Original Article
Correlation between soluble CD14 subtype, platelet activating factor levels, and the severity of sepsis

Wen Yang, Yanbing Liang, Hao Tang, Zhenyu Li, Jingguo Wu, Lijin Zeng, Zhongfu Ma

Department of General Internal Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou City, Guangdong Province, P. R. China

Abstract: Objective: To investigate the correlation between soluble CD14 subtype (sCD14-ST), platelet activating factor (PAF) levels, and the severity of sepsis. Methods: Sixty-five septic patients admitted to our hospital from January 2015 to April 2017 were enrolled in this study. They were randomly assigned to the sepsis group (n=30), the severe sepsis group (n=20) or the septic shock group (n=15) in terms of the severity of disease. The plasma sCD14-ST and PAF levels, and the APACHE II scores were compared among the patients in the three groups. Correlations among the plasma sCD14-ST and PAF levels, and the APACHE II score were detected with the use of Pearson correlation analysis. In addition, the plasma sCD14-ST and PAF levels were measured in 50 healthy volunteers, and the value of the plasma sCD14-ST and PAF levels for diagnosis of sepsis was tested by the receiver operating characteristic (ROC) curves. Results: Plasma sCD14-ST and PAF levels, and the APACHE II scores were increased sequentially and significantly among the sepsis group, the severe sepsis group, and the septic shock group (all P<0.05). There were positive correlations of APACHE II scores with plasma sCD14-ST levels (r=0.805, P=0.003) and the PAF levels (r=0.712, P=0.014). The ROC curve analysis indicated that plasma sCD14-ST (P=0.015) and PAF (P=0.011) levels were of value in differentiating patients with sepsis, and the differences were statistically significant. Conclusion: The plasma sCD14-ST and PAF levels can be used as one of the markers for early sepsis diagnosis, and is favorable for assessing the severity in sepsis.

Keywords: Sepsis, severe sepsis, septic shock, soluble cluster of differentiation 14 subtype, platelet activating factor

Introduction

Sepsis, one of the common conditions encountered in intensive care units, is severe and has high mortality in patients. Poorly controlled sepsis may progress into severe sepsis, septic shock, or multiple organ failure [1]. Accordingly, early diagnosis and identification of sepsis and timely formulation of reasonable protocols are of great significance for good prognosis. Recently, biomarkers used in combination or alone in clinical diagnosis of sepsis include procalcitonin (PCT), interleukin and C-reactive protein (CRP). However, the sensitivity and specificity of these biomarkers are not high or it takes a long time to get the results, therefore all these factors are unfavorable for early assessment of the disease [2]. The PCT level has been shown to be elevated in patients with non-septic conditions such as large-area trauma, organ transplantation, or pancreatitis [3]. Blood cul-

ture is the gold standard for septic diagnosis, yet it takes 48 to 72 hours to get the result of a blood culture and it is time-consuming, and has a low positive rate [4]. It is noteworthy that it is of great clinical value to find a highly efficient and simple biomarker for early diagnosis of sepsis.

Recent studies have found that soluble cluster of differentiation 14 subtype (sCD14-ST) concentration in the peripheral blood increases dramatically in septic patients, and increases earlier and faster than PCT and CRP [5, 6]. Other studies indicate platelet-activating factor (PAF) plays a crucial role in the onset and progression of sepsis [7, 8]. However, no previous reports have been involved in the correlations among the plasma sCD14-ST, PAF levels, and septic shock in patients. Therefore, the purpose of this study was to clarify the value of differentiation, diagnosis, and prognosis of the plasma

sCD14-ST and PAF levels in septic patients, with an aim to bring some insights into clinical treatment.

Materials and methods

Patients

Between January 2015 and April 2017, 65 septic patients admitted to our hospital were recruited in this study. The enrolled patients included 42 male and 23 female patients, with a mean age of 58±7.9 years. In accordance with the severity of the disease, the patients were assigned to the sepsis group (n=30), the severe sepsis group (n=20) or the septic shock group (n=15). Patients older than 18 years old were eligible for enrollment if they met the new criteria for diagnosis of sepsis specified in the guidelines of ACCP/SCCM International Sepsis Definition Conference [9]. Sepsis was defined as the presence of clinically-confirmed infection and systemic inflammatory response syndrome (SIRS). Severe sepsis was defined as the presence of sepsis, organ dysfunction, hypoperfusion, or sepsis-induced hypotension. Septic shock was defined as sepsis-induced persistent hypotension, with a systolic blood pressure < 90 mmHg or over 40 mmHg lower than basic blood pressure. Patients were excluded if they had immunodeficiency disease, autoimmune disease, granulocytisis (<0.5*10^9), tumor, or had received immunosuppressive therapy within 3 months before enrollment. Patients who were pregnant, hospitalized for less than 48 hours, or provided incomplete clinical data were also excluded from this study. The eligible patients provided written informed consent, and the Hospital Ethics Committee reviewed and approved the protocol of this study.

