Table 1. Patients' underlying diseases

Underlying disease		PCT value (ng/ml)			
		Systemic and localized bacterial infection groups combined		Nonbacterial infection and Non-infectious group combined	
	n	*n*	Range	*n*	Range
Circulatory disease	38	10	0–10.08	28	0–1.70
Respiratory disease	10	5	0–21.04	5	0–0.42
Gastroenterological disease	14	11	0.60–373.46	3	0–0.91
Hepatobiliary disease	7	4	0–205.79	3	0–0.41
Renal disease	3	2	2.02–212.18	1	0.33
Neurological disease	3	3	0–7.98	0	–
Diabetes mellitus	7	6	0.34–82.29	1	0
Malignant disease	6	3	0.42–1.73	3	0
Trauma	15	10	0–82.48	5	0–0.38
Burns	7	7	0–34.53	0	–
Kawasaki disease	12	1	4.89	11	0–1.91
Others	17	7	0–20.59	10	0–8.72
None	37	21	0–93.29	16	0–3.67
Total	176	90		86	

Statistical analysis

The statistical significances of differences were determined using the Mann-Whitney *U*-test and receiver operating characteristic (ROC) analysis, carried out with StatFlex Ver. 5.0 (AHTEKKU, Osaka, Japan). *P* values of less than 0.05 were considered significant.

Results

Serum PCT, endotoxin, β-D-glucan, IL-6, and CRP concentrations in patient groups

The patterns of distribution of PCT, endotoxin, IL-6, and CRP concentrations in the systemic bacterial infection group, localized bacterial infection group, nonbacterial infection group, and noninfectious disease group are shown in Fig. 1. The median ages of the patients with nonbacterial and suspected bacterial infections were lower than those of the other groups (Table 2). Previous studies have reported that there were no differences in PCT values by age,[15,16] with the exception of neonates.[17] Table 3 summarizes serum concentrations of PCT, endotoxin, IL-6, and CRP in patients in the five groups and in the healthy volunteers. Table 4 shows statistical analysis using the criteria for the diseases. Serum PCT concentrations were significantly higher in both the systemic bacterial infection and localized bacterial infection groups than in both the nonbacterial infection and noninfectious disease groups ($P < 0.05$). Serum PCT concentrations did not differ significantly between the systemic bacterial infection and localized bacterial infection groups ($P = 0.770$). The systemic bacterial infection and localized bacterial infection groups were therefore combined as the bacterial infectious disease group. In the same fashion, no significant difference in serum PCT concentration was observed between the nonbacterial infection group and the noninfectious disease groups ($P = 0.174$), and the nonbacterial infection and noninfectious disease groups were therefore combined as the nonbacterial infectious disease group. The patterns of distribution of PCT, endotoxin, IL-6, and CRP concentrations for these two groups are shown in Fig. 2. Serum PCT, endotoxin, IL-6, and CRP concentrations were significantly higher in the bacterial infectious disease group than in the nonbacterial infectious disease group ($P < 0.001$, $P < 0.005$, $P < 0.001$, and $P < 0.001$).

Cutoff value and diagnostic accuracy of serum PCT concentration

Table 5 shows the sensitivity, specificity, positive predictive values, and negative predictive values for the serum markers. When 0.5 ng/ml was used as the cutoff value for PCT, the sensitivity, specificity, positive predictive value, and negative predictive value were 64.4%, 86.0%, 82.9%, and 69.8%, respectively. Figure 3 presents the receiver operating characteristic curves of four serum markers used to discriminate the bacterial infectious disease group from the nonbacterial infectious disease group. The area under the receiver operating characteristic curve (AUC) for PCT was 0.84, which was significantly higher than that for endotoxin (0.60; $P < 0.001$), and tended to be higher than those for IL-6 (0.77; $P = 0.22$) and CRP (0.78; $P = 0.32$).

Sensitivities of serum markers for the type of infection

Table 6 shows the sensitivities of PCT, endotoxin, β-D-glucan, IL-6, and CRP with regard to the type of infection determined by culture. The difference in PCT serum con-

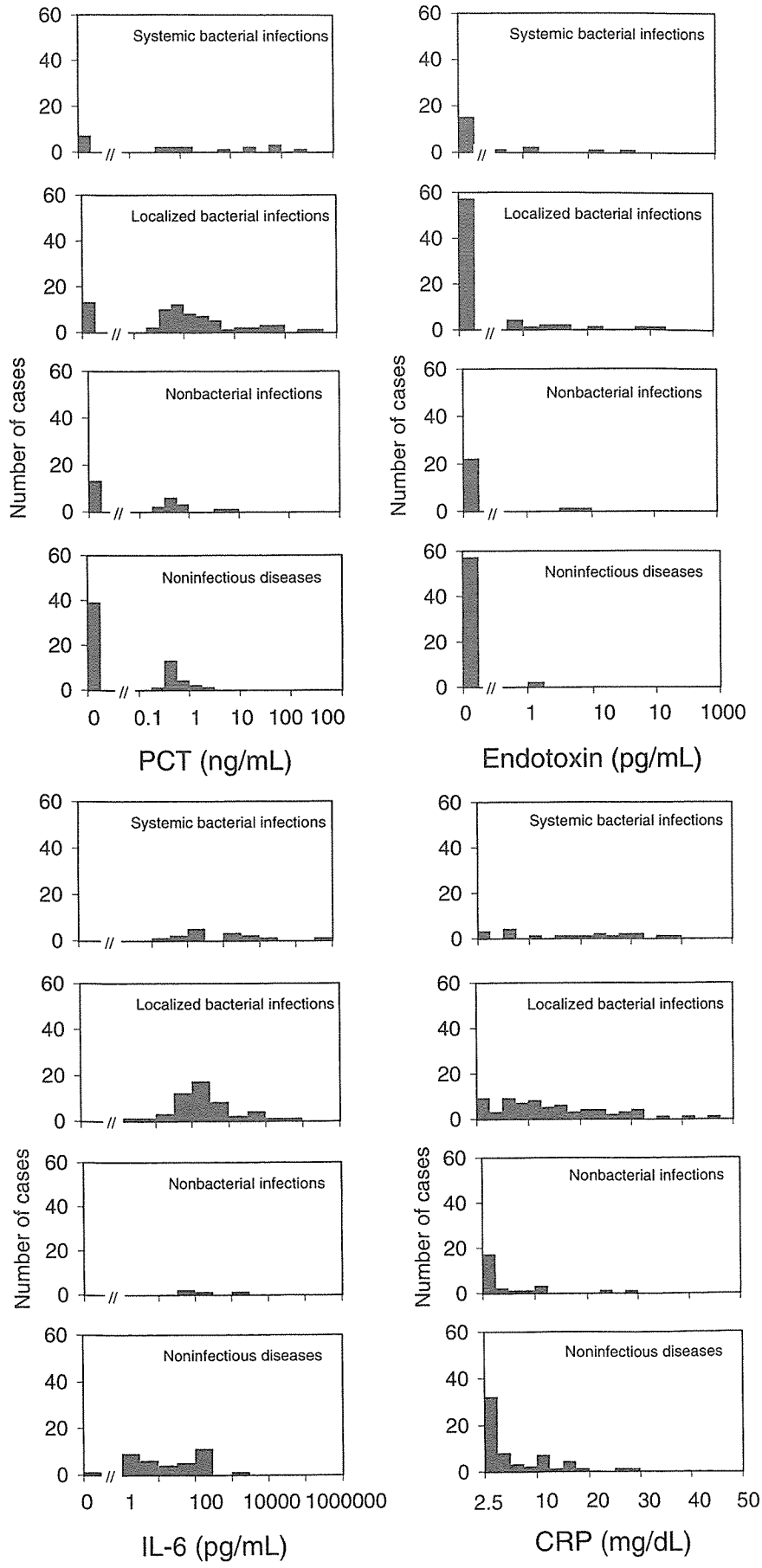

Fig. 1. Distribution patterns of procalcitonin (*PCT*), endotoxin, interleukin-6 (*IL-6*) and C-reactive protein (*CRP*) in patients with systemic bacterial infections, localized bacterial infections, nonbacterial infections, and noninfectious diseases

Table 2. Patient demographics

	n	Sex	Age (years)
		Male/Female	Median (range)
Systemic bacterial infection	20	7/13	58 (1–81)
Localized bacterial infection	70	44/26	53 (0.1–92)
Nonbacterial infection	26	13/13	4 (0.1–72)
Suspected bacterial infection	69	45/24	5 (0.1–85)
Noninfectious disease	60	38/22	48 (0.1–87)
Healthy volunteers	20	16/4	22 (22–27)

