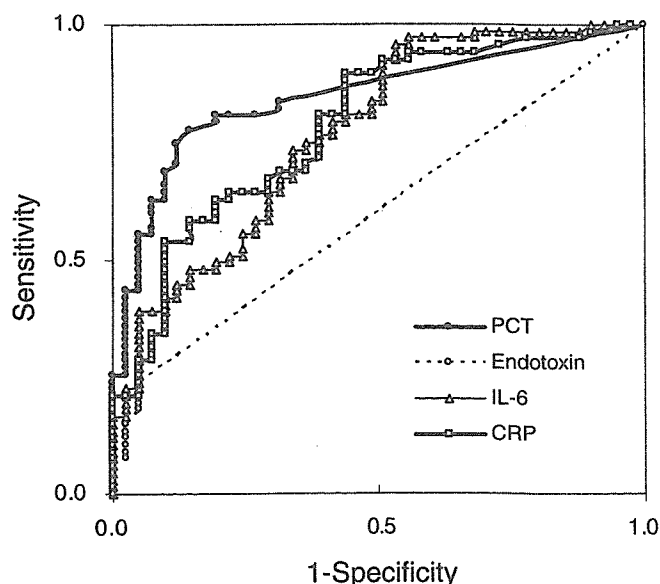


Table 6. Sensitivity of PCT, endotoxin, β -D-glucan, IL-6, and CRP with respect to the type of infection

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

	<i>n</i>	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.

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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

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

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Table 1. Medical histories of the subjects in this study

Sepsis (<i>n</i> = 66)		SIRS (<i>n</i> = 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).

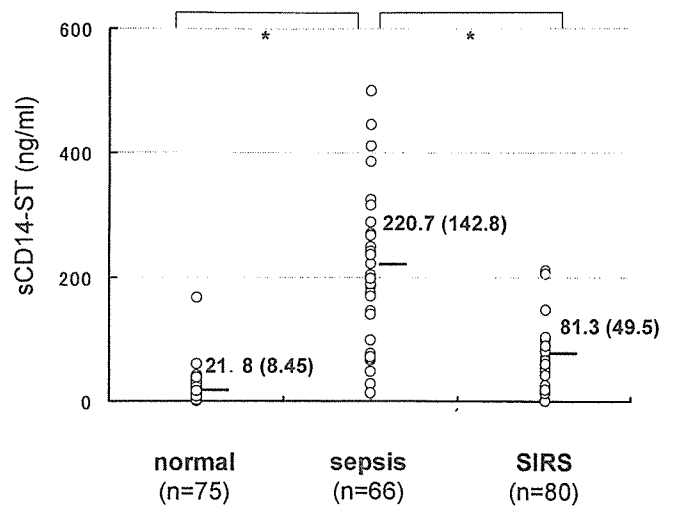


Fig. 1. The concentrations of soluble CD14 subtype (sCD14-ST) measured in plasma samples from normal controls, sepsis patients, and systemic inflammatory response syndrome (SIRS) patients. Data values are expressed as medians and interquartile ranges. Mann-Whitney tests were used to compare the results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