sCD14-ST and PAF determination

Before antibiotic therapy within 24 h after admission, 5 mL of blood was collected by an endotoxin-free anticoagulation tube containing ethylenediaminetetraacetic acid (EDTA, anticoagulation agent) from the medial cubital vein of each patient, followed by plasma isolation from the collected blood at 2500 r/min for 10 min and detection of the plasma sCD14-ST levels with the use of a PATHFAST analyzer (Mitsubishi, Japan) for chemiluminescent immunoassay. The PAF levels of patients were detected by the enzyme-linked immunosorbent assay (ELISA), strictly following the instructions on the ELISA kits (R&D Science, US).

Outcome measures

The sCD14-ST and PAF levels in plasma and the Acute Physiology and Chronic Health Evaluation (APACHE) II scores of patients were compared among the three study groups. The APACHE II scores included the acute physiological scores (0-48 points), healthy condition scores (2-5 points) and age (0-6 points). Scores range from 0 to 59, with higher score indicating more severe disease. Moreover, the correlations among the sCD14-ST and PAF levels, and the APACHE II scores were analyzed among the three study groups.

Additionally, 50 healthy volunteers were assigned as normal controls, and their plasma sCD14-ST and PAF levels were measured. The receiver operating characteristic (ROC) curve was established according to the plasma sCD14-ST and PAF levels of healthy volunteers and septic patients, and the value of the plasma sCD14-ST and PAF levels in diagnosing sepsis was assessed based on the area under the curve (AUC).

Statistical analysis

All the data analyses were performed with SPSS software, version 21.0. Measurement data are presented as the mean ± standard deviation, with one-way analysis of variance (ANOVA) with post hoc Bonferroni's test utilized for the comparisons across the three groups. Count data are described as percentages, with Chi-square tests for comparisons across the three groups, and Chi-square segmentation tests for comparisons between two groups. Correlations among the sCD14-ST and PAF levels, and the APACHEII scores were identified by Pearson correlation analysis. The ROC curve was established, and the ROC was utilized to evaluate the value of the sCD14-ST and PAF levels for diagnosing sepsis. A P value less than 0.05 were deemed as statistically significant.

Results

Patient characteristics at baseline

There were 30 patients in the sepsis group, 20 in the severe sepsis group and 15 in the septic

Table 1. Patient characteristics at baseline

Variable	Case	Age (year)	M/F (n)	BT (°C)	RR (bpm)	HR (bpm)	LA (mmol/L)	WBC (10^9/L)
SG	30	55.6±7.2	19/11	37.6±1.2	24.5±8.3	103.4±15.9	2.1±1.1	12.1±5.1
SSG	20	60.4±8.1	14/6	37.4±1.0	23.7±7.4	108.7±16.5	2.5±1.0	16.9±9.2
SSGR	15	59.7±7.8	9/6	37.8±0.8	24.8±8.7	109.4±16.9	2.7±1.2	17.3±6.8
F/χ^2		0.339	0.415	0.132	0.015	0.119	0.198	0.480
P		0.725	0.813	0.879	0.986	0.889	0.826	0.640

Note: M/F denotes male/female, BT body temperature, RR respiratory rate, HR heart rate, LA lactic acid, WBC white blood cell count, SG sepsis group, SSG severe sepsis group, and SSGR septic shock group.
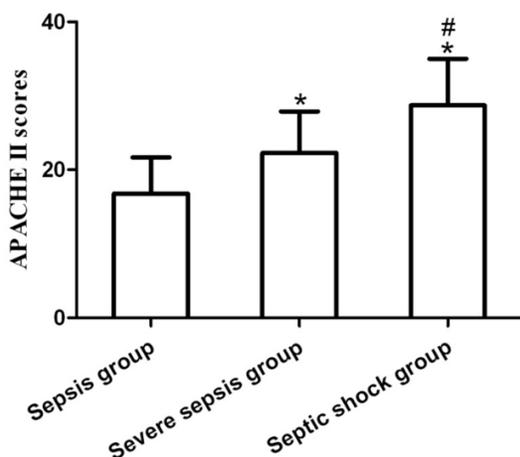

Figure 1. Comparison of the APACHE II scores among the three groups. Compared to the sepsis group, *P<0.001; Compared to the severe sepsis group, #P<0.001.