Table 3. Serum concentrations of PCT, endotoxin, IL-6 and CRP in patients with systemic bacterial infection, localized bacterial infection, nonbacterial infection, suspected bacterial infection, and noninfectious diseases, and healthy volunteers

	n	PCT (ng/ml)	Endotoxin (pg/ml)	IL-6 (pg/ml)	CRP (mg/dl)
		Median (range)	Median (range)	Median (range)	Median (range)
Systemic bacterial infection	20	0.66 (0.00–212.18)	0.0 (0.0–39.4)	199.5 (22.3–592000.0)	20.0 (0.1–38.2)
Localized bacterial infection	70	0.94 (0.00–373.46)	0.0 (0.0–135.4)	141.2 (1.6–38922.0)	11.9 (0.2–46.7)
Nonbacterial infection	26	0.16 (0.00–8.72)	0.0 (0.0–7.0)	152.6 (54.3–2550.0)	1.9 (0.3–28.4)
Suspected bacterial infection	69	0.38 (0.00–85.93)	0.0 (0.0–29.1)	17.1 (10.3–1086.0)	2.5 (0.1–26.8)
Noninfectious disease	60	0.00 (0.00–1.91)	0.0 (0.0–1.3)	17.1 (0.0–1350.0)	2.1 (0.0–28.1)
Healthy volunteers	20	0.00 (0.00–0.00)	0.0 (0.0–0.6)	1.8 (1.5–4.5)	0.1 (0.0–0.1)

Table 4. Statistical analysis according to the disease criteria

	p value			
	PCT	Endotoxin	IL-6	CRP
Systemic bacterial infection vs localized bacterial infection	0.770	0.469	0.131	0.244
Systemic bacterial infection vs nonbacterial infection	0.026	0.149	0.317	<0.001
Systemic bacterial infection vs noninfectious disease	<0.001	0.004	<0.001	<0.001
Localized bacterial infection vs nonbacterial infection	<0.001	0.323	0.766	<0.001
Localized bacterial infection vs noninfectious disease	<0.001	0.011	<0.001	<0.001
Nonbacterial infection vs noninfectious disease	0.174	0.317	0.104	0.756

centrations between Gram-negative and Gram-positive bacterial infections was not significant (13.79 ± 28.18 ng/ml for Gram-negative and 9.91 ± 35.20 ng/ml for Gram-positive bacterial infections; $P = 0.673$). The sensitivity of PCT for mixed Gram-negative and Gram-positive bacterial infections was 64.3% (9/14 cases). The sensitivities of PCT and endotoxin for Gram-negative bacterial infections in systemic infections were 100% (3/3) and 67% (2/3), respectively. On the other hand, the sensitivities of PCT and endotoxin for localized Gram-negative bacterial infections were 50% (6/12) and 0% (0/12), respectively. In a patient with confirmed fungal infection, the PCT result was negative, below the cutoff value. Four of 24 samples from patients with viral infections (16.7%) exhibited PCT concentrations exceeding the cutoff value. One patient with malaria showed a high PCT concentration, of 8.7 ng/ml.

Sensitivity of serum PCT compared with blood culture

The sensitivities of serum PCT and blood culture were compared in the combined systemic bacterial infection group and the localized bacterial infection group. The sensitivity of PCT was 70.2% (33/47 cases) in this combined group, but it was 42.6% (20/47 cases) for blood culture.

Discussion

Sepsis can be difficult to distinguish from other, noninfectious, conditions in critically ill patients admitted with clinical signs and symptoms of various acute inflammatory diseases. This issue is of paramount importance, given that therapies and outcomes differ greatly between patients with and those without bacterial sepsis. Blood culture is the most reliable method of detecting bacterial infections. However, more than 3 days is required to obtain results, and the positive detection rate is low. Although CRP and IL-6 have been suggested to be good indicators of sepsis, elevated CRP and IL-6 concentrations can also be found following surgical procedures and in patients with nonbacterial or noninfectious inflammation alone. Thus, there is an unmet need for clinical tools that distinguish bacterial infections from other inflammatory diseases.

The diagnostic and prognostic importance of PCT in severe inflammatory diseases was first reported for a series of patients with burns, in 1992.[18] Serum PCT values were less than 0.1 ng/ml in healthy individuals, but were markedly increased, mostly as a result of induced extrathyroidal production, in patients with severe infection. However, the roles of PCT and the origin of its production, as well as the

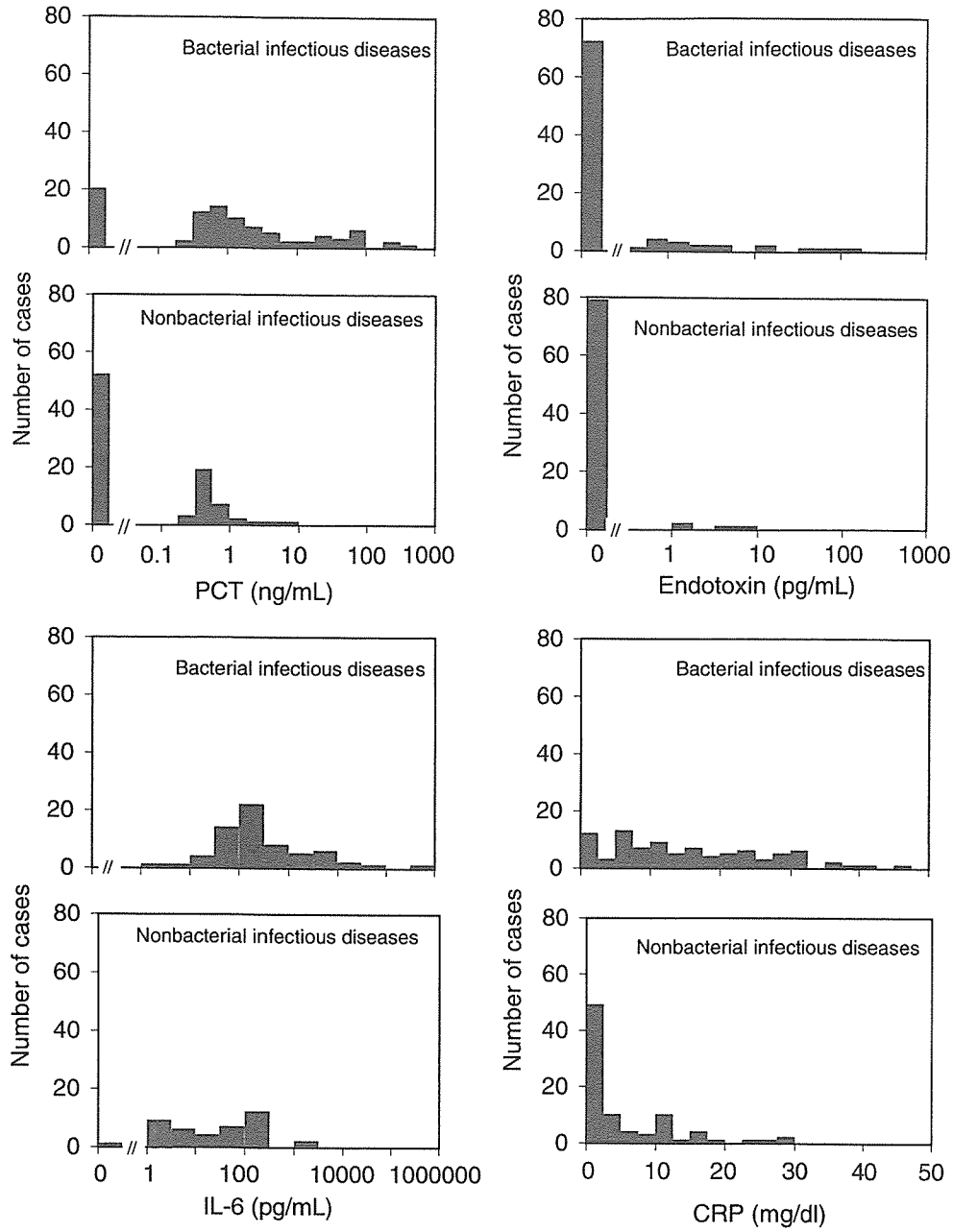

Fig. 2. Distribution patterns of PCT, endotoxin, IL-6, and CRP in patients with bacterial infectious diseases and those with nonbacterial infectious diseases

Table 5. Sensitivity, specificity, positive predictive value and negative predictive value of PCT, endotoxin, IL-6, and CRP in patients with bacterial infectious diseases and those with nonbacterial infectious diseases

	Cutoff value	Sensitivity	Specificity	Positive predictive value	Negative predictive value
PCT	0.5 ng/ml	64.4% (58/90)	86.0% (74/86)	82.9% (58/70)	69.8% (74/106)
PCT	2.0 ng/ml	34.4% (31/90)	97.7% (84/86)	93.9% (31/33)	58.7% (84/143)
Endotoxin	1.0 pg/ml	14.6% (13/89)	95.2% (79/83)	76.5% (13/17)	51.0% (79/155)
IL-6	10 pg/ml	96.9% (63/65)	39.0% (16/41)	71.6% (63/88)	88.9% (16/18)
IL-6	100 pg/ml	70.8% (46/65)	65.9% (27/41)	76.7% (46/60)	58.7% (27/46)
CRP	0.3 mg/dl	97.8% (88/90)	9.3% (8/86)	53.0% (88/166)	80.0% (8/10)
CRP	5.0 mg/dl	83.3% (75/90)	68.6% (59/86)	73.5% (75/102)	79.7% (59/74)

mechanism underlying PCT induction, are still not well known. Recent findings suggest that sources of PCT may include hepatic cells and monocytes/macrophages.[19,20] PCT is consistently increased after endotoxin injection, suggesting an association of endotoxin with septic shock and high PCT serum concentration.[21] Tumor necrosis factor (TNF) and IL-6 concentrations peaked before the appearance of PCT, suggesting that proinflammatory cytokines may play a

