Am J Transl Res 2023;15(6):4164-4171
www.ajtr.org /ISSN:1943-8141/AJTR0146876

Original Article

Prognostic value of coagulation and fibrinolysis function indexes and NETs for sepsis patients

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Received October 8, 2022; Accepted May 11, 2023; Epub June 15, 2023; Published June 30, 2023

Abstract: Objective: To clarify the role of coagulation and fibrinolysis as well as the level of neutrophil extracellular traps (NETs) in patients with sepsis, and to explore their clinical significance in identifying the disease and predicting the prognosis. Methods: In this retrospective study, the clinical data from 120 patients with sepsis admitted to People's Hospital of Changshou from January 2019 to December 2021 were analyzed. The patients were divided into a survival group and a death group according to the survival of patients within 28 days of admission. Another 120 patients with common bacterial infection were selected as the bacterial group and 120 healthy subjects who underwent physical examination in our hospital during the same period were selected as the healthy group. NETs, coagulation and fibrinolysis indexes, prothrombin time (PT), fibrinogen (FIB), D-dimer level, International Normalized Ratio (INR), Acute Physiology and Chronic Health Evaluation (APACHE) II score, and sequential organ failure assessment (SOFA) score of the patients with sepsis were compared with those of bacterial group and healthy group. Correlations between these measures were analyzed, and the predictive value of NETs for survival in patients with sepsis was assessed. Results: Compared with bacterial group and healthy group, the levels of serum NETs, PT, FIB, D-dimer, and INR value in sepsis patients were significantly increased. The level of NETs was positively associated with APACHE II score, SOFA score, PT, FIB, D-dimer, and INR. INR showed good performance in predicting death within 28 days after admission in sepsis patients. Conclusion: The NETs and coagulation indexes have high predictive value for the prognosis of patients with sepsis.

Keywords: Sepsis, NETs, PT, FIB, D-dimer, INR

Introduction

Globally, approximately 31.5 million people are diagnosed with sepsis every year, among them, 19.4 million sufferers develop severe sepsis, and nearly 5.3 million sufferers die from it [1]. The high morbidity and mortality of sepsis not only pose a threat to human life and health, but also increase the burden on society. In order to reduce the damage caused by sepsis, in-depth research on the mechanism of sepsis has become a topic in the medical field. Neutrophils are critical to the pathogenesis of sepsis and are an essential component of the body's innate immune response. They can not only phagocytose invading pathogens, but also directly contribute to the bactericidal effect of antibiotics [2]. In addition, in the process of uncontrolled excessive systemic inflammatory response in sepsis, neutrophils release a variety of cytokines, which lead to coagulation dysfunction and organ function damage through various pathways [3]. Studies have shown that NETs can participate in the occurrence and development of coagulation disorders in sepsis through various pathways [4]. The intricate interaction among inflammation-endothelial injury-coagulation and fibrinolysis disorders is the core mechanism of sepsis-related coagulation dysfunction [5].

In 2004, Brinkmann et al. first reported that activated neutrophils released an extracellular fiber that could limit the spread of various pathogens and named them neutrophil extracellular traps (NETs) [6]. The destruction of the structure of NETs by endonucleases and the phenomenon that NETs can interact with histone antibodies indicate that the main structures of NETs are DNA and histones [7]. Since

Table 1. Comparison of general data among three groups

Group	n	Male/Female (n)	Age ($\bar{x}$±sd, years)	BMI ($\bar{x}$±sd, kg/m^2)	Hypertension history (n)	Diabetes history (n)
Sepsis group	120	66/54	48.65±5.36	23.7±1.53	25	21
Bacterial group	120	65/55	49.12±5.16	23.3±1.49	21	18
Healthy group	120	63/57	48.39±5.51	23.5±1.32	19	15
x^2/F		0.157	0.575	2.284	1.051	1.176
P		0.925	0.563	0.103	0.591	0.555

Notes: BMI: body mass index.