Table 2. Comparison of the sCD14-ST and PAF levels among the three groups

Variable	Case	sCD14-ST (pg/ml)	PAF (ng/L)
SG	30	725.6±216.3	1075.7±90.5
SSG	20	1412.8±635.7*	1256.3±107.6*
SSGR	15	2478.5±732.4*,#	1383.1±117.3*,#
F		28.105	29.358
P		0.000	0.000

Note: compared with the sepsis group, *P<0.001; compared with the severe sepsis group, #P<0.001.

Table 3. Correlations of the APACHE II scores with the sCD14-ST level and the PAF level

Marker	Coefficient	P
sCD14-ST	0.805	0.003
PAF	0.712	0.014

shock group. Age, sex, white blood cell counts, lactic acid levels and vital signs were generally well-balanced among the three groups, so they were comparable (P>0.05, Table 1).

APACHE II scores

The APACHE II score was 16.8±4.9 in the sepsis group, 22.3±5.6 in the severe sepsis group and 28.7±6.3 in the septic shock group; the differences in the APACHE II scores were statistically significant among the three groups (P<0.001), with the highest score in the septic shock group, the second highest score in the severe sepsis group, and the lowest score in the sepsis group (Figure 1).

Plasma sCD14-ST and PAF levels

The plasma sCD14-ST and PAF levels of patients were increased sequentially in the sepsis group, the severe sepsis group, and the septic shock group, with substantial differences among the three groups (Both P<0.001; Table 2).

Correlations of the APACHE II scores with the sCD14-ST level and the PAF level

Pearson correlation analysis demonstrated that the sCD14-ST level was positively correlated with the APACHE II score, and the PAF level was also positively correlated with the APACHE II score, with statistical differences (Both P<0.05), as reported in Table 3.

Fifty healthy volunteers were assigned as normal controls. According to the ROC curve analysis showing the value of the markers sCD14-ST and PAF for sepsis diagnosis, the AUC for sCD14-ST was 0.906, with the best cut-off value at 850.8 pg/mL. The AUC for PAF was 0.779, with the best cut-off value at 987.6 ng/L, suggesting that the markers sCD14-ST and PAF were of value in diagnosing sepsis and

Table 4. ROC curve analysis for sepsis diagnosis by the markers sCD14-ST and PAF

Marker	AUC	SE	P	95% CI
sCD14-ST (pg/ml)	0.906	0.058	0.015	0.459-0.681
PAF (ng/L)	0.779	0.064	0.011	0.561-0.907

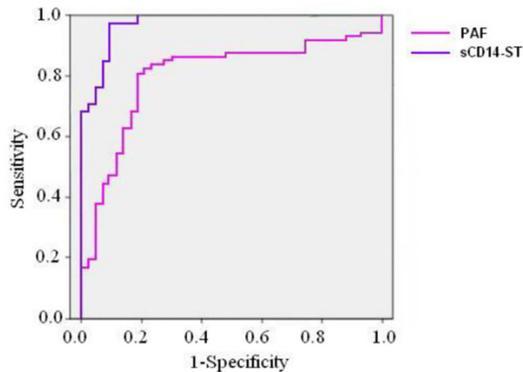Note: AUC denotes area under the curve, SE standard error, and CI confidence interval.

Figure 2. The ROC curves for sepsis differentiation by the markers sCD14-ST and PAF.

dramatically different in sepsis diagnosis (P<0.05), as illustrated in **Table 4** and **Figure 2**.

Discussion

Sepsis is an infection-induced systemic inflammatory response syndrome and one of the major causes of death in critically ill patients. Early differentiation of high-risk patients is favorable for clinicians to formulate timely treatment measures, and improve the recovery rate of patients with sepsis. PCT, CRP, and WBC are the primary biomarkers for evaluating the conditions and prognosis of septic patients. Among them, studies on PCT are the most and used most widely. Previous studies reveal that significant increases in PCT concentrations are observed in patients with severe trauma or undergoing operations [10, 11]. Of note, the prediction of prognosis, and differentiation and diagnosis of sepsis by PCT or other markers alone tend to result in missed diagnosis or misdiagnosis. Therefore, it is of significance to find effective biomarkers for monitoring the patients with sepsis [12].