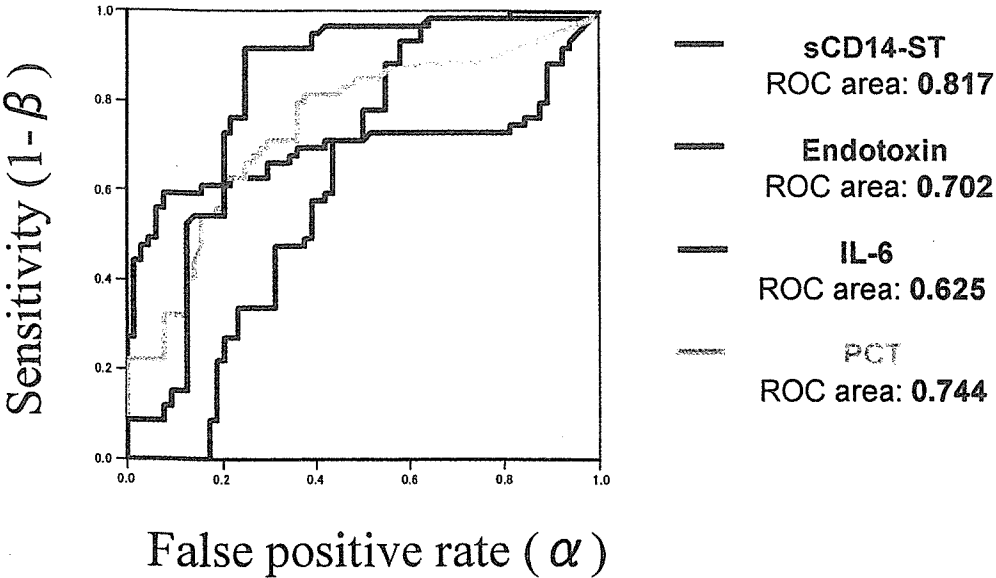


Table 2. Correlation of sCD14-ST levels with those of other markers

	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.

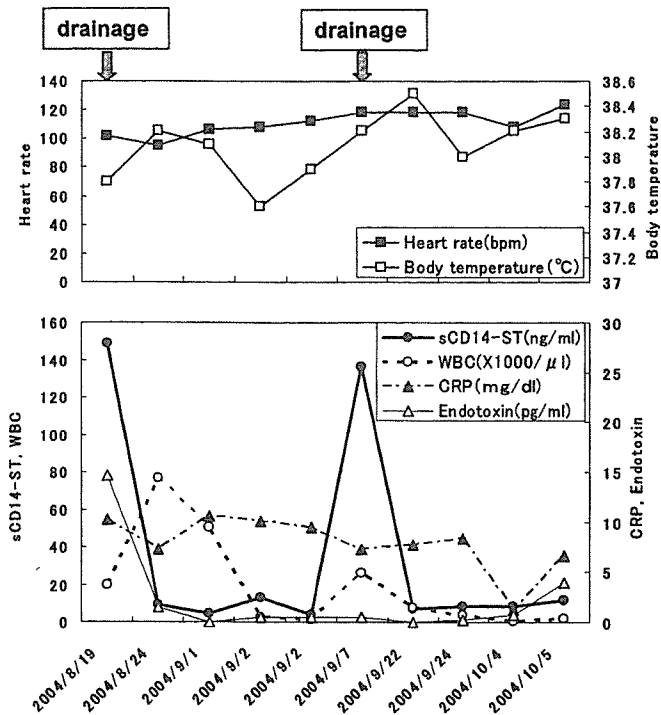


Fig. 3. Changes in sCD14-ST, endotoxin, and C-reactive protein (CRP) concentrations in a patient with cholangiocarcinoma, after surgery. sCD14-ST concentrations were measured by enzyme immunoassay (EIA). Endotoxin and CRP were measured with commercially available kits. Drainage was performed after computed 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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[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、血中にも可溶性蛋白質として存在することが知られている。われわれは可溶性 CD 14 分子のうち従来の CD 14 と分子量が異なる、すなわち可溶性 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 2000 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		

