Soluble CD14 Subtype in Peripheral Blood is a Biomarker for Early Diagnosis of Sepsis

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ABSTRACT

Objective: To study the value of serum soluble CD14 subtype (sCD14-ST) in early diagnosis of sepsis.

Methods: Seventy-two patients were diagnosed with systemic inflammatory response syndrome, sepsis, or septic shock. Peripheral blood was collected at 0, 12, 24, and 48 hours after admission to the hospital. Levels of sCD14-ST, procalcitonin (PCT), hypersensitive C-reactive protein (CRP), and white blood cells (WBC) were determined.

Results: Levels of sCD14-ST in the patients with septic shock were higher than those in the other patients ($P < .01$) and peaked at 48 h. PCT and CRP levels were similar in the patients at admission but increased by 5 times to 10 times in the next 48 h, especially in the patients with septic shock. WBC levels remained high and did not change dramatically. Receiver operating characteristic analysis revealed that the area under the curve, sensitivity, and specificity values of sCD14-ST to diagnose sepsis were much higher than those of the other markers.

Conclusion: Compared with PCT, CRP, and WBC, sCD14-ST is a better biomarker for the early diagnosis of sepsis.

Keywords: soluble CD14 subtype, sepsis, procalcitonin, C-reactive protein, white blood cell, diagnosis, biomarker

Sepsis is a systemic inflammatory response caused by local infection, which develops rapidly and can lead to death with multiple tissue damage and multiple organ failure.[1,2] The mortality rate of sepsis can be up to 40%, and the incidence of sepsis has increased by 1.5% per year.[3,4] For every 1-hour delay in effective antibiotic treatment during the first 6 hours of sepsis, the death rate of patients increases by about 7.6%,[5] and antibiotic treatment after 24 hours of the onset of sepsis is generally not effective.[6] A 1991 consensus conference developed the initial definition of sepsis, which included an excessive focus on inflammation and on the inadequate specificity and sensitivity of the systemic inflammatory response syndrome (SIRS) criteria.[7] The Third International Consensus Conference defined sepsis as "life-threatening organ dysfunction caused by a dysregulated host response to infection."[8]

Early diagnosis of sepsis is very important to reduce mortality. Currently, procalcitonin (PCT) is used as a biomarker in the clinical diagnosis of sepsis. The level of PCT increases significantly in sepsis, especially in patients with positive blood culture or septic shock.[9] However, false-negative results might occur in some patients with sepsis, especially in the early stage when the PCT level is low.[10,11] Furthermore, PCT can be nonspecifically elevated in certain clinical contexts, such as some end-organ dysfunctions, postsurgical anastomotic leaks, acute kidney injury, cardiogenic shock, anaphylaxis, and complications after intracerebral hemorrhage.[12,13] Blood culture is the "gold standard" for the diagnosis of sepsis; however, it usually

Abbreviations:
sCD14-ST, soluble CD14 subtype; PCT, procalcitonin; CRP, C-reactive protein; WBC, white blood cells; SIRS, systemic inflammatory response syndrome; LPS, lipopolysaccharide; mCD14, membrane bound-type CD14; ICU, intensive care unit; ROC, receiver operating characteristic; SOFA, sequential organ failure assessment; AUC, area under the curve; 95% CI, 95% confidence interval.

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takes 48 to 72 hours, and its sensitivity is low.[14] In addition, C-reactive protein (CRP) and white blood cells (WBC) are commonly used markers to screen for infection; however, in the diagnosis of sepsis, their specificity and reliability are low.[15] Moreover, besides infection, high levels of CRP and WBC are found in noninfectious stress, trauma, aseptic inflammation, and tumors.[15]

