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ORIGINAL ARTICLE

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Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis

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Abstract CD14, a high-affinity receptor for lipopolysaccharide (LPS), is a glycoprotein expressed on the surface membranes of monocytes/macrophages. We have identified a previously unknown form of soluble CD14, named soluble CD14 subtype (sCD14-ST), that is increased in patients with sepsis. To measure sCD14-ST concentrations in plasma, we prepared anti-sCD14-ST antibodies and developed an enzyme immunoassay (EIA) for this soluble form of CD14. With this assay, quantitative measurements are available within 4 h, and we compared the levels of sCD14-ST in plasma from normal subjects (healthy controls), patients with systemic inflammatory response syndrome (SIRS), and sepsis patients. The level of sCD14-ST in subjects with sepsis was much higher than the levels in subjects with SIRS and the healthy controls. Additionally, when a subject's sCD14-ST level was used as a diagnostic marker for sepsis, the area under the receiver operating characteristic (ROC) curve was 0.817, thereby demonstrating that elevated sCD14-ST levels were a better marker for sepsis than the other molecular markers we tested. sCD14-ST levels also correlated with procalcitonin (PCT) levels and with sequential organ failure assessment (SOFA) scores. Finally, changes in sCD14-ST concentration correlated with the severity of sepsis. Taken together, these results indicate that sCD14-ST is a useful marker for the rapid diagnosis of sepsis and for monitoring the severity of the disease.

Key words Sepsis · SIRS · CD14 · EIA · Diagnosis

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Introduction

CD14, a cluster-of-differentiation (CD) marker protein expressed by bone-marrow cells, is found on the surface membranes of mononuclear cells, where it serves as a specific high-affinity receptor for lipopolysaccharide (LPS).[1,2] It was previously reported that membrane-bound CD14 was absent in patients with paroxysmal nocturnal hemoglobinuria (PHN), whereas soluble CD14 (sCD14) was detected in the plasma of patients with PHN.[3] In normal plasma, sCD14 has been detected at microgram concentrations as both a 49-kD and a 55-kD molecule.[4,5] Interestingly, several diseases, including sepsis, AIDS, acute respiratory distress syndrome, and systemic lupus erythematosus, have been associated with elevated sCD14 plasma levels.[6–9]

What is the function of sCD14? In mice, sCD14 has been shown to reduce the mortality rate caused by endotoxin shock and the severity of gram-negative bacterial infections.[10] Moreover, an increased serum sCD14 concentration has been correlated with interleukin (IL)-8 levels and poor outcomes for patients with sepsis.[11] However, because increased levels of sCD14 are not disease-specific, sCD14 is not an ideal marker for sepsis.

We have developed an enzyme immunoassay (EIA) to measure sCD14-subtype (ST) levels in plasma. In this study, we report on our use of this assay to determine the levels of sCD14-ST in plasma from healthy subjects, subjects with systemic inflammatory response syndrome (SIRS), and subjects with sepsis. Receiver Operating Characteristic (ROC) analysis suggested that sCD14-ST could be used as a diagnostic marker for sepsis.

Subjects, materials, and methods

Plasma samples

All of the subjects in this study were inpatients at the Critical Care and Emergency Center of Iwate Medical University. Healthy volunteers served as control subjects. Cases of

Table 1. Medical histories of the subjects in this study

Sepsis (n = 66)		SIRS (n = 80)	
Appendicitis	10	Myocardial infarction	18
Perforation of duodenum	10	Carbon monoxide poisoning syndrome	10
Perforation of colon	12	Craniocerebral trauma	23
Pyelonephritis	4	Liver trauma	5
Cholangitis	9	Fat embolism	2
Mesenteric vascular occlusion	7	Pelvic fracture	5
Perforation of stomach	1	Others	17
Perforation of small intestine	4		
Others	9		

sepsis and SIRS without infection were defined by the criteria delineated by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference Committee.[12] Blood was collected before medical treatment was administered.