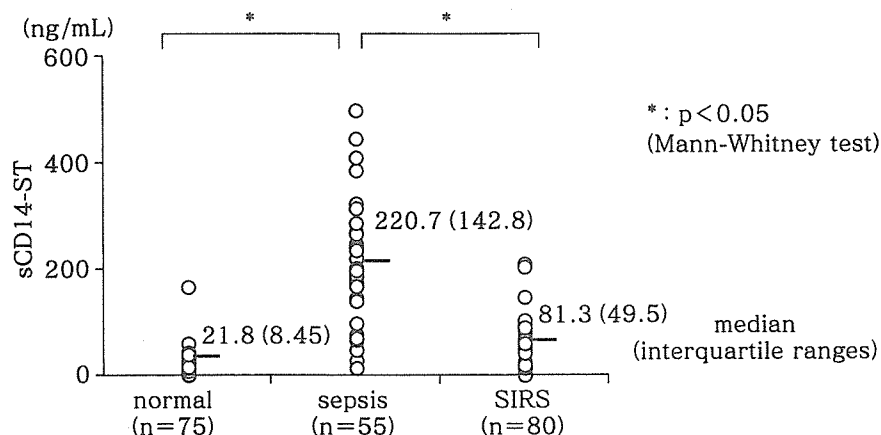


図 1 健常者，敗血症患者，感染を伴わない SIRS 患者の sCD 14-ST 値

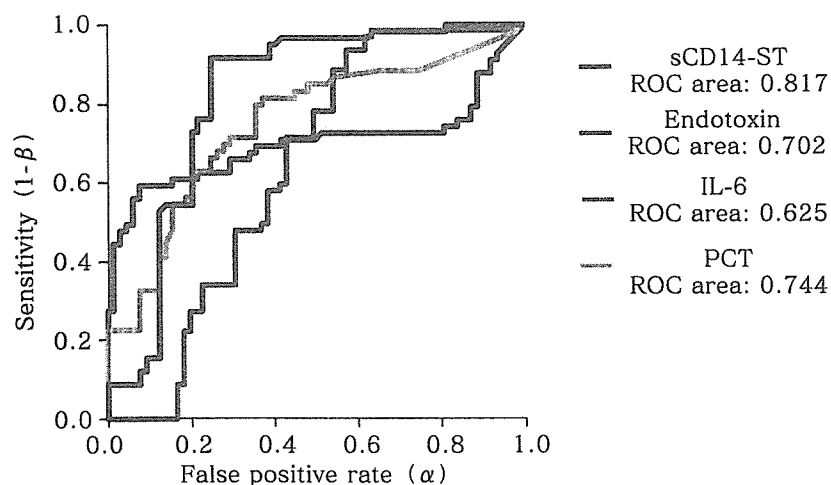


図 2 それぞれの液性因子の敗血症診断能力

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)

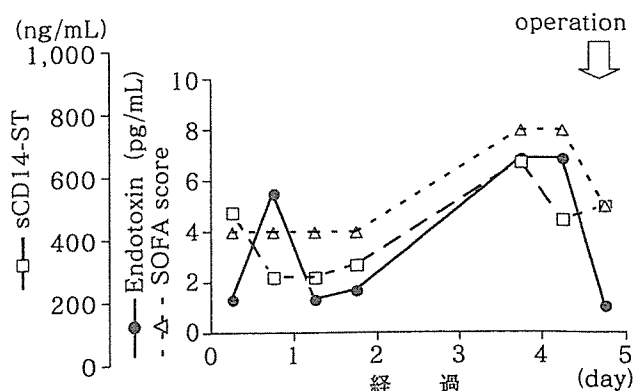


図 3 十二指腸潰瘍穿孔症例の sCD 14-ST の経時的変化

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局限しており, 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 に平行して推移を認め, 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

