.

Risk factors associated with the early prognosis of sepsis patients

In order to analyze the risk factors impacting the early prognosis of sepsis patients, 40 patients were included in this study and categorized into two groups: the survival group (n = 31) and the severe sepsis group (n = 9) based on their 28-day treatment outcomes. Several factors, such as age (≥ 65 years), PCT levels (≥ 10 ng/L), PLT ($< 50 \times 10^9/L$), platelet adhesion rate ($\geq 60\%$), PAR ($< 60\%$), and the use of vasoactive drugs, were analyzed. Logistic regression analysis was applied to evaluate the significance of these factors, and the results indicate that all these risk factors were statistically significant in both patient groups ($P < 0.05$) (Table 3). Additional details are available in Table 4.

Discussion

Blood platelets, which originate from non-nucleated cells derived from megakaryocytes, play pivotal roles in both coagulation and immune responses.¹⁰ Previous research has revealed that in patients with sepsis, the overutilization

Table 3 Comparison of coagulation function between the two groups of patients.

Project	Survival group (n = 31)	Group with severe sepsis (n = 9)	P
PT(s)	13.6 (74.2)	14.1 (44.4)	0.083
APTT (s)	31.5 (52~79)	29.7 (64~89)	0.412
TT (s)	15.2 \pm 1.6	17.8 \pm 2.3	0.001
FIB (g/L)	3.72 \pm 1.36	2.42 \pm 1.02	0.372
FDP ($\mu g/mL$)	6.42 (3.50~16.72)	9.72 (5.61~46.73)	0.472
PLT ($\times 10^9/L$)	183 (147~269)	167 (125~273)	0.381
PAR induced by AA (%)	78.3 (70.3~85.9)	50.2 (37.1~61.7)	0.012
PAR induced by ADP (%)	74.2 (62.3~76.3)	53.7 (30.6~67.2)	0.017

Table 4 Risk factors for early prognosis of patients with sepsis.

Risk factors	Regression coefficient	Standard error	Wald value	OR value	95% CI	P
Age \geq 65 years old	1.327	0.623	4.528	4.015	1.027~13.729	0.047
PCT \geq 10 ng/L	1.703	0.769	18.62	57.81	6.735~372.931	<0.001
PLT count $<$ $50 \times 10^9/L$	1.797	0.684	5.329	6.361	1.293~29.416	0.023
Platelet adhesion rate \geq 60%	1.524	0.634	5.681	4.619	1.582~19.491	0.020
Platelet aggregation rate \geq 60%	2.475	0.725	9.421	9.450	2.603~45.810	0.001
Vasoactive drugs	1.753	0.623	6.148	6.203	1.736~26.921	0.015

of platelets because of an exaggerated inflammatory response disrupts platelet production. During the early stages of sepsis, a substantial influx of inflammatory mediators and pathogenic microorganisms invades the host, triggering excessive activation, adhesion, and aggregation of platelets, thereby increasing their release function. This cascade of events can lead to the formation of local microthrombi, resulting in the consumption of a significant quantity of platelets and coagulation factors. In severe cases, it may even culminate in disseminated intravascular coagulation, leading to the development of multiple organ failure syndrome.¹¹

Early investigations conducted by Yaguchi et al. revealed that in patients with sepsis who had experienced a significant reduction in PLT, their PAR levels were decreased significantly.⁷ Furthermore, it was suggested that platelet aggregation is particularly sensitive to cyclooxygenase isoenzymes, COX-1 and COX-2, which includes AA, and can be substantially influenced by it. Additionally, in patients with normal PLT, it was observed that cyclooxygenase could inhibit platelet aggregation by as much as 50%. Additionally, Davies et al.¹² reported that the PAR induced by AA and ADP in patients with severe sepsis was significantly lower, compared to those with systemic inflammatory response syndrome.

Logistic regression analysis was employed to identify independent protective factors for sepsis patients. The results demonstrated that the prognosis of sepsis patients was closely associated with age, PCT levels, PLT count, PLT adhesion rate, PAR, and the utilization of vasoactive drugs. It is known that the aging process often leads to a weakened immune function in patients, diminishing their ability to combat external pathogens, contributing to disease progression, worsening its severity, and ultimately resulting in a poorer prognosis. PCT, as an inflammatory biomarker, was found to significantly increase in sepsis patients, making it a valuable indicator for predicting the severity of sepsis, and PLT plays a crucial role in sepsis by activating inflammatory factors, such as interleukin 6 (IL-6), to enhance their adhesion to endothelial cells, initiates coagulation responses in damaged endothelial cells, regulates vascular function, releases essential nutrients, and counteracts the harmful effects of oxygen free radicals. Hence, PLT can be considered a protective factor for sepsis patients.

The administration of vasoactive drugs during treatment can effectively inhibit platelet aggregation, thereby preventing unfavorable prognostic outcomes. Research has demonstrated that platelet aggregation function is significantly impaired in sepsis patients with a PAR induced by

ADP of less than 60%. Furthermore, these patients have a notably higher mortality rate and exhibit poorer responses to treatment.¹³

Conclusion

This study highlighted several significant findings related to sepsis patients. Age, PCT levels, PLT count, PLT adhesion rate, PAR, and the use of vasoactive drugs were identified as factors influencing sepsis patient prognosis. Aging was associated with weakened immunity and more severe disease outcomes. Elevated PCT levels indicated higher severity of sepsis, PLT was identified to have a protective role in sepsis by modulating inflammation and coagulation, use of vasoactive drugs was associated with improved outcomes, and impaired platelet aggregation, particularly a PAR induced by ADP of less than 60%, was linked to higher mortality and diminished treatment response.

Competing interests

The authors stated that there were no conflicts of interest to declare.

Consent to participate statement

Written informed consent was obtained from legally authorized representative(s) for anonymized patient information to be published in this article.

Data availability

The authors declare that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon request.

Author Contributions

Ting Chen and Song Zhong: designed and conducted the study. Ting Chen, Haohao Yang, Zheren Zhao, and Hui Wang: supervised data collection. Ting Chen, Haohao Yang, Zheren Zhao, and Hui Wang: analyzed the data. Ting Chen,

Haohao Yang, Zheren Zhao, and Hui Wang: interpreted the data. Ting Chen and Song Zhong: prepare the manuscript for publication and reviewed draft of the manuscript. Ting Chen, Haohao Yang, and Zheren Zhao contributed equally to the work and should be considered first co-authors. All authors read and approved the final manuscript.

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