Assays

The plasma concentrations of C-reactive protein (CRP), IL-6, procalcitonin (PCT), and endotoxin were measured using commercially available kits (CRP, Immunoticles auto CRP; A&T, Tokyo, Japan; IL-6, Biosource, Camarillo, CA, USA; PCT, LUMItest; B·R·A·H·M·S, Berlin, Germany; endotoxin, LAL IES; Wako Pure Chemicals, Osaka, Japan). Furthermore, the plasma levels of several markers were measured over time in patients with sepsis. Routine laboratory parameters such as leukocyte counts and body temperature and heart rate were determined.

sCD14-ST EIA

To study the level of sCD14-ST in plasma, we developed an EIA for sCD14-ST, using two sCD14-ST-specific antibodies. Briefly, rabbit anti-sCD14-ST polyclonal antibodies were used to capture the target protein and peroxidase-labeled mouse anti-sCD14-ST monoclonal antibodies were used to detect the captured protein in sandwich EIA. Using this assay, quantitative measurements are available within 4 h. The standard curve was linear from 3 to 150 ng/ml, and the intraassay and interassay variations were less than 10%.

Statistical analysis

Mann-Whitney tests were used to compare the results from each group. Correlations between markers were analyzed using Spearman's rank correlation test. All of the analyses were two-sided, and P values less than 0.05 were considered significant. ROC analyses were used to examine the capability of markers to diagnose sepsis (SPSS., Chicago, IL, USA).

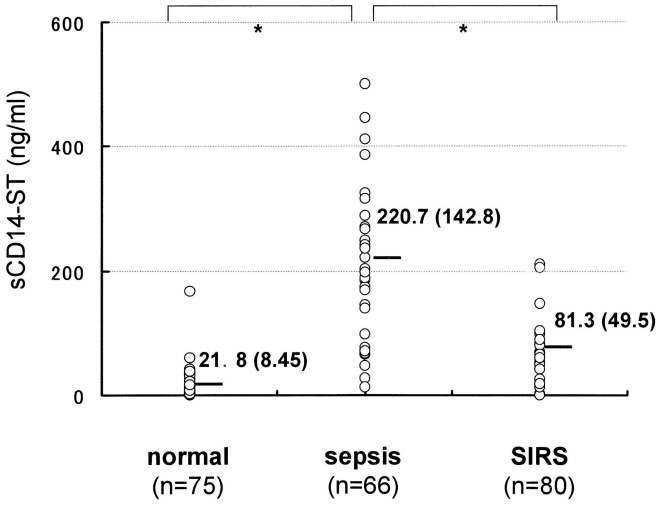

Fig. 1. The concentrations of soluble CD14 subtype (*sCD14-ST*) measured in plasma samples from normal controls, sepsis patients, and systemic inflammatory response syndrome (*SIRS*) patients. Data values are expressed as medians and interquartile ranges. Mann-Whitney tests were used to compare the results from each group. $*P < 0.05$, significant increase compared with the normal controls and SIRS without infection. Numbers in parentheses are interquartile ranges

Results

sCD14-ST concentrations

The medical histories of the patients with sepsis and those with SIRS are shown in Table 1. The concentration of sCD14-ST in plasma samples was determined by sCD14-ST EIA (Fig. 1). The median sCD14-ST concentrations in plasma from healthy individuals (75 samples), subjects with sepsis (66 samples), and subjects with SIRS (80 samples) were 21.8 ng/ml, 220.7 ng/ml, and 81.3 ng/ml, respectively. sCD14-ST levels were significantly higher in the sepsis group than in the SIRS and healthy control groups, demonstrating the specific elevation of sCD14-ST in patients with sepsis.

ROC analysis

ROC analysis revealed the area under the curve (AUC) for sCD14-ST was 0.817, which was the highest among the measurement markers (Fig. 2). The AUC values for