Table 6. Sensitivity of PCT, endotoxin, β-D-glucan, IL-6, and CRP with respect to the type of infection

Type of infection	PCT	Endotoxin	β-D-glucan	IL-6	CRP
	0.5 ng/ml	1.0 pg/ml	11 pg/ml	100 pg/ml	5 mg/dl
Gram-negative infection	65.2% (15/23)	21.7% (5/23)	17.4% (4/23)	58.8% (10/17)	87.0% (20/23)
Gram-positive infection	61.0% (25/41)	7.5% (3/40)	16.2% (6/37)	69.0% (20/29)	85.4% (35/41)
Mixed Gram-negative and -positive infection	64.3% (9/14)	21.4% (3/14)	16.7% (2/12)	72.7% (8/11)	71.4% (10/14)
Mixed bacterial and fungal infections	87.5% (7/8)	25.0% (2/8)	57.1% (4/7)	83.3% (5/6)	100.0% (8/8)
Fungal infection	0.0% (0/1)	0.0% (0/1)	100.0% (1/1)	0.0% (0/1)	100.0% (1/1)
Mycoplasmal infection	0.0% (0/1)	0.0% (0/1)	0.0% (0/1)	–	0.0% (0/1)
Viral infection	16.7% (4/24)	4.5% (1/22)	0.0% (0/19)	50.0% (1/2)	20.8% (5/24)
Malarial infection	100.0% (1/1)	100.0% (1/1)	0.0% (0/1)	100.0% (1/1)	100.0% (1/1)

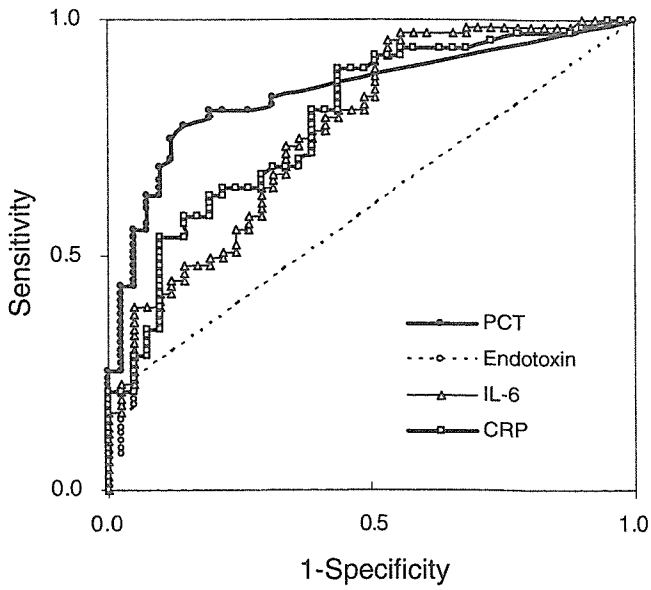

Fig. 3. Receiver operating characteristic curves (ROCs) of serum parameters (PCT, endotoxin, IL-6, and CRP) in patients with bacterial infectious diseases and those with non-bacterial infectious diseases

role in inducing PCT release.[22] Many studies have established that the determination of serum PCT concentrations can be used to differentiate bacterial from viral infections and to identify bacterial infections in patients admitted to intensive care units because of systemic inflammatory response syndrome (SIRS). Some studies have compared the diagnostic value of PCT with those of other parameters of inflammation, such as CRP and cytokine concentrations.[23,24] Thus, we conducted a prospective, multicenter study in patients diagnosed with or suspected of having infections. We obtained a cutoff value of 0.5 ng/ml for the PCT concentration, with acceptable sensitivity and high specificity. When assessed in 90 patients diagnosed with localized bacterial infectious disease and 86 patients diagnosed with nonbacterial infectious disease, the sensitivity, specificity, positive predictive value, and negative predictive value of PCT were 64.4%, 86.0%, 82.9%, and 69.8%, respectively. Al-Nawas et al.[25] showed that PCT determination in adult patients with sepsis had a lower specificity

Table 7. Sensitivity of PCT and blood culture in patients with systemic bacterial infections and localized bacterial infections

	n	PCT	Blood culture
Systemic bacterial infections	20	11 (55.0%)	20 (100%)
Localized bacterial infections	70	47 (67.1%)	0 (0.0%)
Blood culture negative	27	22 (81.5%)	0 (0.0%)
Blood culture not performed	43	25 (58.1%)	No test

(79%) and higher negative predictive value (78%), but lower sensitivity (60%) and positive predictive value (61%) than in our study, as above. The study by Liaudat et al.[26] intended to evaluate PCT concentration as an early predictive marker of bacteremia. In their hospital, where the prevalence of bacteremia was 8%, they found that PCT evaluation had a negative predictive value of 96%. Gendrel et al.[5] pointed out that low PCT serum concentrations in bacteremic patients may be due to previous administration of antibiotics. In the present study, 17 of 32 patients with bacterial infectious disease with a PCT concentration of less than 0.5 ng/ml had received antibiotics within 2 days of the testing. With the diagnostic criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference,[27] we classified 18 of these 32 patients as having non-SIRS ($n = 6$) or sepsis ($n = 12$), but none of them were classified as having severe sepsis. The AUC for PCT differed significantly from that for endotoxin, and tended to be higher than those for IL-6 and CRP. PCT is specific for bacterial infectious disease, but CRP and IL-6 may have elevated values in patients with SIRS.

The sensitivity of PCT was compared with respect to the classification of bacteria. No significant difference was observed for PCT serum concentrations between Gram-negative and Gram-positive bacterial infections, similar to already published data.[26]

The study by Assicot et al.[4] indicated that patients with viral infection had normal or only slightly increased concentrations of PCT. In the present study, 4 of 24 patients with viral infection had PCT concentrations higher than 0.5 ng/ml, and the mean PCT concentration in the viral infection group was 0.36 ± 0.76 ng/ml, while the highest concentration was 3.67 ng/ml.

PCT yielded negative results in one patient with fungal infection. The sensitivity of PCT for mixed bacterial and

fungal infection was 87.5% (7/8 cases). Endo et al.[28] have suggested that blood PCT does not increase in patients with deep-seated mycoses. Thus, although additional studies are necessary, PCT could be useful to distinguish bacterial from fungal infections.

In a patient with malaria, the PCT concentration was 8.72 ng/ml. Chiwakata et al.[29] showed that patients with severe and complicated *Plasmodium falciparum* malaria had significantly higher concentrations of serum PCT than those with uncomplicated malaria.

In conclusion, serum PCT concentration is specific for bacterial infection. The PCT concentration may contribute to a decision to withhold antibiotic treatment. Thus, it may be useful in detecting febrile patients suffering from severe focal infections or culture-negative sepsis who should be treated promptly with antibiotics, as well as patients suffering from benign occult bacteremia (who could avoid hospitalization), irrespective of the results of blood cultures.