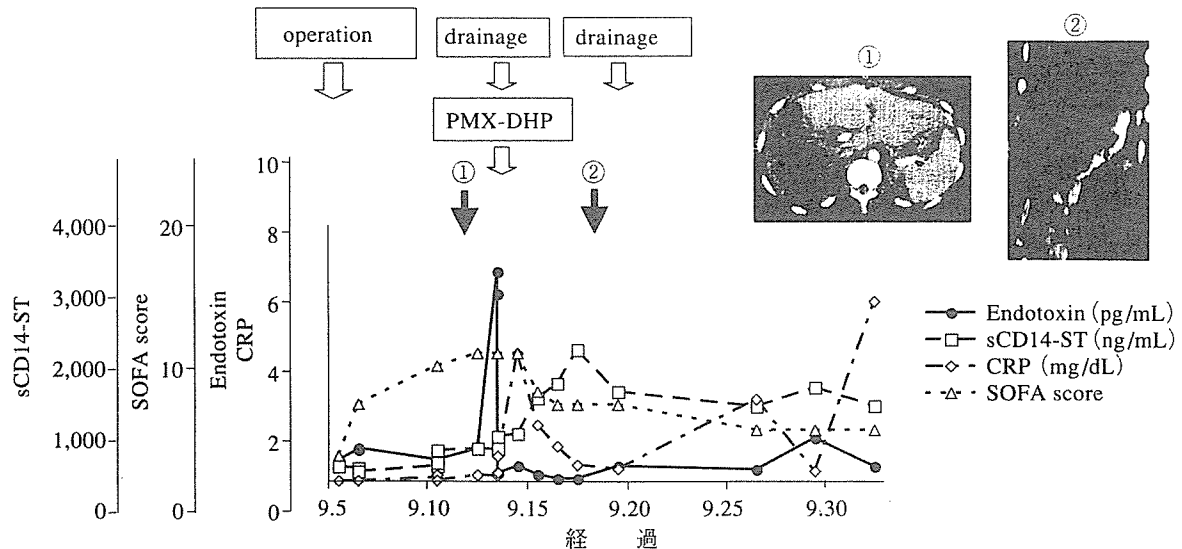


図 4 肝臓腫瘍患者症例の sCD 14-ST の経時的変化

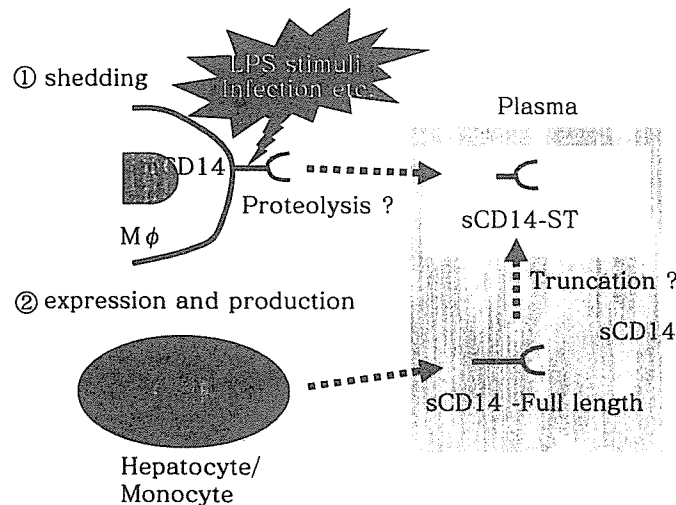


図 5 sCD 14-ST の由来について

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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[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