Soluble cluster of differentiation cell surface molecules disappear or newly-emerge in the process when hematopoietic stem cells are dif-

ferentiated into different lineages, at differentiating stages, or in the course of activation. CD14 is a receptor for lipopolysaccharide-lipopolysaccharide binding protein, and exists as soluble CD14 and membrane CD14 in the human body. Recent studies have indicated that sCD14-ST has certain advantages in the diagnosis and prognosis of sepsis [13, 14]. According to our current study, the sCD14-ST levels were elevated sequentially in the sepsis group, the severe sepsis group, and the septic shock group, and was remarkably different among the three groups, consistent with the results reported in previous studies [15, 16]. In another study, elevated sCD14-ST level is significantly better than PCT, WBC, and CRP at differentiating sepsis, which is attributed to the low specificity of PCT, WBC, and CRP, and the fact that their expression levels affected by multiple factors [17]. The results of Pearson correlation analysis revealed a positive correlation between the sCD14-ST and APACHE II scores. The ROC curve analysis demonstrated that the AUC showing the value of the sCD14-ST level in diagnosing sepsis was greater than 0.5, which was substantially different, suggesting that sCD14-ST level is valuable in the diagnosis of patients with sepsis, and correlated with the disease progression in septic patients.

PAF, a potent bioactive phospholipid, produced from leukocytes, platelets, endotheliocytes, and various cells and tissues in the lungs, liver, and kidneys [18, 19]. PAF also promotes the secretion of the inflammatory cytokines including TNF-1 and interleukin [20]. It is reported that the accumulation of PAF in the body further aggravates the inflammatory response, resulting in visceral injuries [21]. PAF has been shown to be associated with the severity of infectious diseases in patients in the ICUs [22]. The results of our current study indicated that the PAF levels were strikingly different among the three groups (P<0.05), with the highest PAF level in patients with septic shock and the lowest PAF level in those with sepsis. This might be explained by the fact that PAF is involved in septic shock caused by gram-positive bacteria and endotoxin, and that the ischemia reperfusion injuries develop in the process of septic shock which stimulates synthesis of PAF [23, 24]. The results of the ROC curve analysis in our current study reveal that PAF is of great value in the differentiation and diagnosis of sepsis

and is positively correlated with the APACHE II score. It is noteworthy that the PAF level in septic patients is a potential marker for assessment of the prognosis of patients.

In conclusion, sCD14-ST and PAF can be applied as markers for diagnosis of sepsis, which is conducive to evaluating the severity of sepsis. However, additional studies with large samples are needed for further investigation, with an aim to provide more experimental and theoretical evidence for clinical guidance of the treatment of sepsis.

Disclosure of conflict of interest

None.

Address correspondences to: Zhongfu Ma, Department of General Internal Medicine, The First Affiliated Hospital, Sun Yat-sen University, No. 58, Zhongshan Second Road, Guangzhou City 510080, Guangdong Province, P. R. China, Tel: +86-020-28823388, Fax: +86-020-28823388, E-mail: zhongfuma682@163.com

References

[1] Emami-Razavi SH, Mohammadi A, Alibakhshi A, Jalali M and Ghajarzadeh M. Incidence of post-operative sepsis and role of charlson co-morbidity score for predicting postoperative sepsis. Acta Med Iran 2016; 54: 318-322.

[2] Yaguchi A, Yuzawa J, Klein DJ, Takeda M and Harada T. Combining intermediate levels of the Endotoxin Activity Assay (EAA) with other biomarkers in the assessment of patients with sepsis: results of an observational study. Crit Care 2012; 16: R88.

[3] Sager R, Kutz A, Mueller B and Schuetz P. Procalcitonin-guided diagnosis and antibiotic stewardship revisited. BMC Med 2017; 15: 15.

[4] Dinc F, Akalin H, Ozakin C, Sinirtas M, Kebabci N, Iscimen R, Kelebek Girgin N and Kahveci F. Comparison of blood culture and multiplex real-time PCR for the diagnosis of nosocomial sepsis. Minerva Anestesiol 2016; 82: 301-309.

[5] Yaegashi Y, Shirakawa K, Sato N, Suzuki Y, Kojika M, Imai S, Takahashi G, Miyata M, Furusako S and Endo S. Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. J Infect Chemother 2005; 11: 234-238.

[6] Endo S, Suzuki Y, Takahashi G, Shozushima T, Ishikura H, Murai A, Nishida T, Irie Y, Miura M, Iguchi H, Fukui Y, Tanaka K, Nojima T and Oka-mura Y. Usefulness of presepsin in the diagnosis of sepsis in a multicenter prospective study. J Infect Chemother 2012; 18: 891-897.

[7] Yost CC, Weyrich AS and Zimmerman GA. The platelet activating factor (PAF) signaling cascade in systemic inflammatory responses. Biochimie 2010; 92: 692-697.

[8] Teixeira-da-Cunha MG, Gomes RN, Roehrs N, Bozza FA, Prescott SM, Stafforini D, Zimmerman GA, Bozza PT and Castro-Faria-Neto HC. Bacterial clearance is improved in septic mice by platelet-activating factor-acetylhydrolase (PAF-AH) administration. PLoS One 2013; 8: e74567.