CD14 is the receptor for the lipopolysaccharide (LPS)–LPS binding protein complex and exists in 2 forms, the membrane bound type (mCD14) and the soluble type (sCD14).[16] sCD14 exists in plasma and originates from secreted mCD14.[16–18] CD14 mediates the cell response to LPS and activates the Toll-like receptor 4-specific proinflammatory signaling cascade.[19] CD14 is also involved in the recognition of a wide variety of other bacterial products, such as peptidoglycans of gram-positive bacteria, and it reaches high levels in the early stage of sepsis.[17–19] During systemic inflammation or bacterial infections, circulating plasma proteases cleave the soluble fraction of CD14 to generate a truncated form named sCD14 subtype (sCD14-ST), also known as presepsin.[20] The release of sCD14-ST is a very fast response. Chenevier-Gobeaux et al[21] constructed an in vitro experimental model of sepsis by challenging THP-1 cells, a human monocytic cell line, with LPS, and found that sCD14 could be detected at 1 h and peaked at 3 h after LPS exposure. To observe the changes in sCD14-ST in patients with sepsis and explore its potential value in the early diagnosis of sepsis, we detected the serum sCD14-ST levels in patients with SIRS or sepsis at 0, 12, 24, and 48 hours after admission to the intensive care unit (ICU) and compared its diagnostic value for sepsis with that of WBC, CRP, and PCT using receiver operating characteristic (ROC) analysis. We found sCD14-ST levels increased in the very early stage, especially in patients with septic shock. The sensitivity and specificity of sCD14-ST to diagnose sepsis were better than those of PCT, CRP, and WBC.

Materials and Methods

Patients

Seventy-two patients were recruited from the ICU, emergency, trauma surgery, and infection medicine departments at Shengzhou People's Hospital (the First Affiliated Hospital of Zhejiang University Shengzhou Branch) from September 2017 to April 2019. Twenty-eight patients were diagnosed as having SIRS on the basis of the following clinical features: temperature of >38 °C or <36 °C; heart rate >90 beats/min; respiratory frequency >20 breaths/minute or $Paco_2$ of <32 mm Hg (4.27 kPa); and WBC > 12*10 E9/L or <4*10 E9/L. Twenty patients were diagnosed as having sepsis with a (sepsis-related) sequential organ failure assessment (SOFA) score of 2 points or more. Twenty-four patients were diagnosed as having septic shock with acute circulatory failure characterized by hypotension that could not be attributed to other reasons. The exclusion criteria were severe hypohepatia, severe renal dysfunction, autoimmune diseases, history of malignant tumor and diabetes, and age <18 years or >70 years. This study received approval from the ethics review boards of Shengzhou People's Hospital (the First Affiliated Hospital of Zhejiang University Shengzhou Branch). All subjects were volunteers and provided written informed consent to participate in the study.

Laboratory Tests

Ten milliliters of each patient's blood was drawn into 3 EDTA-coated tubes (2 mL), 1 common tubes (2 mL/tube), and a blood culture bottle, at 0, 12, 24, and 48 hours after admission to the hospital.

Mitsubishi PATHFAST and sCD14-ST (Presepsin) kits (Tokyo, Japan) were used to measure sCD14-ST concentrations. The whole blood sample was directly analyzed using a chemiluminescence enzyme-linked immunosorbent assay in the PATHFAST Presepsin fully automated chemiluminescence immunoassay system. The detection time was 21 min. The assay allowed the fast and convenient detection of sCD14-ST (presepsin). The reference interval for the sCD14-ST level was 55 to 184 pg/mL. PCT was measured using the Roche Elecsys BRAHMS PCT system (Mannheim, Germany) with a reference interval of 0.1 to 0.5 ng/mL. CRP levels were measured using a Lifotronic PA-990 analyzer (Shenzhen, China) with a reference interval of 0 to 10 mg/mL. The WBC counts were determined using a Sysmex XN-9000 hematology analyzer (Kobe, Japan) with a reference interval of 4×10^9/L to 10×10^9/L.

Statistical Analysis

Statistical analyzes were performed using SPSS for Windows version 20.0 (IBM Corp, Armonk, NY, USA). A 1-way analysis of variance was used to compare the data from more than 3 groups. The data from all time points were used for ROC analysis. All tests were 2-tailed, and *P* values of <.05 were considered statistically significant.