* * *

[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

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

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。

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

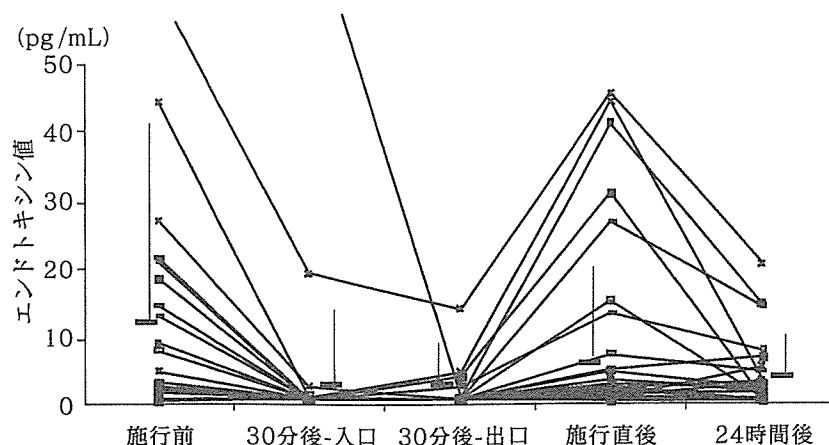


図 1 PMX 施行時のエンドトキシン値の経時的変化

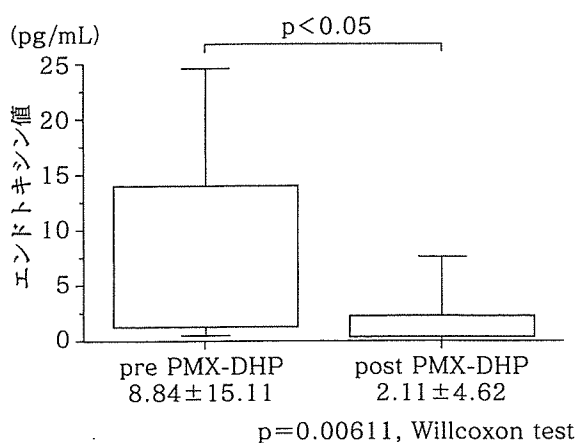


図 2 PMX-DHP 施行前と 24 時間後のエンドトキシン値

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。

臨床的效果としては, PaO₂/FiO₂ (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 μ g/kg/min から 4.48 ± 2.33 μ g/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)。