histones are positively charged, NETs can bind to some negatively charged macromolecules such as heparin, thereby exerting biological effects. In terms of regulating the body's inflammatory response, NETs can promote the body's inflammatory response to inhibit the growth of pathogenic microorganisms, but are essential for the pathogenesis of some immune diseases, showing a "double-edged sword" effect [8]. Therefore, we speculate that coagulation and fibrinolysis function and NETs may have a potential impact on the prognosis of sepsis. At present, most of the related researches on sepsis and NETs are based on the basic research at cell and animal levels, and there is still a lack of relevant research on the clinical significance of plasma NETs in sepsis patients. The aim of this study was to observe the levels of coagulation and fibrinolysis function indexes and NETs in sepsis patients and to explore their clinical value in predicting the prognosis, in order to provide new ideas for early prognosis prediction and treatment optimization of sepsis.

Materials and methods

Clinical information

In this retrospective study, 120 sepsis patients treated in People's Hospital of Changshou from January 2019 to December 2021 were enrolled as study subjects. Inclusion criteria: (1) Those who met the diagnostic criteria for sepsis revised by the American College of Critical Care Medicine in 2016 [9]. (2) Those with an age ≥18 years. (3) Those with predicted hospital stay ≥28 days. Exclusion criteria: (1) Patients complicated with human immunodeficiency virus (HIV) infection. (2) Those who had been treated with radiotherapy and chemotherapy for a long time. (3) Patients associated with autoimmune disease or congenital immune deficiency and genetic metabolic disease. (4) Patients with severe heart, liver and kidney insufficiency. (5) Patients who had received immunosuppression or glucocorticoid therapy shortly before the admission. (6) Patients with blood system diseases.

Another 120 patients with common bacterial infection during the same period were selected as the bacterial group, and 120 healthy subjects who underwent physical examination in our hospital during the same period were selected as the healthy group, in which the inclusion and exclusion criteria for the bacterial group were the same as those for sepsis (except for the first inclusion criteria). The gender, age, BMI, hypertension history, and diabetes history of the 3 groups were well balanced (all P>0.05, **Table 1**). This study was approved by the Ethics Committee of the People's Hospital of Changshou.

Laboratory index detection

Blood samples were collected from patients with sepsis and common bacterial infection within 24 hours of admission, and healthy patients in the morning of the day of physical examination. Fasting venous blood of 9 mL was collected and injected into a dry tube, sodium citrate anticoagulation tube, and EDTA anticoagulation tube of 3 mL each.

After the blood samples in the dried test tube were coagulated, the supernatant was centrifuged (Sorvall Legend Micro17 centrifuge, Thermo Fisher Scientific) at 3000 r/min for 15 min, 4°C, with a centrifugal radius of 10 cm, and the serum was stored at -20°C for testing. The level of NETs was detected by myeloperoxidase (MPO)-DNA complex capture enzyme-linked immunosorbent assay. Serum samples were taken, 100 μL (1:50) diluted serum was added to each well of a 96-well plate, and anti-MPO monoclonal antibody (5 μg/mL, Bio-rad, USA) was added and incubated overnight at

4°C followed by PBS wash for three times; then, peroxidase substrate (Bio-rad, USA) was added, and the captured MPO-DNA complexes were detected according to the instructions of the apoptosis kit (Roche Diagnostics GmbH). Mark microplate absorbance spectrophotometer (American Bio-rad Company) was used to detect the absorbance value at 450 nm. Total bilirubin and serum creatinine levels were detected by Roche Cobas8000. Samples from EDTA anticoagulant test tube were taken. Mindray BC-30S automatic blood cell analyzer was used to check blood routine, and record white blood cell count. Blood samples from sodium citrate anticoagulation test tube were centrifuged at 3000 r/min for 10 min with a centrifugal radius of 10 cm, and then Sysmex CS5100 automatic coagulation analyzer (Shanghai Dacheng Medical Equipment Co., Ltd.) was used to detect prothrombin time (PT) (kit lot numbers: No. 2400069, Sichuan Food and Drug Administration (license) 2012), fibrinogen (FIB) (kit lot numbers: No. 2400100, Anhui Food and Drug Administration (license) 2011), and D-dimer (kit lot numbers: No. 2400458, Su Food and Drug Supervision (license) 2014), and the International Normalized Ratio (INR) was calculated. The kits were purchased from Suzhou Liangchen Biomedical Technology Co., Ltd.