[9] Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL and Ramsay G; SCCM/ESICM/ACCP/ATS/SIS. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Crit Care Med 2003; 31: 1250-1256.

[10] Hu L, Shi Q, Shi M, Liu R and Wang C. Diagnostic calue of PCT and CRP for detecting serious bacterial infections in patients with fever of unknown origin: a systematic review and meta-analysis. Appl Immunohistochem Mol Morphol 2017; 25: e61-e69.

[11] Andriolo BN, Andriolo RB, Salomao R and Atallah AN. Effectiveness and safety of procalcitonin evaluation for reducing mortality in adults with sepsis, severe sepsis or septic shock. Cochrane Database Syst Rev 2017; 1: Cd010959.

[12] Giannakopoulos K, Hoffmann U, Ansari U, Bertsch T, Borggrefe M, Akin I and Behnes M. The use of biomarkers in sepsis: a systematic review. Curr Pharm Biotechnol 2017; 18: 499-507.

[13] Matera G, Quirino A, Peronace C, Settembre P, Marano V, Loria MT, Marascio N, Galati L, Barreca GS, Giancotti A, Amantea B, Liberto MC and Foca A. Soluble CD14 subtype-a new biomarker in predicting the outcome of critically III septic patients. Am J Med Sci 2017; 353: 543-551.

[14] de Guadiana Romualdo LG, Torrella PE, Acebes SR, Oton MDA, Sanchez RJ, Holgado AH, Santos EJ and Freire AO. Diagnostic accuracy of presepsin (sCD14-ST) as a biomarker of infection and sepsis in the emergency department. Clin Chim Acta 2017; 464: 6-11.

[15] Godnic M, Stubljar D, Skvarc M and Jukic T. Diagnostic and prognostic value of sCD14-ST-presepsin for patients admitted to hospital intensive care unit (ICU). Wien Klin Wochenschr 2015; 127: 521-527.

[16] Mussap M, Puxeddu E, Puddu M, Ottonello G, Coghe F, Comite P, Cibecchini F and Fanos V. Soluble CD14 subtype (sCD14-ST) presepsin in premature and full term critically ill new-

borns with sepsis and SIRS. Clin Chim Acta 2015; 451: 65-70.

[17] Enguix-Armada A, Escobar-Conesa R, Garcia-De La Torre A and De La Torre-Prados MV. Usefulness of several biomarkers in the management of septic patients: C-reactive protein, procalcitonin, presepsin and mid-regional pro-adrenomedullin. Clin Chem Lab Med 2016; 54: 163-168.

[18] Mariano F, Bussolati B, Migliori M, Russo S, Triolo G and Camussi G. Platelet-activating factor synthesis by neutrophils, monocytes, and endothelial cells is modulated by nitric oxide production. Shock 2003; 19: 339-344.

[19] Corl CM, Contreras GA and Sordillo LM. Lipoxygenase metabolites modulate vascular-derived platelet activating factor production following endotoxin challenge. Vet Immunol Immunopathol 2010; 136: 98-107.

[20] Han SJ, Ko HM, Choi JH, Seo KH, Lee HS, Choi EK, Choi IW, Lee HK and Im SY. Molecular mechanisms for lipopolysaccharide-induced biphasic activation of nuclear factor-kappa B (NF-kappa B). J Biol Chem 2002; 277: 44715-44721.

[21] Mazereeuw G, Herrmann N, Bennett SA, Swardfager W, Xu H, Valenzuela N, Fai S and Lanctot KL. Platelet activating factors in depression and coronary artery disease: a potential biomarker related to inflammatory mechanisms and neurodegeneration. Neurosci Biobehav Rev 2013; 37: 1611-1621.

[22] Poeze M, Froon AH, Ramsay G, Buurman WA and Greve JW. Decreased organ failure in patients with severe SIRS and septic shock treated with the platelet-activating factor antagonist TCV-309: a prospective, multicenter, double-blind, randomized phase II trial. TCV-309 Septic Shock Study Group. Shock 2000; 14: 421-428.

[23] Iwase M, Yokota M, Kitaichi K, Wang L, Takagi K, Nagasaka T, Izawa H and Hasegawa T. Cardiac functional and structural alterations induced by endotoxin in rats: importance of platelet-activating factor. Crit Care Med 2001; 29: 609-617.

[24] Claus RA, Russwurm S, Dohrn B, Bauer M and Losche W. Plasma platelet-activating factor acetylhydrolase activity in critically ill patients. Crit Care Med 2005; 33: 1416-1419.