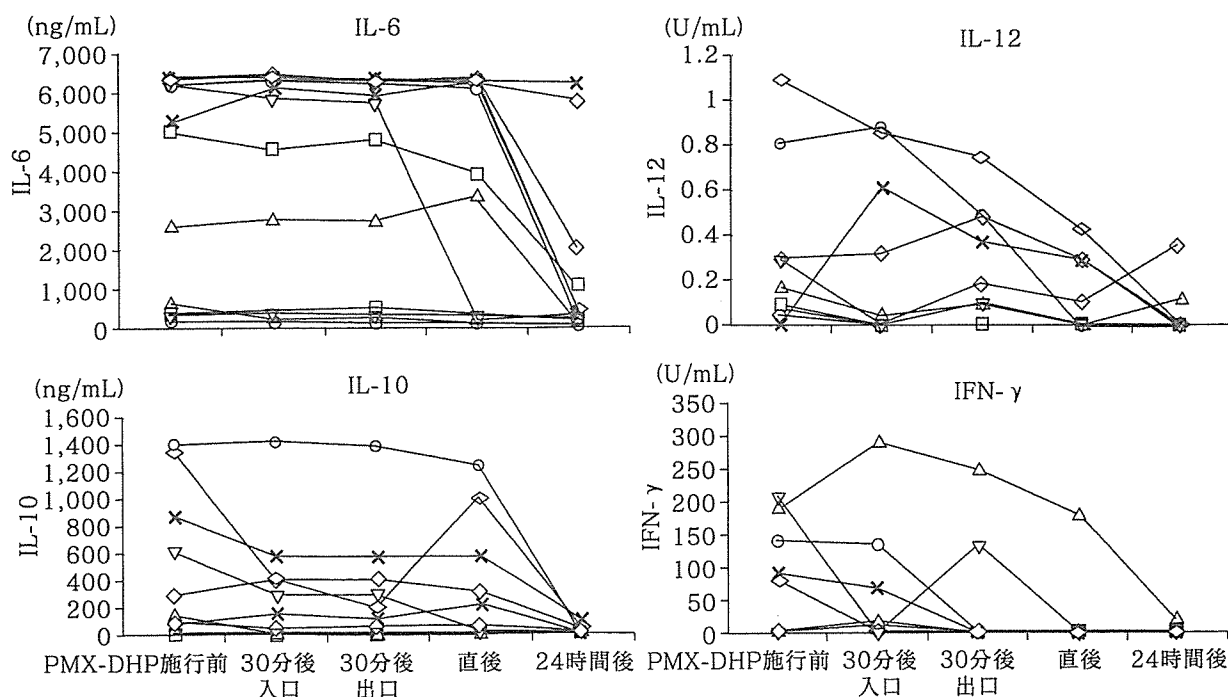


図 3 各サイトカイン値の経時的変化

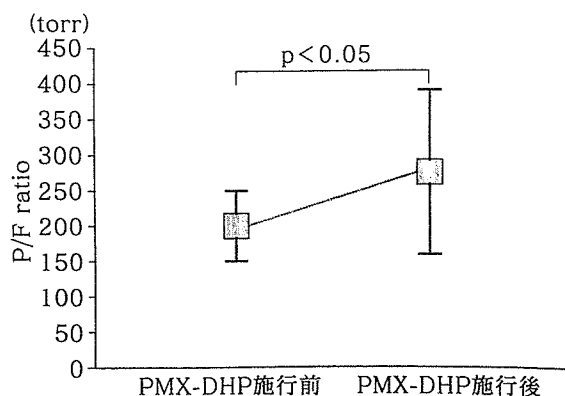


図4 PMX-DHP 施行前後の呼吸状態の変化 (P/F 比)

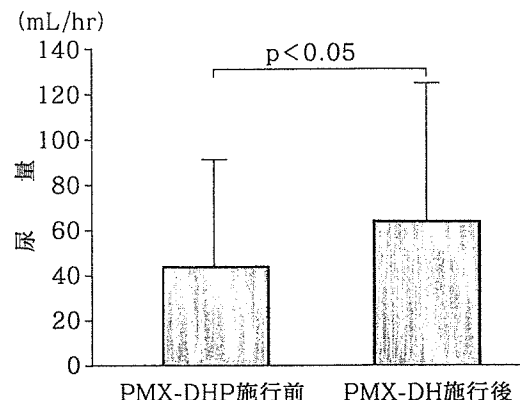


図6 PMX-DHP 施行前後の時間尿の変化

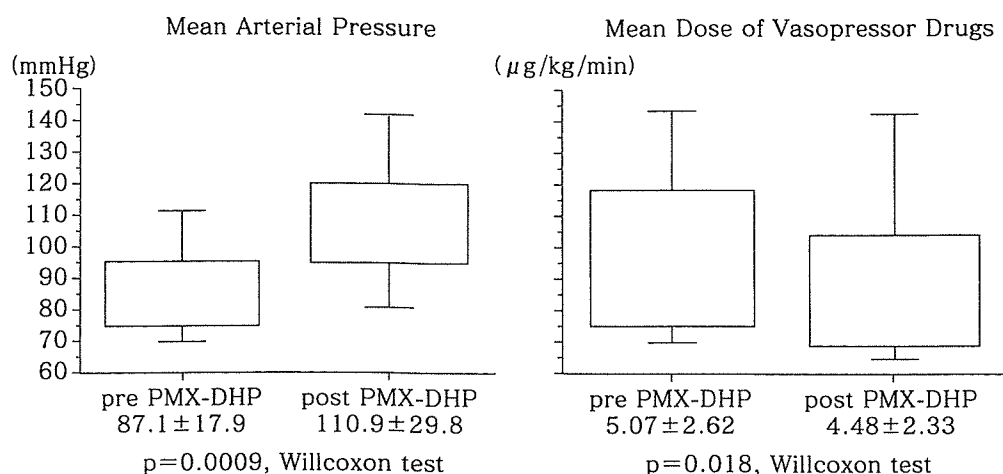


図5 PMX-DHP 施行前後の平均血圧と昇圧剤の変化

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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[症例報告]

長時間 PMX-DHP 療法が奏功した 重症 *Klebsiella pneumoniae* 敗血症患者の 1 例

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要 旨

グラム陰性桿菌敗血症の治療においてエンドトキシン吸着療法は、特に敗血症性ショックを呈したとき重要な治療法として施行されている。今回、われわれは敗血症性ショックを伴った重症 *Klebsiella pneumoniae* 感染症患者に長時間 PMX-DHP を施行し、14 時間で昇圧効果を認めた。エンドトキシン値も 200 pg/mL から 3.1 pg/mL まで改善を認めた。昇圧効果が認められたのは、開始後 3 時間経過してからである。今後、PMX-DHP 施行時間についてはその有用性が示唆され、さらなる検討が必要と思われた。