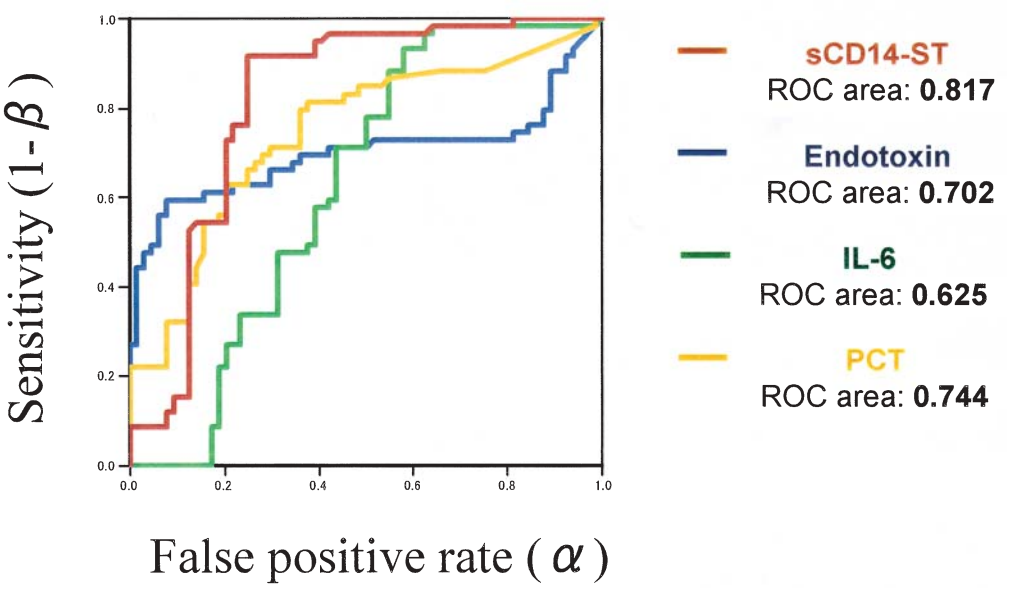

Fig. 2. Receiver operating characteristic (*ROC*) analysis of sCD14-ST, endotoxin, interleukin 6 (*IL-6*), and procalcitonin (*PCT*) for the diagnosis of sepsis (sepsis vs normal + SIRS without infection). The areas under the ROC curves (AUC) were calculated, using SPSS software (SPSS, Chicago, IL, USA). The AUC in the ROC analysis for sCD14-ST was 0.817, better than that for the other markers

Table 2. Correlation of sCD14-ST levels with those of other markers

	Endotoxin	IL-6	CRP	PCT	SOFA
sCD14-ST	0.118	0.095	0.610*	0.597*	0.750*

* $P < 0.01$ (Spearman test)

endotoxin, PCT, and IL-6 were 0.702, 0.744, and 0.625, respectively. The ROC curves also show that sCD14-ST is a sensitive marker for sepsis, whereas endotoxin is a specific marker.

Correlation with other markers

sCD14-ST levels correlated with PCT levels, CRP levels, and sequential organ failure assessment (SOFA) scores, which describe the severity of organ dysfunction (Table 2). In particular, the correlation between the concentration of sCD14-ST and the SOFA score was high, at 0.750. On the other hand, endotoxin and IL-6 concentrations did not strongly correlate with sCD14-ST levels.

Case study

To test whether or not sCD14-ST can be used as a diagnostic marker for sepsis, we measured sCD14-ST levels over time in a 65-year-old male patient with cholangiocarcinoma (Fig. 3). Three days after the left lobe of the liver, the duodenum, and the head of the pancreas had been resected, portal thrombus formation was detected, and a hepatectomy was performed. Seven days later, computed tomography (CT) scanning detected necrosis of the liver and the onset of sepsis. Local drainage was performed and the symptoms of sepsis subsided. Fifteen days after the initial drainage, an abscess was detected in the liver. A second drainage was performed and the clinical condition of the patient improved.