Table 1. Clinical Information for Patients with SIRS and Sepsis

Patient Clinical Characteristic	SIRS	Sepsis	Septic shock
Age, mean ± SE, y	51.83 ± 5.32	47.57 ± 6.12	53.96 ± 8.74
Sex (male/female), No.	12/16	9/11	13/11
SOFA score, mean ± SE	2.15 ± 0.74	3.26 ± 0.93	5.43 ± 0.85
Location of infection, No. (%)			
Respiratory tract	13 (46.43)	9 (45.00)	9 (37.50)
Gastrointestinal tract	4 (14.29)	3 (15.00)	9 (37.50)
Genitourinary tract	5 (17.86)	2 (10.00)	4 (16.67)
Other sites	6 (21.43)	6 (30.00)	2 (8.33)
Blood culture, No. (%)			
Klebsiella pneumoniae (n = 9)	2 (7.14)	2 (10.00)	5 (20.83)
Escherichia coli (n = 6)	1 (3.57)	3 (15.00)	2 (8.33)
Adenoid bacterial (n = 4)	2 (7.14)	0 (0.00)	2 (8.33)
Enterococcus faecalis (n = 2)	1 (3.57)	1 (5.00)	0 (0.00)
Staphylococcus aureus (n = 4)	1 (3.57)	1 (5.00)	2 (8.33)

SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment.

Results and Discussion

A total of 72 sepsis patients were recruited in this study, including 34 males and 38 females, aged 36 years to 69 years (average, 52.3 years ± 8.57 years). Infection sites included the respiratory system in 31 cases, the digestive system in 16 cases, the urinary system in 11 cases, and other sites in 14 cases. Twenty-five cases provided positive blood cultures, including 9 cases of *Klebsiella pneumoniae*, 6 cases of *Escherichia coli*, 4 cases of *adenoid bacterial*, 2 cases of *Enterococcus faecalis*, and 4 cases of *Staphylococcus aureus*. The treatment of patients included basic and symptomatic treatments and antibiotics. The use of antibiotics was based on blood culture and drug sensitivity tests or on empirical medicine. The detailed clinical information is shown in **Table 1**.

Biomarkers such as WBC, CRP, and PCT are widely used in the clinical diagnosis of sepsis; however, their specificity and sensitivity are not high enough, especially in the early stage of sepsis.[15,22] Here, we compared the values of WBC, CRP, PCT, and sCD14-ST in the early diagnosis of sepsis. We found that WBC levels were significantly increased in all patients with SIRS or sepsis upon admission, and there was no significant difference among the 3 groups of patients. WBC levels remained high, and there were no significant changes within 48 hours after admission (**Figure 1**). ROC curve analysis showed that the area under the curve (AUC) of WBC for the diagnosis of sepsis was only 0.579 ± 0.076 ($P > .05$) (**Table 2**). Poor sensitivity (42.4%) and specificity (39.8%) scores indicated that WBC had poor diagnostic value in the early diagnosis of sepsis. CRP is another indicator used to diagnose infection; however, it also lacks specificity.[23] In the present study, we found that patients with sepsis had a high level of CRP when they were admitted to hospital, with no significant difference among the 3 groups. CRP levels gradually increased within 48 hours after admission, and the increases in the severe sepsis and septic shock groups were significantly higher than that in the general sepsis group ($P < .05$) (**Figure 1**). ROC curve analysis showed that the AUC of CRP in the diagnosis of sepsis was 0.710 ± 0.067 ($P < .05$). The sensitivity of CRP in the diagnosis of sepsis was high (89.3%); however, its specificity was poor (52.8%) (**Table 2**).