References

1. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. Clin Microbiol Rev 1997;10:444–65.
2. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303–10.
3. Christ-Crain M, Jaccard-Stolz D, Bingisser R, Gencay MM, Huber PR, Tamm M, et al. Effect of procalcitonin-guided treatment on antibiotics use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. Lancet 2004;363:600–7.
4. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341:515–8.
5. Gendrel D, Raymond J, Coste J, Moulin F, Lorrot M, Guerin S, et al. Comparison of procalcitonin with C-reactive protein, interleukin 6 and interferon-alpha for differential of bacterial vs viral infections. Pediatr Infect Dis 1999;18:875–81.
6. Delevaux I, Andre M, Colombier M, Albuisson E, Meylheuc F, Begue RJ, et al. Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? Ann Rheum Dis 2003;62:337–40.
7. Gendrel D, Bohuon C. Procalcitonin in pediatrics for differentiation of bacterial and viral infections. Intensive Care Med 2000;26:S178–81.
8. Ugarte H, Silva E, Mercan D, Mendonca AD, Vincent JL. Procalcitonin used as a marker of infection in the intensive care unit. Crit Care Med 1999;27:498–504.
9. Meisner M, Tschaikowsky K, Schmidt J, Schuttler J. Procalcitonin (PCT) – Indications for a new diagnostic parameter of severe bacterial infection and sepsis in transplantation, immunosuppression and cardiac assist devices. Cardiovasc Eng 1996;1:67–76.
10. Nylen ES, Whang KT, Snider RH, Steinwald PM, White JC, Becker KL. Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. Crit Care Med 1998;26:1001–6.
11. Reinhart K, Karzai W, Meisner M. Procalcitonin as a marker of the systemic inflammatory response to infection. Intensive Care Med 2000;26:1193–200.
12. Kambayashi J, Yokota M, Sakon M, Shiba E, Kawasaki T, Mori T, et al. A novel endotoxin-specific assay by turbidimetry with Limulus amoebocyte lysate containing β-glucan. J Biochem Biophys Methods 1991;22:93–100.
13. Mori T, Ikemoto H, Matsumura M, Yoshida M, Inada K, Endo S, et al. Evaluation of plasma (1 3)-β-D-glucan measurement by the kinetic turbidimetric Limulus test, for the clinical diagnosis of mycotic infections. Eur J Clin Chem Clin Biochem 1997;35:553–60.
14. Mori T, Matsumura M. Clinical evaluation of diagnostic methods using plasma and/or serum for three mycoses: *Aspergillosis*, *Candidosis*, and *Pneumocystosis*. Jpn J Med Mycol 1999;40:223–30.
15. Schwarz S, Bertram M, Schwab S, Andrassy K, Hacke W. Serum procalcitonin levels in bacterial and abacterial meningitis. Crit Care Med 2000;28:1828–32.
16. Gendrel D, Raymond J, Assicot M, Moulin F, Iniguez JL, Lebon P, et al. Measurement of procalcitonin levels in children with bacterial or viral meningitis. Clin Infect Dis 1997;24:1240–2.
17. Chiesa C, Panero A, Rossi N, Stegagno M, De Giusti D, Osborn JF, et al. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis 1998;26:664–72.
18. Nylen ES, O'Neill W, Jordan MH, Snider RH, Moore CF, Lewis M, et al. Serum procalcitonin as an index of inhalation injury in burns. Horm Metab Res 1992;24:439–42.
19. Russwurm S, Wiederhold M, Oberhoffer M, Stonans I, Zipfel PF, Reinhart K. Molecular aspects and natural source of procalcitonin. Clin Chem Lab Med 1999;37:789–97.
20. Muller B, White JC, Nylen ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-I gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab 2001;86:396–404.
21. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, et al. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994;79:1605–8.
22. Oberhoffer M, Stonans I, Russwurm S, Stonane E, Vogelsang H, Junker U, et al. Procalcitonin expression in human peripheral blood mononuclear cells and its modulation by lipopolysaccharides and sepsis related cytokines in vitro. J Lab Clin 1999;134:49–55.
23. Fleischhack G, Kambeck I, Cipic D, Hasan C, Bode U. Procalcitonin in paediatric cancer patients: its diagnostic relevance is superior to that of C-reactive protein, interleukin 6, interleukin 8, soluble interleukin 2 receptor and soluble tumor necrosis factor receptor II. Br J Haematol 2000;111:1093–102.
24. Schroder J, Staubach KH, Zabel P, Stuber F, Kremer B. Procalcitonin as marker of severity in septic shock. Langenbecks Arch Surg 1999;384:33–8.
25. Al-Nawas B, Krammer I, Shah PM. Procalcitonin in diagnosis of severe infections. Eur J Med Res 1995/96;1:331–3.
26. Liaudat S, Dayer E, Praz G, Bille J, Troillet N. Usefulness of procalcitonin serum level for the diagnosis of bacteremia. Eur J Microbiol Infect Dis 2001;20:524–7.
27. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit. Care Med,1992;20:864–74.
28. Endo S, Inada K, Okamoto K, Kuboi M, Kasai T, Yamada Y, et al. Procalcitonin level was not elevated in patients with deep-seated mycosis (in Japanese). Jpn Soc Surg Infect 2000;12:141–5.
29. Chiwakata CB, Manegold C, Bonicke L, Waase I, Julch C, Dietrich M. Procalcitonin as a parameter of disease severity and risk of mortality in patients with *Plasmodium falciparum* malaria. J Infect Dis 2001;183:1161–4.

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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).

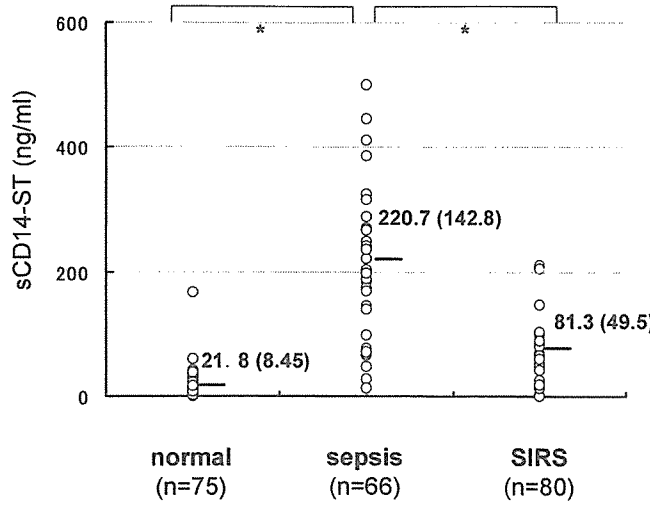

Fig. 1. The concentrations of soluble CD14 subtype (*sCD14-ST*) measured in plasma samples from normal controls, sepsis patients, and systemic inflammatory response syndrome (*SIRS*) patients. Data values are expressed as medians and interquartile ranges. Mann-Whitney tests were used to compare the results 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

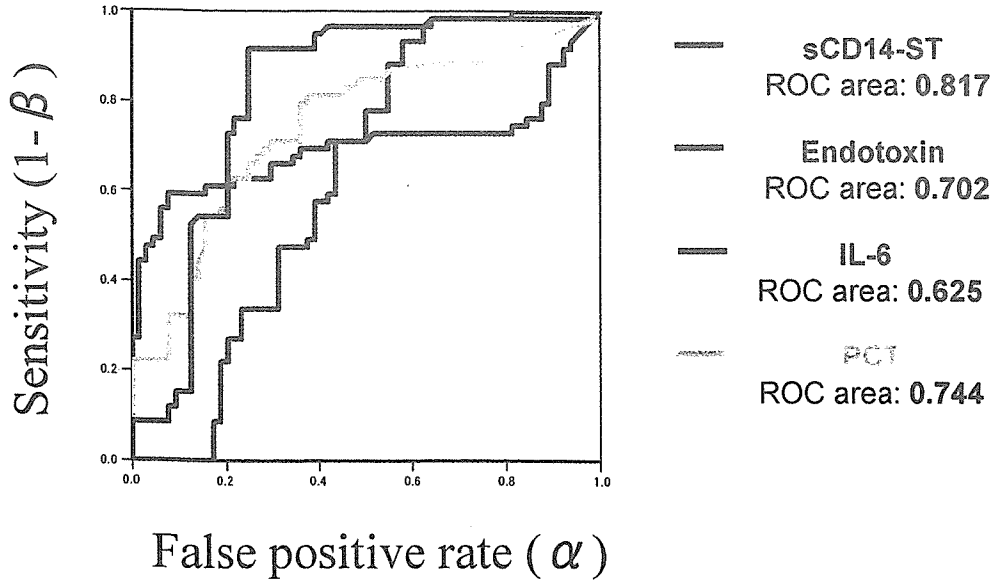

Fig. 2. Receiver operating characteristic (*ROC*) analysis of sCD14-ST, endotoxin, interleukin 6 (*IL-6*), and procalcitonin (*PCT*) for the diagnosis of sepsis (sepsis vs normal + SIRS without infection). The areas under the ROC curves (AUC) were calculated, using SPSS software (SPSS, Chicago, IL, USA).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

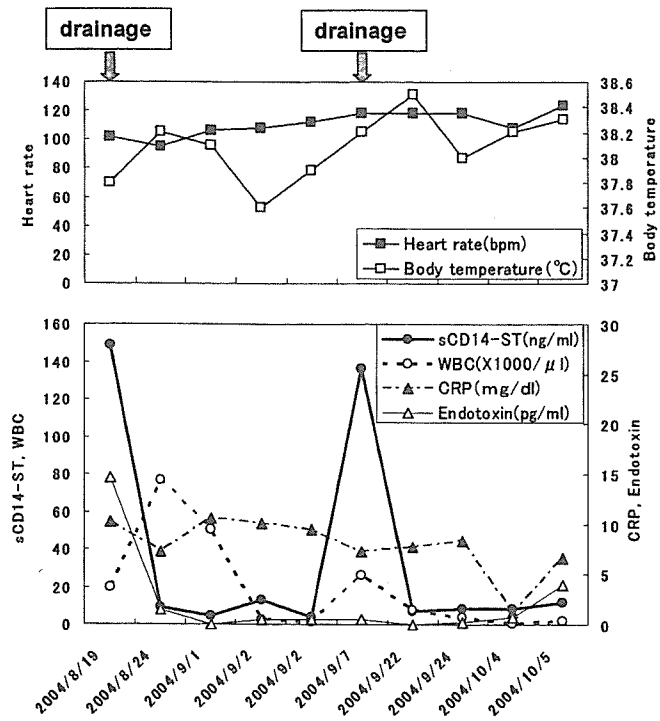

Fig. 3. Changes in sCD14-ST, endotoxin, and C-reactive protein (*CRP*) concentrations in a patient with cholangiocarcinoma, after surgery. sCD14-ST concentrations were measured by enzyme immunoassay (EIA). Endotoxin and CRP were measured with commercially available kits. Drainage was performed after 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 6h 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 20min 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.