索引用語：長時間 PMX-DHP、敗血症性ショック、*Klebsiella pneumoniae* 感染症、エンドトキシン

はじめに

エンドトキシン吸着療法（ポリミキシン B 固定化ファイバーカラム（PMX）による直接血液灌流法（DHP）；以下 PMX-DHP と略す）は、グラム陰性桿菌敗血症治療において重要な役割を果たし、その有用性は多く報告されてきている。その作用機序として、ポリミキシン B の陰性桿菌に対する強い抗菌作用によって、細菌の細胞壁外膜成分である LPS に結合することにより細菌細胞膜構造の乱れから溶菌作用をきたすとされている¹。この PMX-DHP は通常施行時間が 2 時間とされているが、臨床的観点からその施行時間についての検討はまだ十分に行われていない。

今回われわれは重症 *Klebsiella pneumoniae* 敗血症患者に長時間 PMX-DHP を計 14 時間施行し、救命された症例を経験したので報告する。

I. 症 例

症例：73 歳，男性

既往歴：3 年前，急性心筋梗塞。高血圧症にて近医加療中。

家族歴：特記事項なし

現病歴：平成 16 年 9 月某日，急性発症の腹痛で前医を受診。上腸間膜血栓症を疑い精査するが所見に乏しく，急性胃腸炎の診断で入院となる。絶食の上，中心静脈栄養管理下となり腹痛も消失し入院 5 日目に流動食が開始となる。経口摂取後より嘔吐がみられ，再度消化管精査を行うが器質的閉塞性病変は認めなかった。入院 13 日目より 39°C の発熱がみられ，動脈血培養で *Klebsiella pneumoniae* が検出された。IPM/CS，CLDM の抗生剤併用が施行されたが，徐々に血圧低下（75/42 mmHg）をきたし，PMX-DHP を 2 時間施行するが昇圧効果がなく，また

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表 1 搬入時検査所見

TP	5.6 g/dL	WBC	24,590/ μ L
Alb	2.9 g/dL	RBC	3.22×10^6 / μ L
Na	136 mEq/L	Hb	10.3 g/dL
K	4.8 mEq/L	Ht	30.40%
Cl	105 mEq/L	Plt	71×10^3 / μ L
BUN	68.6 mg/dL	APTT	91 秒
Cr	2.7 mg/dL	PT	13.4 秒
AST	128 IU/L	Fibrinogen	622 mg/dL
ALT	56 IU/L	AT-III	61%
LDH	295 IU/L	FDP	7.7 μ g/mL
T-Bil	0.9 mg/dL		
CRP	26.6 mg/dL		

徐々に意識レベル低下と呼吸困難が出現したため、16 日目に当センターに救急搬送となった。

入院時現症および検査所見：JCS 2, 血圧 75/45 mmHg (DOA 4 μ g/kg/min, DOB 5 μ g/kg/min 使用), 心拍数 110/分, 体温 38.4°C。両側呼吸音は減弱し, 動脈血ガス分析で pH 7.265, pCO₂ 39.3 mmHg, pO₂ 91.4 mmHg, HCO₃ 17.4 mmol/L, BE -8.9 (酸素 10 L/min リザーバマスク) と高度アシドーシスと低酸素血症を認め, 直ちに気管内挿管, 人工呼吸器管理下とした。血液一般, 生化学データ等は図 1 に示す。