PCT is a stable inflammatory indicator with a long half-life of up to 30 hours.[22] In recent years, it has been widely used to diagnose sepsis. In this study, we found that patients with sepsis had a high level of PCT on admission, with no significant difference among the 3 groups. The PCT levels gradually increased within 48 hours after admission, and the increase in the septic shock group was significantly higher than that in the other 2 groups ($P < .05$) (**Figure 1**). ROC curve analysis showed that the AUC of PCT in the diagnosis of sepsis was 0.824 ± 0.096 ($P < .05$), and the sensitivity (84.2%) and specificity (85.7%) of PCT were significantly higher than those of WBC and CRP, which was consistent with the results of a previous study.[24] Although PCT levels in the severe sepsis and septic shock groups were significantly higher than those in the general sepsis group, there was no difference in PCT levels among the 3 groups at

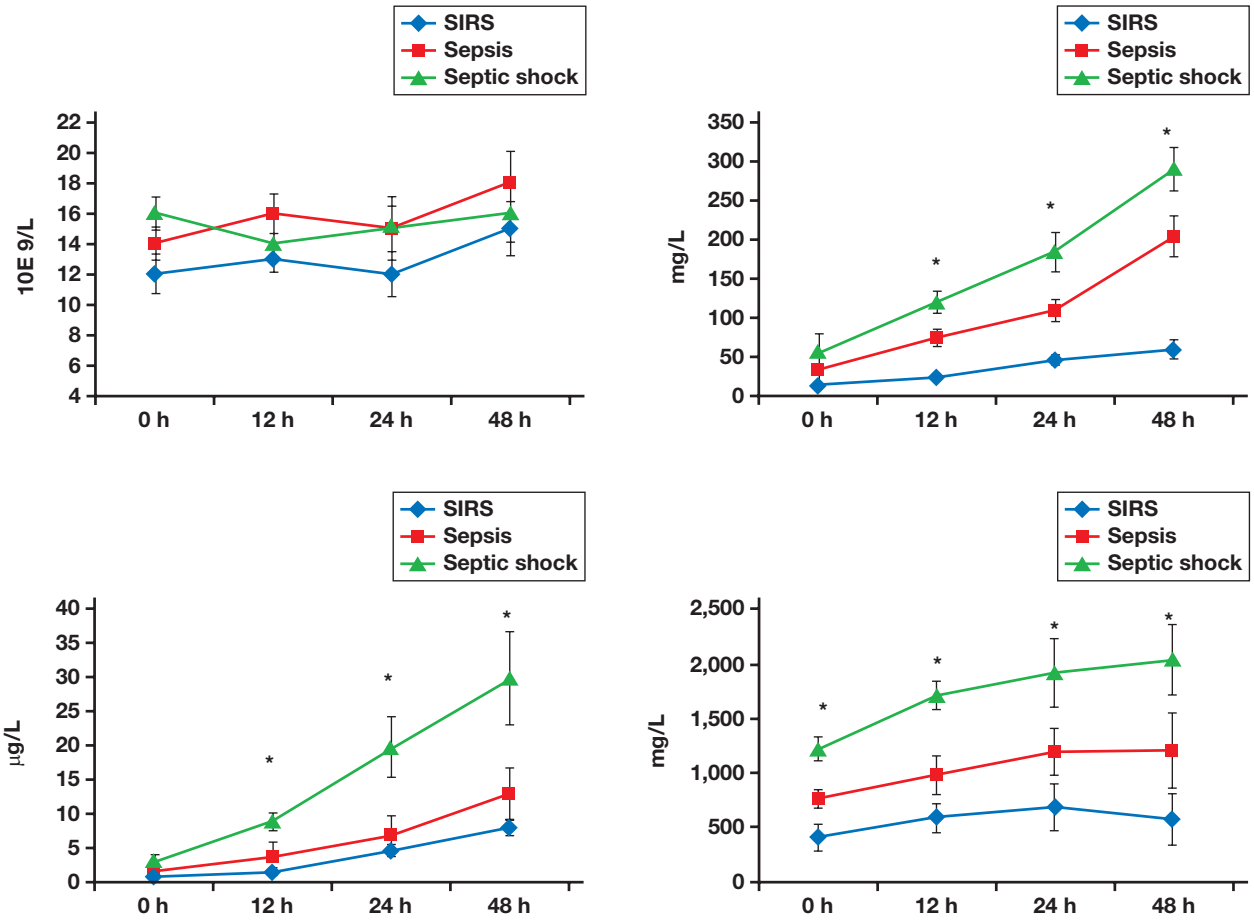

Figure 1

Serum levels of WBC, CRP, PCT, and sCD14-ST of patients with sepsis at 48 hours after admission. Data are shown as the mean ± standard error; *, $P < 0.05$. WBC, white blood cells; CRP, C-reactive protein; PCT, procalcitonin; sCD14-ST, soluble CD14 subtype.