Condition assessment

Sepsis patients were scored with Acute Physiology and Chronic Health Evaluation (APACHE) II within 24 hours after admission to the intensive care department. The APACHE II score was calculated according to age, acute physiology, and chronic health. According to the APACHE II score, they were divided into 0-10 points group, 10-20 points group and >20 points group. In addition, sepsis patients were scored with sequential organ failure assessment (SOFA). SOFA was calculated according to daily respiratory system, blood system, circulatory system, nervous system indexes, bilirubin, creatinine and urine volume. Based on the SOFA score, they were divided into 0-5 points group, 5-10 points group and >10 points group.

Clinical intervention, outcome tracking and related data collection

The pathogenic bacteria in all sepsis patients were identified at an early stage, and sensitive antibiotics were applied according to drug susceptibility tests. In addition, goal-directed fluid resuscitation, vasoactive drugs to maintain blood pressure, and tissue and organ function support were given. The survival status of the sufferers within 28 d of admission was counted, and the sufferers were divided by a survival group and a death group. The related factors influence prognosis of sufferers with sepsis were collected, including age, gender, infection site, blood gas indexes, laboratory indexes, APACHE II score, SOFA score, etc.

Statistical methods

SPSS 22.0 was adopted for statistical analysis. Measurement data in line with normal distribution were expressed as ($\bar{x}\pm sd$), and the comparison was conducted using one-way ANOVA or independent sample t test. Enumeration data were expressed as cases (%) and compared using the x^2 test. Pearson or Spearman correlation analysis was applied to analyze the correlation of NETs with coagulation and fibrinolysis indexes, APACHE II and SOFA score. Logistic stepwise regression analysis was conducted to analyze risk factors for death within 28 days after admission in sepsis patients. ROC curve was used to analyze the predictive value of NETs and SOFA scores in predicting the death within 28 days after admission in sepsis patients. The test level was $\alpha=0.05$, and $P<0.05$ indicated significant difference.

Results

Comparison of NETs, coagulation and fibrinolysis index levels among 3 groups

Compared with bacterial group and healthy group, sepsis group presented significantly higher expression levels of Serum NETs, PT, FIB, D-dimer, and INR (all $P<0.05$, **Table 2**).

Comparison of serum NETs levels among sepsis patients with different APACHE II and SOFA scores

There were 34 patients with APACHE II score of 0-10 points, 54 with the score of 11-20 points, and 32 with the score of >20 points. There were 40 cases with SOFA score of 0-5 points, 55 cases with 6-10 points, and 25 cases with >10 points. There were obvious distinctions in serum NETs levels among sepsis patients with

Table 2. Comparison of NETs, coagulation and fibrinolysis index among three groups ($\bar{x}\pm sd$)

Group	n	NETs (ng/mL)	PT/s	FIB (g/L)	D-dimer (mg/L)	INR
Sepsis group	120	1.34±0.35*,#	13.51±2.68*,#	4.98±1.31*,#	1.12±0.31*,#	1.74±0.28*,#
Bacterial group	120	0.36±0.43	12.33±1.02	2.91±0.87	0.36±0.11	1.08±0.24
Healthy group	120	0.43±0.12	12.26±1.14	2.73±0.65	0.34±0.07	1.04±0.11
F		334.400	18.650	194.400	629.400	375.600
P		<0.001	<0.001	<0.001	<0.001	<0.001

Notes: *represents comparison with healthy group, *P*<0.05; #represents comparison with bacterial group, *P*<0.05. NETs: neutrophil extracellular traps; PT: prothrombin time; FIB: fibrinogen; INR: international normalized ratio.