Acknowledgments This study was partially supported by grants from the Mutual Aid Corporation for Private Schools of Japan; and the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Ferrero E, Goyert SM. Nucleotide sequence of the gene encoding the monocyte differentiation antigen, CD14. Nucleic Acids Res 1988;16:4173.
2. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complex of lipopolysaccharide (LPS) and LPS binding protein. Science 1990;249:1431–33.
3. Golenbock DT, Bach RR, Lichenstein H, Juan TS, Tadavarthy A, Moldow CF. Soluble CD14 promotes LPS activation of CD14-deficient PNH monocytes and endothelial cells. J Lab Clin Med 1995;125:662–71.
4. Grunwald U, Krüger C, Westermann J, Lukowsky A, Ehlers M, Shütt C. An enzyme-linked immunosorbent assay for the quantification of solubilized CD14 in biological fluids. J Immunol Methods 1992;155:225–32.
5. Landmann R, Zimmerli W, Sansano S, Link S, Hahn A, Glauser MP, et al. Increased circulating soluble CD14 is associated with high mortality in gram-negative septic shock. J Infect Dis 1995;171: 639–44.
6. Endo S, Inada K, Kasai T, Takakuwa T, Nakae H, Kikuchi M, et al. Soluble CD14 (sCD14) levels in patients with multiple organ failure (MOF). Res Commun Chem Pathol Pharmacol 1994;84:17–25.
7. Lien E, Aukrust P, Sundan A, Muller F, Froland SS, Espevik T. Elevated levels of serum-soluble CD14 in human immunodefiency virus type 1 (HIV-1) infection: correlation to disease progression and clinical events. Blood 1998;92:2084–92.
8. Martin TR, Rubenfeld GD, Ruzinski JT, Goodman RB, Steinberg KP, Leturcq DJ, et al. Relationship between soluble CD14, lipopolysaccharide binding protein, and the alveolar inflammatory response in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med. 1997;155:937–44.
9. Nockher WA, Wigarnd R, Schoeppe W, Scherberich JE. Elevated levels of soluble CD14 in serum of patients with systemic lupus erythematosus. Clin Exp Immunol 1994;96:15–9.
10. Lee JW, Paape MJ, Zhao X. Recombinant bovine CD14 reduces severity of experimental *Escherichia coli* mastitis in mice. Vet Res 2003;34:307–16.
11. Landmann R, Reber AM, Sansano S, Zimmerli W. Function of soluble CD14 in serum from patients with septic shock. J Infect Dis 1996;173:661–8.
12. Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee: American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definition for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20:864–74.

13. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341:515–18.
14. Bufler P, Stieger G, Schuchmann M, Hess S, Krüger C, Stelter F, et al. Soluble lipopolysaccharide receptor (CD14) released via two different mechanisms from human monocytes and CD14 transfectants. Eur J Immunol 1995;25:604–10.
15. Bazil V, Strominger JL. Shedding as a mechanism of down-modulation of CD14 on stimulated human monocytes. J Immunol 1991;147:1567–74.
16. Dellinger RP, Carlet JN, Masur H, Gerlach H, Calandra T, Cohen J, et al. Surviving sepsis campaign guidelines for management of severe sepsis and septic shock. Intensive Care Med 2004;30:536–55.
17. Oliver S, Hartmut H, Michael M, Andreas K, Wilfried B, Jörg K. Discrimination of sepsis and systemic inflammatory response syndrome by determination of circulating plasma concentrations of procalcitonin, protein complement 3a, and interleukin-6. Crit Care Med 2000;28:2793–98.
18. Holzheimer RG, Capel P, Cavaillon JM, Cainzos M, Frileux P, Haupt W, et al. Immunological surrogate parameters in a prognostic model for multi-organ failure and death. Eur J Med Res 2000;5:283–94.

エンドトキシン血症救命治療研究会誌　第9巻第1号　2005；46-50

[原　著]

新しい敗血症の診断マーカーである可溶性CD 14サブタイプの有用性について

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要　旨

CD 14はエンドトキシンとLBP複合体の細胞膜上のレセプターであり，エンドトキシンの細胞内シグナル伝達を担っているが，血中にも可溶型蛋白質として存在することが知られている。われわれは可溶型CD 14分子のうち従来のCD 14と分子量が異なる，すなわち可溶型CD 14サブタイプ(sCD 14-ST，49 kD)を発見し，このsCD 14-STのELISA法による定量法を開発した。本法により，敗血症患者のsCD 14-ST値を測定することが，ROC曲線による検討で，CRP，エンドトキシン，IL-6，プロカルチトニンなどに比べて敗血症の診断能力において優れていることが判った。

索引用語：敗血症，sCD 14-ST，診断，プロカルチトニン，CRP

はじめに

可溶性CD 14（soluble CD 14；以下sCD 14と略す）はエンドトキシンとLBP複合体の細胞膜上のレセプターであり，低濃度エンドトキシンの細胞内シグナル伝達を担うとされている。そして，血漿中にはその可溶分画が存在し，それが49 kDと55 kDの二つの形態に分けられることがBasilらによって報告されている[1]。われわれは，これまで55 kDのsCD 14が多臓器不全症で上昇することを報告した[2]。一方，49 kD形態そのものの由来は，感染症などの刺激で膜表面上のCD 14が切り離されて出てくるものと考えられている。今回，われわれは49 kD形態そのものをsCD 14-STと呼び，これを特異的に測定するenzyme-linked absorbent assay (ELISA) 測定キットを新たに作成した[3]。今回，sCD 14-STを測定し，敗血症の診断法としての有用性について検討した。

Ⅰ．対象と方法

本研究は岩手医科大学倫理委員会の承認を得て，さらに本人あるいはその家族の同意を得た。

Prospective cohort studyとして，岩手医科大学高度救命救急センターに入院した患者において20001年1月から12月までの12カ月の検討である。

敗血症患者55名である。敗血症の診断はACCP/SCCM Consensus Conferenceに拠った[4]。感染を合併しない全身性炎症反応症候群（systemic inflammatory response syndrome；以下SIRSと略す）患者は80名で，それぞれに背景因子は**表1**に示す。併せて健常者75名についても検討した。

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表 1　対象症例の背景

Sepsis (n=55)		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		

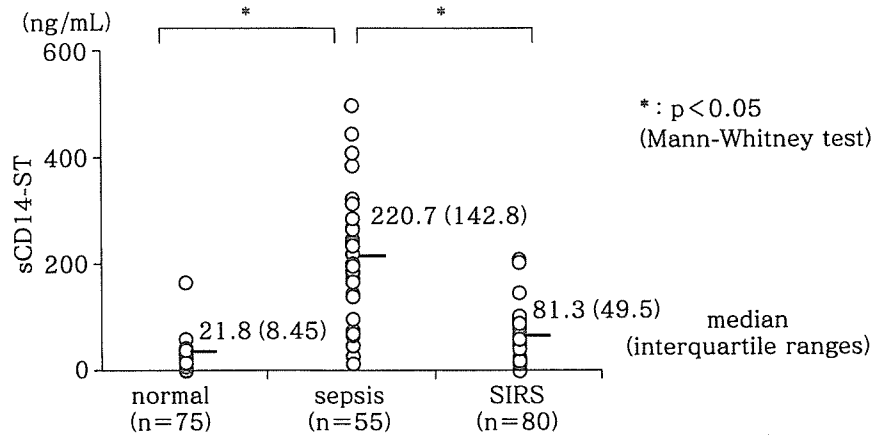

図 1　健常者，敗血症患者，感染を伴わない SIRS 患者の sCD 14-ST 値

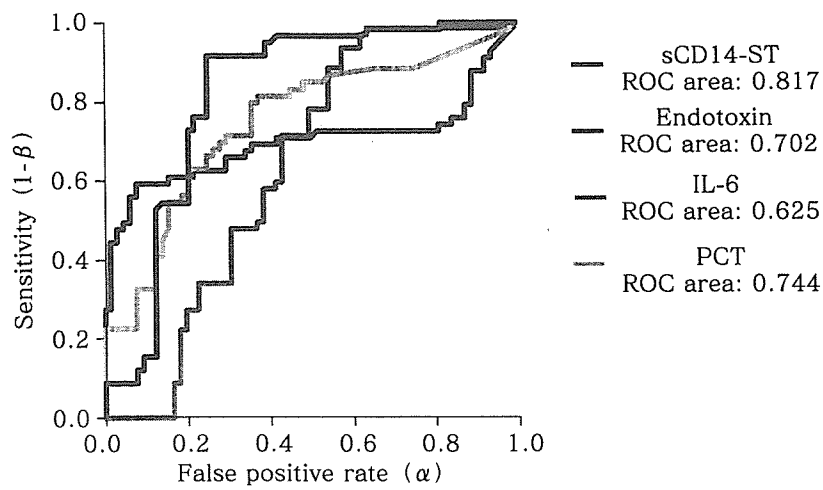

図 2　それぞれの液性因子の敗血症診断能力

検体はヘパリン加のエンドトキシンフリーのシリンジで採血し，直ちに 3,000 rpm で 40 秒間遠心後に，多血小板血漿 (platelet-rich plasma：PRP) を得て，測定まで−80 度で保存した。