Table 2. ROC Curve Analysis of WBC, CRP, PCT, and sCD14 to Diagnose SIRS and Sepsis

Diagnosis	Detection	Cutoff	AUC, mean ± SE	*P* Value	Sensitivity	Specificity	95% CI
SIRS	WBC	13.79	0.56 ± 0.38	.13	0.52	0.31	0.41–0.70
	CRP	42.09	0.63 ± 0.24	.05	0.81	0.42	0.47–0.86
	PCT	1.78	0.79 ± 0.08	.00	0.76	0.64	0.56–0.97
	sCD14-ST	463.84	0.72 ± 0.01	.18	0.93	0.79	0.58–0.90
Sepsis	WBC	16.76	0.61 ± 0.10	.02	0.42	0.40	0.50–0.79
	CRP	63.81	0.70 ± 0.13	.00	0.79	0.68	0.51–0.80
	PCT	2.13	0.83 ± 0.07	.00	0.84	0.75	0.71–0.94
	sCD14-ST	512.34	0.91 ± 0.05	.00	0.92	0.94	0.69–0.98
Septic shock	WBC	18.93	0.58 ± 0.08	.08	0.41	0.35	0.52–0.68
	CRP	78.46	0.75 ± 0.05	.01	0.86	0.54	0.53–0.91
	PCT	2.75	0.81 ± 0.01	.00	0.88	0.77	0.62–0.87
	sCD14-ST	682.42	0.96 ± 0.08	.00	0.95	0.90	0.73–1.00

ROC, receiver operating characteristic; WBC, white blood cells; CRP, C-reactive protein; PCT, procalcitonin; sCD14-ST, soluble CD14 subtype; AUC, area under curve; 95% CI, 95% confidence interval.

admission, suggesting that PCT cannot be used to assess the severity of sepsis in the early stage.

Studies have found that sCD14-ST could be used to evaluate the disease severity and prognosis of patients with sepsis;[25,26] however, no studies have used sCD14-ST to evaluate sepsis in the early stage. In this study, we found that the levels of sCD14-ST in different sepsis groups were significantly different upon admission, with the highest level in patients with septic shock, a middle-high level in the severe sepsis group ($P < .05$), and the lowest level in the general sepsis group. Within 48 hours after admission, sCD14-ST levels increased in all 3 groups, with the largest increase in the patients with septic shock ($P < .05$) (**Figure 1**). The ROC curve showed that the AUC was 0.948 ± 0.063 ($P < .05$), the sensitivity was 91.7%, and the specificity was 92.9% (**Table 2**), suggesting that sCD14-ST has good diagnostic value for patients with sepsis. Meanwhile, the different sCD14-ST levels in the early stage among the different septic groups suggested that sCD14-ST is a good predictor of the disease severity and prognosis of patients with sepsis, which is consistent with the study of Kweon et al.[27] Animal experiments showed that the concentration of sCD14-ST in rabbit plasma began to rise at 2 hours after infection and peaked at 3 hours, with half-life of 4 hours to 5 hours.[28] The peak time and half-life of the PCT levels were much longer than those of sCD14-ST, indicating the higher value of sCD14-ST than that of PCT for the early diagnosis of sepsis.

In conclusion, the results of our study indicated that sCD14-ST has a higher sensitivity and specificity than those of the conventional laboratory indexes and might be a reliable biomarker for the early diagnosis of sepsis. However, currently, sCD14-ST is not Food and Drug Administration-approved test for the diagnosis of sepsis. Physicians must evaluate the conditions and clinical indices of patients comprehensively to make an overall assessment for sepsis diagnosis rather than focusing only on a single biomarker. Moreover, dynamic monitoring of sCD14-ST during the course of the disease is advisable. **LM**

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