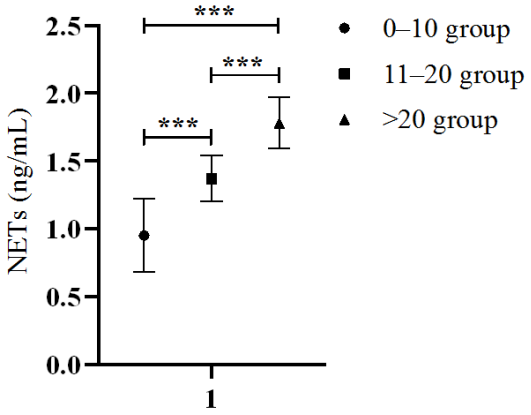

Figure 1. Serum NETs levels in sepsis patients with different APACHE II scores. Notes: NETs: neutrophil extracellular traps; APACHE: Acute Physiology and Chronic Health Evaluation. *** indicates *P*<0.001.

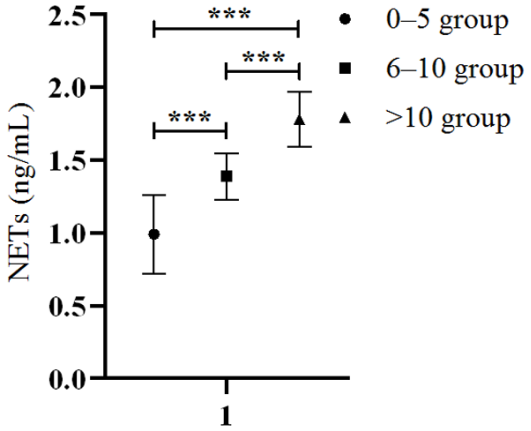

Figure 2. Serum NETs levels in sepsis patients with different SOFA scores. Notes: NETs: neutrophil extracellular traps; SOFA: sequential organ failure assessment. *** indicates *P*<0.001.

different APACHE II and SOFA scores (F1=131.500, F2=113.500, both P<0.001, **Figure 1**). The level of serum NETs in the group with APACHE II score >20 points (1.78±0.19 ng/mL) was higher than that in the group with the score of 11-20 points (1.37±0.17 ng/mL) and the group with the score of 0-10 points (0.95±0.27 ng/mL; t1=10.350, t2=14.360, t3=8.966, all P<0.001). The level of serum NETs in the group with SOFA score >10 points (1.78±0.19 ng/mL) was higher than that in the group with the score of 6-10 points (1.39±0.16 ng/mL) and the group with the score of 0-5 points (0.99±0.27 ng/mL; t1=9.522, t2=12.770, t3=9.031, all P<0.001, **Figure 2**).

Table 3. Correlation analysis of APACHE II score, SOFA score, coagulation and fibrinolysis function indexes with NETs

Index	r	*P*
APACHE II score	0.901	<0.001
SOFA score	0.911	<0.001
PT	0.421	<0.001
FIB	0.437	<0.001
D-dimer	0.453	<0.001
INR	0.420	<0.001

Notes: APACHE: Acute Physiology and Chronic Health Evaluation; SOFA: sequential organ failure assessment; PT: prothrombin time; FIB: fibrinogen; INR: international normalized ratio.

Correlation analysis of NETs with APACHE II score, SOFA score, coagulation and fibrinolysis function indexes

NETs was positively correlated with APACHE II score, SOFA score, PT, FIB, D-dimer, and INR (all P<0.05, **Table 3**); and it's associations with APACHE II and SOFA score were high (all r>0.9) while the associations with others were moderate (all r<0.05).

Comparison of NETs and coagulation and fibrinolysis function indexes among sepsis patients with different prognosis

Among the 120 sepsis patients, 34 (28.33%) died. Compared with the survival group, the

Table 4. Analysis of clinical characteristics of sepsis patients with different prognosis

Factor	Survival group (n=86)	Death group (n=34)	x^2/t	*P*
Gender			0.015	0.903
Male	47	19		
Female	39	15		
Age (years)	48.74±5.18	48.41±5.86	0.303	0.763
BMI (kg/m^2)	23.61±1.62	23.81±1.32	0.640	0.523
Infection site			0.339	0.953
Lung	29	13		
Abdomen	22	8		
Urethra	26	9		
Other	9	4		
APACHE II score (points)	11.79±3.88	20.18±6.46	8.728	<0.001
SOFA score (points)	6.28±2.27	11.74±3.53	10.050	<0.001
NETs (ng/mL)	1.23±0.30	1.60±0.33	5.917	<0.001
PT/s	12.69±2.39	15.59±2.14	6.163	<0.001
FIB (g/L)	4.61±1.09	5.93±1.32	5.622	<0.001
D-dimer (mg/L)	1.02±0.24	1.39±0.28	7.253	<0.001
INR	1.64±0.24	1.97±0.25	6.708	<0.001