血漿中の sCD 14-ST は ELISA で測定した[3]。

プロカルチトニン (procalcitonin；以下 PCT と略す) は化学発光免疫測定法 (LUMI test PCT™, B・R・A・H・M・S DIAGNOSTICA GmbH, Berlin, Germany, 和光純薬工業, 東京；Lumico Analyzer SA-300, マイクロテック, ニチオン, 東京) により測定した[5]。その測定限界値は 0.1 ng/mL であった。

IL-6 は ELISA (TFB, 東京) で測定し，その測

表 2　それぞれの液性因子の相関関係

	Endotoxin	IL-6	CRP	PCT	SOFA
sCD 14-ST	0.118	0.095	0.610*	0.597*	0.750*

*：$p<0.01$ (Spearman test)

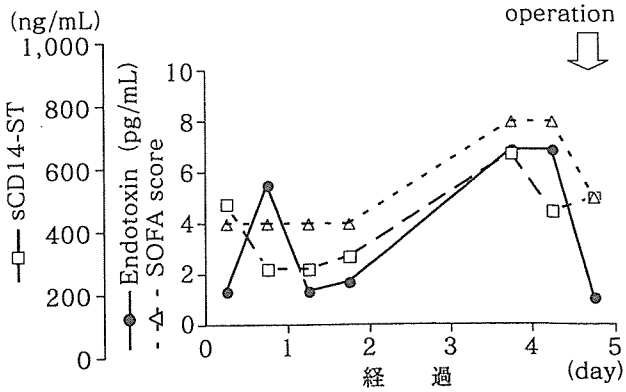

図 3　十二指腸潰瘍穿孔症例の sCD 14-ST の経時的変化

定限界値は 10 pg/mL であった。

エンドトキシンは高感度法で測定した。敗血症におけるエンドトキシン値は 1.1 pg/mL 以上である[6,7]。

CRP は血清を分離後直ちにラテックス凝集比濁法（イムノテイクルスオート CRP，エイアンドティー，東京）で測定した。正常値は 0.3 mg/dL 未満であった。

重症度の指標としては sequential organ failure assessment (SOFA)[8] score を用いた。

II. 結　果

敗血症群の sCD 14-ST の中央値は 220.7 ng/mL，健常者群の sCD 14-ST の中央値は 21.8 ng/mL，感染を合併しない SIRS 群の sCD 14-ST の中央値は 81.3 ng/mL と，敗血症群の sCD 14-ST 値は健常者群および感染を合併しない SIRS 群いずれに対しても有意に高値であった（**図 1**）。

各炎症マーカーの敗血症の診断能力として ROC area での比較では，sCD 14-ST が最も良好な結果が得られた（**図 2**）。

SCD 14-ST とそれぞれの炎症マーカーとの相関関係を**表 2** に示す。

代表的な症例を以下に示す。

症例 1：78 歳，女性。突然に発症した腹痛で救急センターへ搬送された。緊急内視鏡検査で十二指腸潰瘍穿孔による腹膜炎と診断した。この時腹膜炎は上腹部に限局しており，手術をせずに抗生剤や抗潰瘍剤投与による保存治療を行った。しかし，入院 7 日目に腹膜炎の範囲が広がり，状態の悪化を認めたため手術へと方針を代えた。この症例における sCD 14-ST は，発症わずか 3 時間後に 500 ng/mL と上昇していた。その後 200 ng/mL 程度で推移したが，手術前の sCD 14-ST は 700 ng/mL へ上昇して，手術後速やかに低下した。エンドトキシンと SOFA score にパラレルに推移を認め，病態をよく反映しているものと考えられた（**図 3**）。

症例 2：22 歳，男性。オートバイ運転中に立ち木へ衝突して受傷した。搬送時にすでに肝損傷などの多発外傷による出血性ショックの状態であり，緊急手術と TIA 治療を行った。肝臓右葉に広範囲の損傷を認めた。手術後 8 日目に肝臓膿瘍と敗血症性ショックを認め，排膿のためドレーンを挿入した。しかし，排膿が不十分で敗血症から離脱できず，追加のドレーンを挿入した。その後次第に軽快方向へ向かった。sCD 14-ST は，入院当初は 150 ng/mL と軽度の上昇であった。しかし，肝臓膿瘍の判明する 2 日前には 700 ng/mL へと上昇した。そして，1 回目の排膿治療後も sCD 14-ST は上昇し続け 2,500 ng/mL 以上の極めて高い値を示した。1 回目の治療が不十分で，膿瘍が遺残したため敗血症の進行を反映した。2 回目の排膿治療後は下降し，SOFA score と平行するように徐々に下がった。このように sCD 14-ST は敗血症の病態を鋭敏に反映した（**図 4**）。

III. 考　察

今回，われわれは健常者，敗血症患者，および感染を合併しない SIRS 患者の sCD 14-ST について検討した。敗血症患者の sCD 14-ST 値は健常者と感染症を合併しない SIRS 患者の sCD 14-ST 値に対して有意に高値であった。また，sCD-14 値は，PCT 値，CRP 値，SOFA score と有意の相関関係がみられ，敗血症の有用な診断のツールに成り得るこ

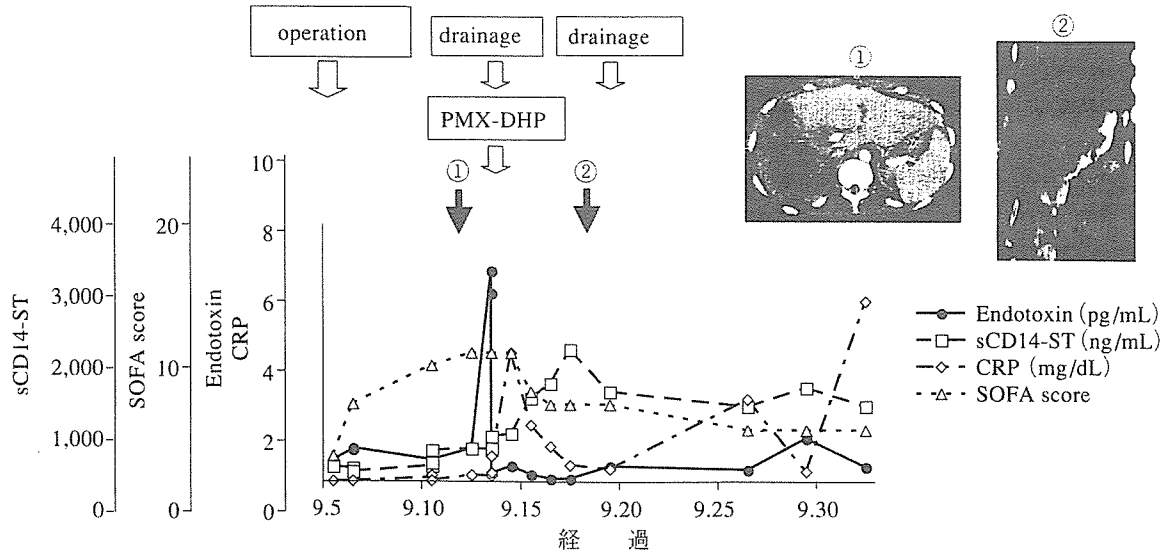

図 4　肝臓膿瘍患者症例の sCD 14-ST の経時的変化

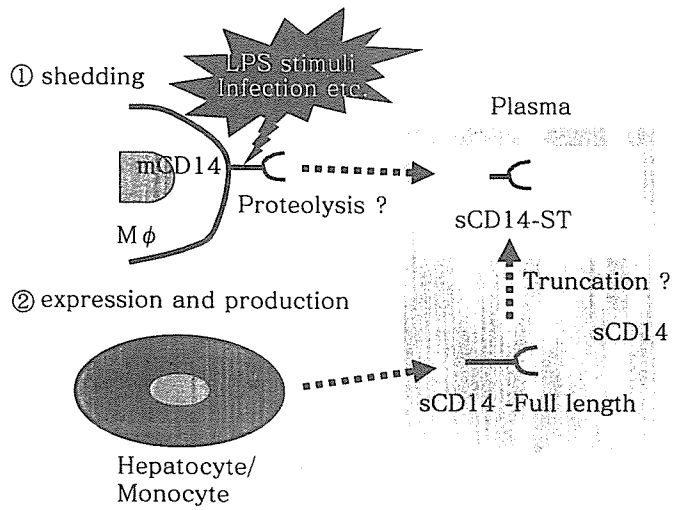

図 5　sCD 14-ST の由来について

とが示された。

ROC 解析では，sCD 14-ST は敗血症の診断能力として他の液性因子と比較して最も優れたものであることも示された。さらに，sCD 14-ST は患者の病態の推移をもよく反映していた。一方，CRP，IL-6 は敗血症から離脱しても高い値で推移することがあり，敗血症の診断マーカーとしては不十分のように思われた。