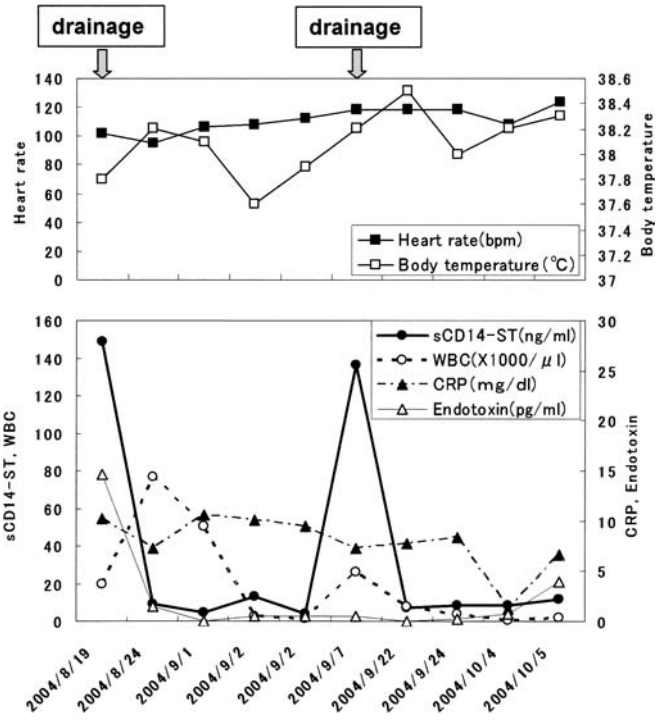

Fig. 3. Changes in sCD14-ST, endotoxin, and C-reactive protein (*CRP*) concentrations in a patient with cholangiocarcinoma, after surgery. sCD14-ST concentrations were measured by enzyme immunoassay (EIA). Endotoxin and CRP were measured with commercially available kits. Drainage was performed after computed tomography (CT) scanning detected necrosis of the liver and the onset of sepsis

We monitored the levels of several markers throughout the course of the infection and found that the sCD14-ST concentration was a reliable marker for sepsis. The concentration of sCD14-ST rose to 150 ng/ml after the onset of the infection, and descended promptly after the first drainage. Subsequently, the concentration of sCD14-ST returned to 150 ng/ml, before the second drainage. In contrast, the con-

centration of endotoxin increased during the first infection but not during the second infection. Furthermore, the concentration of CRP did not reflect the course of the infection. Overall, increases and reductions in the concentrations of sCD14-ST correlated with the clinical diagnosis of sepsis and the success of therapy, respectively.

Discussion

We measured sCD14-ST levels in healthy individuals, SIRS patients, and sepsis patients. In healthy subjects, sCD14-ST levels were an order of magnitude lower than the levels of other forms of sCD14 measured by conventional CD14-EIA.[4] Although we found a small elevation of sCD14-ST in the SIRS group, the levels of sCD14-ST in sepsis patients were significantly higher than those in patients with SIRS or the healthy control subjects. These data demonstrate that the concentration of sCD14-ST is specifically increased during sepsis. Furthermore, the correlation between sCD14-ST concentrations and PCT levels, endotoxin levels, and SOFA scores indicates that measuring sCD14-ST levels would be valuable for the diagnosis of sepsis.

The AUC calculated from the ROC analysis of elevated sCD14-ST levels as a test for sepsis was 0.817, and the ROC curve showed that the sCD14-ST concentration was a significantly more sensitive indicator of sepsis than the concentrations of the other markers tested. Moreover, the time course of the plasma levels of sCD14-ST in a surgery patient reflected the increasing and decreasing severity of the patient's infection. Conversely, CRP levels remained high even when the condition of the patient was stable, demonstrating that the concentration of sCD14-ST was more strongly correlated than the other parameters tested with the clinical course of sepsis. Although the half-life of sCD14-ST is unknown, our data showed that, after treatment, sCD14-ST levels decreased within a few days. Furthermore, sCD14-ST levels increased in the first 6 h after the onset of sepsis (data not shown). These changes in concentration occurred on a much faster time scale than those observed for PCT or CRP. PCT is a diagnostic marker for sepsis that has been used in the European Union.[13] We found that, compared with PCT, sCD14-ST was induced at an earlier stage of sepsis, was present at higher concentrations in plasma, and was a more sensitive indicator of sepsis. Taken together, these results suggest that measuring plasma levels of sCD14-ST should facilitate the rapid diagnosis of sepsis and the assessment of the effectiveness of any administered therapy.

The physiological role of sCD14-ST during sepsis and the mechanisms that induce the production of sCD14-ST are unclear. Bufler et al.[14] reported that sCD14 was released from human monocytes and CD14 transfectants via two different mechanisms: a shedding mechanism and a secretion mechanism. Moreover, Bazil et al.[15] found that sCD14 was shed from stimulated human monocytes. Because we observed an increase in sCD14-ST levels within a few hours of the onset of sepsis, we believe that sCD14-ST is produced by shedding rather than by secretion, which requires protein synthesis.

To reduce the mortality rate of patients with sepsis, rapid diagnosis and therapy are required.[16] To rapidly diagnose sepsis and monitor the severity of the infection, it is currently necessary to use a combination of parameters, including clinical signs, the SOFA scoring system, and the levels of endotoxin, IL6, and PCT.[17,18] A simple immunochromatography-based method that produces results in 20 min has been developed to facilitate sepsis diagnosis. The results from our present study indicate that sCD14-ST is the most suitable marker for sepsis and that using sCD14-ST levels as an indicator of sepsis may decrease the mortality rate in sepsis patients.

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