Notes: BMI: body mass index; APACHE: Acute Physiology and Chronic Health Evaluation; SOFA: sequential organ failure assessment; NETs: neutrophil extracellular traps; PT: prothrombin time; FIB: fibrinogen; INR: international normalized ratio.

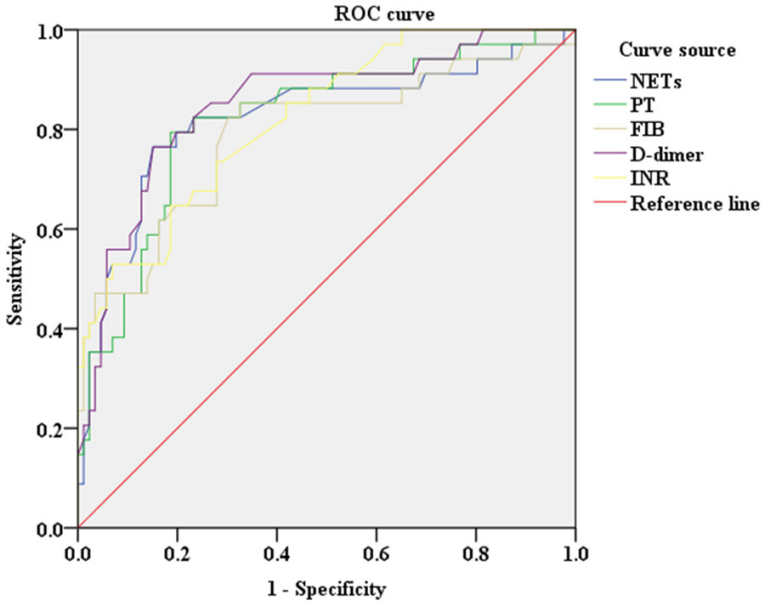

Figure 3. ROC analysis of NETs and coagulation and fibrinolysis function indexes in predicting death within 28 d after admission in sepsis patients. Notes: NETs: neutrophil extracellular traps; ROC: receiver operator characteristic.

death group presented significantly higher expression levels of APACHE II score, SOFA score, NETs level, PT, FIB, D-dimer and INR (all P<0.05, **Table 4**).

The value of NETs and coagulation and fibrinolysis function indexes in predicting the prognosis of sepsis patients

The areas under ROC curves of NETs, PT, FIB, D-dimer, and INR for predicting death within 28 d after admission in sepsis patients were 0.822, 0.820, 0.791, 0.851, and 0.821, respectively (**Figure 3**), indicating they all had high good predictive value (**Table 5**).

Discussion

In the process of sepsis, neutrophils are activated and generate a large number of reactive oxygen species (ROS) through the reduced coenzyme II (NADPH) oxidase pathway. The nuclear membrane of neutrophils is first dissolved, following with the release of nuclear contents into the cytoplasm. The cell membrane is lysed, and the chromatin inlaid with various proteins is released outside the cell to form NETs [10-12]. As the core component of NETs, the level of histones can reflect the severity of sepsis; in addition, when pathogens invade the body, the activated histones can undergo various modifications, affecting the body's immune system and causing further damage [13, 14]. At the same time, NETs are also the carriers of multiple anti-infective effects, which aggregate pathogens and granule proteins together, increasing antibacterial activity and synergy between different components.

Table 5. Sensitivity and specificity of NETs and coagulation and fibrinolysis function indexes in predicting the prognosis of sepsis patients

Index	Area	Sensitivity (%)	Specificity (%)	Cut-off value
NETs (ng/mL)	0.822	0.765	0.849	1.475
PT/s	0.820	0.794	0.814	14.86
FIB (g/L)	0.791	0.853	0.674	5.19
D-dimer (mg/L)	0.851	0.765	0.849	1.225
INR	0.821	0.647	0.814	1.815

Notes: NETs: neutrophil extracellular traps; PT: prothrombin time; FIB: fibrinogen; INR: international normalized ratio.