PCT は敗血症の診断マーカーとして用いられているが[5,9]，敗血症の診断マーカーとしては，sCD 14-ST がより優れているデータが得られた。

敗血症において，sCD-14 の生理学的役割と，産生機構は不明である。Bufler らは sCD 14 はヒトの単球から産生される二つのメカニズムがあると報告している[10]。一つは放出されるもので，もう一つは分泌される経路である（図 5）。

Bazil らも放出されると報告している[11]。sCD 14-ST は敗血症を合併すると 2～3 時間で上昇することから，われわれは，sCD 14-ST は分泌されるよりも放出されると考えるのが妥当と考えている。

SCD 14-ST を測定することで敗血症の早期診断が確実に可能となった。今後，さらに多くの患者のさまざまな感染症の sCD 14-ST を測定し，多くの病態と sCD 14-ST との関わりについて検討する必要がある。

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文　献

1. Basil V and Strominger JL. Shedding as a mechanism of down modulation of CD 14 on stimulated human monocytes. J Immunol 1991；147：1567-74
2. Endo S, Inada K, Kasai T, et al. Soluble CD 14 (sCD 14) levels in patients with multiple organ failure (MOF). Res Commun Chem Pathol Pharmacol 1994；84：17-25
3. Yaegashi Y, Shirakawa K, Sato N, et al. Evaluation of a newly soluble CD 14 subtype as a marker for sepsis. J Infect Chemo. (in press)
4. Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee：Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 1992；101：1644-55/Crit Care Med 1992；20：864-74
5. 遠藤重厚，葛西　健，稲田捷也．全身性炎症反応症候群における感染症および重症度診断としてのプロカルチトニン値測定の意義．感染症誌 1999；73：197-203
6. 八重樫泰法，稲田捷也，佐藤信博，ほか．血漿高感度エンドトキシン測定法について．エンドトキシン血症救命治療研究会誌 2003；7：25-8
7. 遠藤重厚，八重樫泰法，佐藤信博，ほか．PMX-DHP治療効果の検討―高感度エンドトキシン測定法を用いた検討―．エンドトキシン血症救命治療研究会誌 2004；8：79-83
8. Vincent JL, Mendonca A, Camtraome F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units：Results of a multicenter, prospective study. Crit Care Med 1998；26：1793-800
9. Assicot M, Gendrel D, Carsin H, et al. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993；341：515-18
10. Bufler P, Stieger G, Schuchamann M, et al. Soluble lipopolysaccharide receptor (CD 14) released via two different mechanisms from human monocytes and CD 14 transfectants. Eur J Immunol 1995；25：604-10
11. Bazil V and Strminger JL. Shedding as a mechanism of down-modulation of CD 14 on stimulated human monocytes. J Immunol 1991；147：1567-74

[Original Article]

Usefulness of Soluble CD 14 Subtype Which as Is a New Diagnostic Marker for Sepsis

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Abstract

CD 14 is a receptor, which exists existing on the cell membrane of endotoxin and LBP complex, and transmits a signal of endotoxin inside the cell, ; but however, it is also known to exist in blood as soluble protein. We discovered soluble CD 14 subtype (sCD 14-ST, 49 kD), the molecular weight of which is different from that of traditional CD 14 among soluble CD 14, and developed an assay for sCD 14-ST using the ELISA technique. In When examining ROC curves, it was clear that the measurement of ing the sCD 14-ST value in septic patients by this method provides a superior ability to diagnose sepsis compared to factors such as CRP, endotoxin, IL-6, and procalcitonin.

Key words：Sepsis, sCD 14-ST, Diagnosis, Procalcitonin, CRP

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エンドトキシン血症救命治療研究会誌　第9巻第1号　2005；118-121

[多施設臨床研究報告]-1

高感度エンドトキシン測定法による PMX-DHPの効果判定について

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はじめに

PMX-DHPは敗血症・敗血症性ショック患者の治療の有効な手段であることはよく知られている。しかし，その作用メカニズムについては十分解明されていない。その一因としてこれまでのエンドトキシン測定法に問題があった。われわれはこれまで高感度エンドトキシン測定法の有用性について報告してきた[1~3]。

今回，多施設において高感度エンドトキシン測定法を用いてエンドトキシン値を測定することによりPMX-DHPの治療効果について検討した。

I．対象と方法

本臨床研究は，岩手医科大学・救急医学，東京女子医科大学・救急医学，昭和大学藤が丘病院・救急医学科，東京医科大学八王子医療センター・救命救急部，刈谷総合病院・救急集中治療部，佐賀県立病院好生館・救命救急センターの6施設が参加した。

調査期間は2004年4月～9月までの6カ月間であった。登録症例は40症例であった。

エンドトキシン値はトキシノメーターを用い，Endotoxin-Single Wako（和光純薬工業，大阪）で測定した高感度法を用いた[4]。本法による敗血症のカットオフ値は1.1 pg/mLである[1,2]。Interleukin 6(IL-6)，interleukin 10(IL-10)，interleukin 12(IL-12)，interferon γ(IFN-γ)はいずれもenzyme-linked immunosorbent assay(ELISA)法で測定した。

表1　PMX-DHP施行前のエンドトキシン値
陽性例（PMX-DHP施行直前）
25/40例（62.5%）

平均値	11.54411
標準偏差	32.83808
中央値	1.35
4分位範囲	0.09

II．結　果

全症例において敗血症のカットオフ値である1.1 pg/mLを超えたものは40例中25例（62.5%）であった（表1）。

PMX-DHP施行時のエンドトキシン値の経時的変化を図1に示す。PMX-DHP施行24時間後のエンドトキシン値はPMX-DHP施行前に対して有意に低下した(図2)。PMX-DHP施行によりエンドトキシン値が低下することが示されている。

各サイトカイン値の経時的変化を図3に示す。IL-6，IL-10，IL-12，IFN-γすべてのサイトカイン

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索引用語：高感度エンドトキシン測定法，多施設研究，エンドトキシン血症，サイトカイン

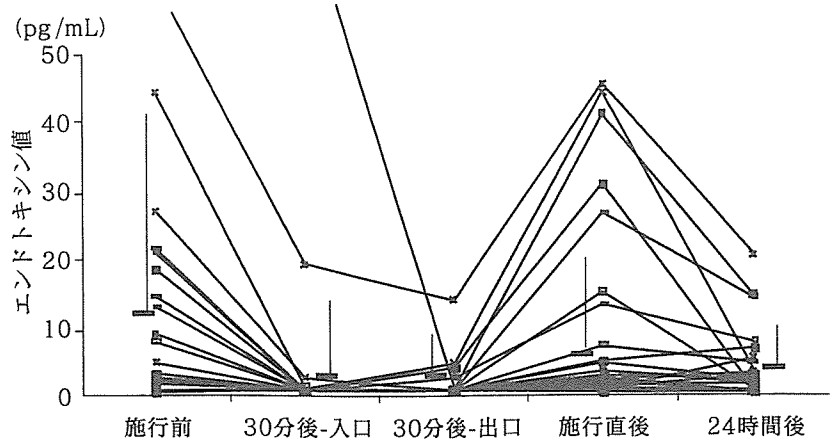

図 1　PMX 施行時のエンドトキシン値の経時的変化

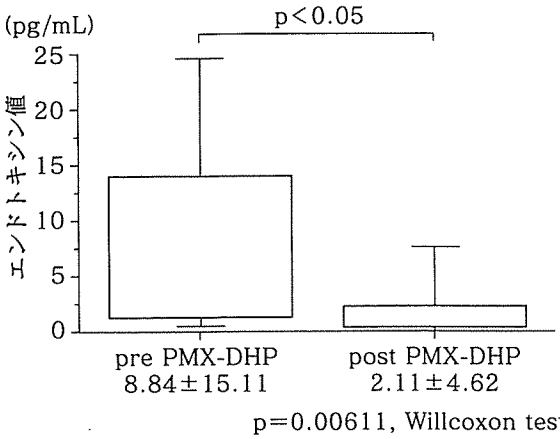

図 2　PMX-DHP 施行前と 24 時間後のエンドトキシン値

が経時的に低下する傾向がみられた。

エンドトキシン値と IL-6 値（r=0.369, p<0.05），エンドトキシン値と IL-10 値(r=0.461, p<0.05），エンドトキシン値と IFN-γ 値（r=0.317, p<0.05）間には有意の相関関係が認められた。

臨床的効果としては，PaO_2/FiO_2（P/F）比が 199±51 から 273±115 へと有意に改善した（**図 4**）。収縮期血圧は 87.1±17.9 mmHg から 110.9±29.8 mmHg へ有意に上昇し，昇圧剤は 5.67±2.62 μg/kg/min から 4.48±2.33 μg/kg/min へと有意に減量できた(**図 5**)。時間尿量も 45±47 mL から 61±63 mL へと有意に上昇した（**図 6**）。