In this study, we found that high level of NETs was associated with sepsis, which is similar to a previous study [15]. NETs are released by neutrophils that can trap and kill microorganisms, suppressing infection. NETs can increase antibacterial activity by modifying histones and can also form a defense network together with elastase, defensin and reactive oxygen species to capture and kill invasive pathogens, thus playing a role in host defense [16]. Studies have shown that the production of intravascular NETs in a mouse model of sepsis can improve the clearance rate of Escherichia coli and inhibit the spread of bacteria [17, 18]. It can be seen that the increased level of NETs indicates the aggravation of infection and the more severe sepsis. NETs can be used as an effective indicator to determine the severity and prognosis of sepsis, which is also confirmed by the results of this study. NETs have prothrombotic properties, and excessive production of NETs can activate endothelial cells, antigen-presenting cells, and platelets, leading to immune-inflammatory responses and triggering thrombosis [19]. Sepsis can further enhance coagulation activity and inhibit fibrinolytic activity, and induce blood hypercoagulability, while coagulation disorders can aggravate disease progression. It can be seen that NETs may participate in the pathogenesis of sepsis by inducing abnormal coagulation mechanism. The specific mechanisms are as follows: First, NETs provide a scaffold structure and a source of stimulation for thrombosis. NETs can capture pathogenic bacteria and platelets, promote platelet activation and aggregation, and induce red thrombus [20]. Second, NETs can also promote the formation of various thrombin such as von Willebrand factor, fibronectin, fibrinogen, coagulation factor XII, tissue factor, etc. by providing scaffolds for erythrocytes and procoagulant molecules, and the histone DNA of NETs. The scaffold enhances the stability of the fibrin in the thrombus and prolongs the clot lysis time [21]. In conclusion, NETs affect the blood supply of multiple vital organs throughout the body of sepsis patients by promoting and reinforcing thrombosis, resulting in multiple organ failure and even death.

We also found that the levels of PT, FIB, D-dimer and INR in sepsis patients increased with the progression of the disease, indicating that the body was in a hypercoagulable state with secondary hyperfibrinolysis. There is a certain correlation between coagulation and fibrinolysis. Moreover, APACHE II score, SOFA score, levels of NETs, PT, FIB, D-dimer and INR value of death patients significantly increased, suggesting that activation of secondary fibrinolytic system is essential for the pathophysiology of sepsis, and hyperfibrinolysis is related to the severity of sepsis. After the cross-linking of fibrin monomer and activating factor, hydrolysis produces a specific fibrinolytic process marker, which is D-dimer. The rise of D-dimer level indicates that there is a secondary fibrinolytic process in the patient's body, which can also indirectly reflect the patient's coagulation state. Especially with the aggravation of sepsis, there is a certain degree of coagulation dysfunction, leading to hyperfibrinolysis, thus D-dimer in the patients significantly increases, suggesting that there is a close correlation between coagulation disorder and sepsis severity [22]. Coagulopathy that usually represents the increase of FIB, PT, TT, APTT, INR and D-dimer, is closely related to the progress of sepsis. It runs through the whole pathological process and is the key to the development and prognosis of sepsis.

The aim of this study was to clarify the levels of coagulation, fibrinolysis and neutrophil extracellular traps (NETs) in patients with sepsis, and to explore their clinical significance in the judgment of disease prognosis, which has certain clinical value. However, this study is a single-center study with a small number of cases that may lead to biased results. Further studies with multi-center and large sample size data are needed to confirm the results in the future. In conclusion, the levels of NETs, PT, FIB, D-dimer, and INR in sepsis patients elevate,

and these indicators are all related to in-hospital death of sepsis patients. NETs and coagulation and fibrinolysis function indexes have high value in predicting the prognosis of sepsis and can be applied as potential indicators for the prognosis judgment of sepsis patients.

Disclosure of conflict of interest

None.

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