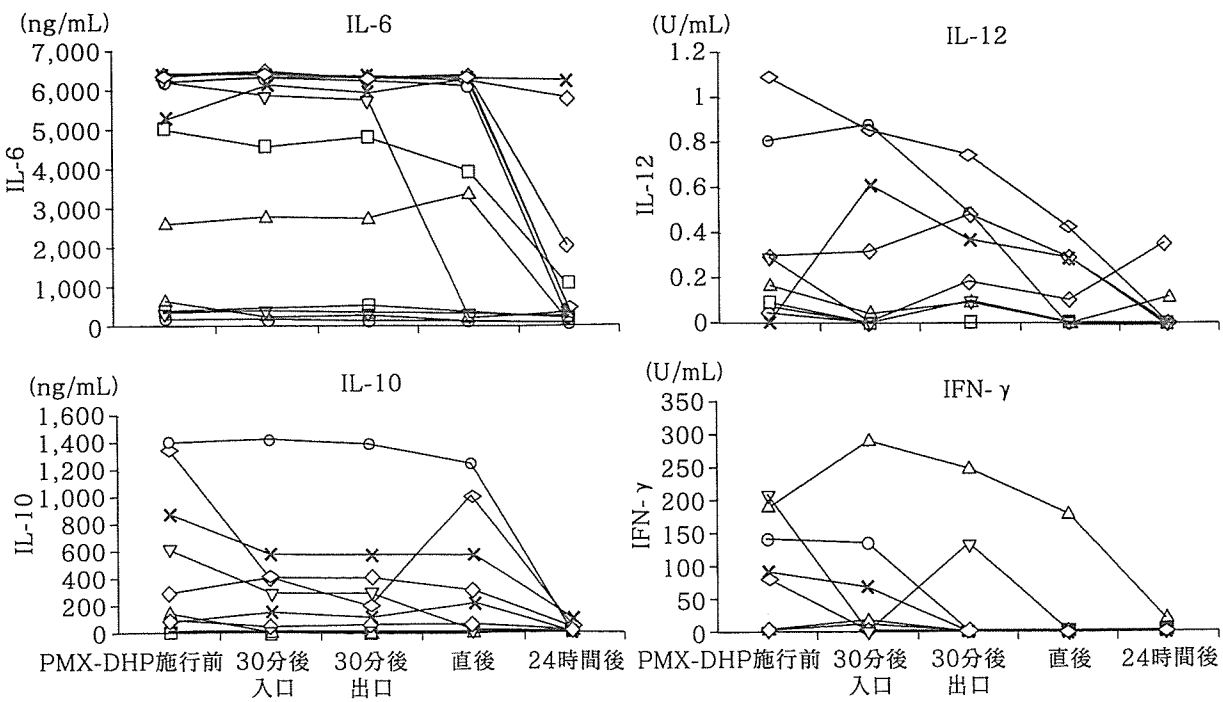

図 3　各サイトカイン値の経時的変化

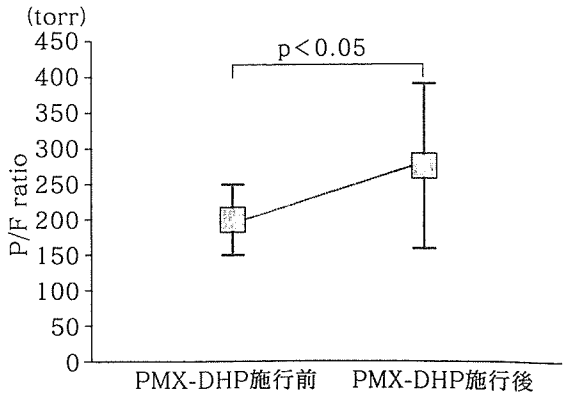

図 4　PMX-DHP 施行前後の呼吸状態の変化(P/F 比)

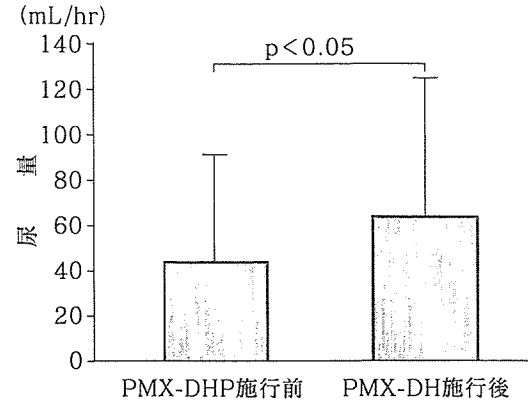

図 6　PMX-DHP 施行前後の時間尿の変化

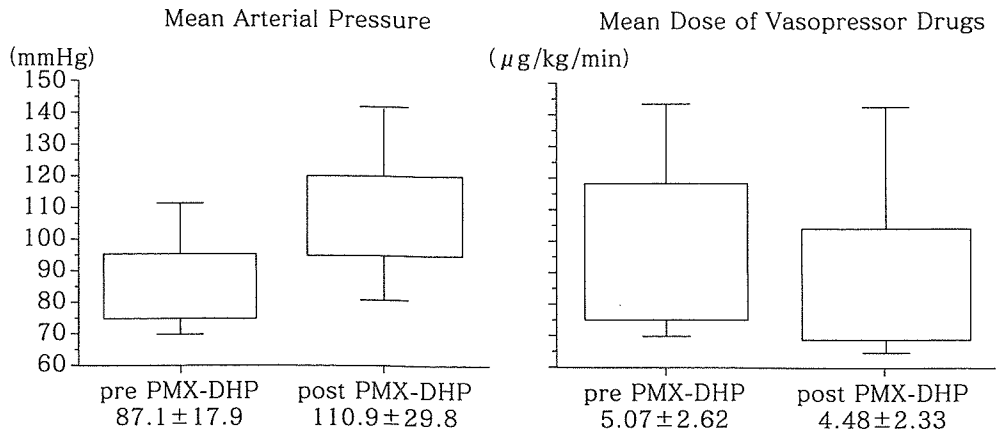

図 5　PMX-DHP 施行前後の平均血圧と昇圧剤の変化

III. 考　察

全敗血症患者の約40%がグラム陰性桿菌による感染症であり，これら患者の約半数が菌血症であることが明らかとなっている[4]。重要なことは，グラム陰性菌血症は，真菌血症やグラム陽性菌血症に比べて敗血症性ショックに至る可能性が顕著に高いことである（50～60%対5～10%）。内科または外科集中治療室（ICU）に入室した患者では，一般病棟における患者に比べて敗血症発症リスクが顕著に高いとの報告がある[5~7]。

これまでのエンドトキシン測定は，特異度と測定時間の短縮を重視し，陽性は3.5～5.0 pg/mL 以上と設定されていた。われわれは，測定時間を200分にすることにより，敗血症におけるエンドトキシンのカットオフ値を1.1 pg/mL 未満とすることにし，その有用性について確認した[1]。

従来，明らかに敗血症の状態であるが，エンドトキシン値が5.0 pg/mL 未満で正常域と判断し，PMX-DHP を施行しなかった症例が多数みられた。本法を用いることにより従来見過ごされてきたエンドトキシン値陽性の症例が見出せることになったことは治療において非常に大きな進歩と思われる。

今回は，この高感度エンドトキシン測定法を用いて多施設における PMX-DHP の有用性について検討したところ，エンドトキシン値の明らかな低下を認めるとともに，エンドトキシンがその強い刺激因子となり産生される IL-6, IL-10, IL-12, IFN-γ などのサイトカインも PMX-DHP 施行により低下していることが確認された。このことは，敗血症においてエンドトキシン値が陽性であることが病態形成に強く関わっていることを示唆するものであろう。言い換えれば，エンドトキシン値が1.1 pg/mL を超える時点で PMX-DHP を施行する意義があると思われる。また，PMX-DHP 施行によりエンドトキシ

ン値の低下，各種サイトカインの低下に伴い，呼吸状態の改善，尿量の増加，収縮期血圧上昇，さらに昇圧剤の減量といずれも有意な臨床症状の改善も得られた。

今回は，短期間の多施設における検討ではあったが，高感度エンドトキシン測定法を用いて敗血症におけるPMX-DHPの有用性について確認した。

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文　献

1. 八重樫泰法，稲田捷也，佐藤信博，ほか．血漿高感度エンドトキシン測定法について．エンドトキシン血症救命治療研究会誌 2003；7(1)：25-8
2. 遠藤重厚，八重樫泰法，佐藤信博，ほか．PMX-DHP治療効果の検討―高感度エンドトキシン測定法を用いた検討―．エンドトキシン血症救命治療研究会誌 2004；8(1)：79-83
3. 遠藤重厚，佐藤信博，小鹿雅博，ほか．エンドトキシン測定法の評価．Current Concept in Infectious Diseases 2005；24(1)：16-7
4. Oishi H, Takaoka A, Hatayama Y, et al. Automated Limulus amebocyte lyste (LAL) test for endotoxin assay using a new Toxinometer ET-201. J Parenter Sci Technol 1981；39：194-200
5. Fridkin SK, Welbel SF, Weinstein RA. Magitude and prevention of nosocomial infections in the intensive care unit. Infect Dis Clin North Am 1997；11：479-96
6. Sands KE, BaBates DW, Lanken PN, et al. Epidemiology of sepsis syndrome in eight academic medical centers. JAMA 1997；278：234-40
7. Martin MA, Itokazu G, Danziger LH. Epidemiology and clinical impact of Gram-negative sepsis. Infect Dis Clin North Am 1991；5：739-54

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