入院後経過：グラム陰性桿菌敗血症性ショックの診断で直ちに PMX-DHP を施行した。抗凝固剤としてメシル酸ナファモスタットを 20~30 mg/hr 持続投与とした。1 本目の PMX-DHP で 7 時間施行 (血流ポンプ 80 mL/min) し, この時点で血圧 130/58 mmHg まで上昇した。引き続き再プライミングの後, 2 本目の PMX-DHP を 7 時間施行し, 最終的に血圧 158/72 mmHg まで上昇が認められた。エンドトキシン値は高感度エンドトキシン測定法^{2,3}で 1 本目開始時 200 pg/mL, 2 本目開始時 6.9 pg/mL, 2 本目終了時 3.1 pg/mL まで著明に減少した。さらに入院 5 日目まで連日 PMMA-CHDF (20 時間/日) を施行し, 抗生剤は MEPM 1.5 g/日, 免疫グロブリン製剤 12.5 g/5 日間の併用を行った。同時に血清クレアチニン値も 2.7 mg/dL から 1.0 mg/dL へ改善し, PMX-DHP から離脱できた。人工呼吸器も離脱となり, リハビリ目的で入院 39 日目に前医へ転院となった。

II. 考 案

Klebsiella pneumoniae は, 腸内細菌に属するグラム陰性の嫌気性桿菌である。*Klebsiella pneumoniae* 感染症は, 一般に健常人には一次感染を発症することは少ないとされているが, 近年糖尿病や慢性閉塞性肺疾患においては, 二次感染として重要であると報告されている⁴。本症例は敗血症性ショックに陥っており, 呼吸器感染が原因と思われた。

一方, グラム陰性桿菌敗血症の病態に対するメカニズムは, 近年の分子生物学的アプローチの手法の発達に伴い徐々に解明されてきている。その中で, PMX-DHP はその有用性が確立されつつある。

PMX-DHP の施行時間については, これまでも長時間施行することの有用性が報告されてきている⁵。特に循環動態安定化という視点からみれば, 重症度の高い敗血症患者では 2 時間では, 十分な循環動態は得られず, 多くの症例で開始 2 時間以降に循環動態が安定してくることを報告している⁶。本症例も血圧の安定化がみられたのは開始後 3 時間であったが, さらに継続することによって十分な循環動態安定化が認められた。また, 1 本目の PMX-DHP 回収後より血圧の低下が一時低下したが, 2 本目を再プライミング後直ちに開始して, 計 14 時間の試行で最終的に十分な昇圧効果が得られている。エンドトキシン吸着の観点からは 1 本目で 200 pg/mL が 6.9 pg/mL まで著明に低下しているが, 施行時間を決定する上では, 血圧がパラメーターになっていた。さらに三浦ら⁵は, 長時間施行症例では肺酸素化能の改善がより有用であることを報告している。また同時に安全性についても検討しているが, 血小板数の減少は 2 時間施行群と長時間施行群では開始時に比較して両群とも減少していたが, 両群間では有意差は認めなかったと報告している。

今後長時間 PMX-DHP については, 臨床症例では本症例のように奏効例は多く報告されてきているが, さらに有効性と安全性について炎症性サイトカインや各種メディエーターの推移を含め検討する必要がある。

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[Case Report]

**A Case with Severe *Klebsiella pneumoniae* Sepsis
who Was Cured with Long-term PMX-DHP Therapy**

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Abstract

It has been demonstrated that PMX-DHP is powerful therapy in patients with septic shock. In this time, we performed long-term treatment PMX-DHP in a patient with *Klebsiella pneumoniae* infection. Circulation was improved after 14 hours. The endotoxin level in peripheral blood decreased from 200 pg/mL before PMX-DHP to 3.1 pg/mL after the second PMX-DHP. These observations were indicated that long-term treatment with PMX-DHP may be useful therapy for patients with severe septic shock.

Key words : Long-term PMX-DHP, Septic shock, *Klebsiella pneumoniae* infection, Endotoxin

* * *

Evaluation of the correlation between the NOx and TGF- β_1 in cases of sepsis complicating diffuse peritonitis with the severity of the morbid condition

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Abstract : In patients of sepsis complicated by diffuse peritonitis, the serum transforming growth factor β_1 levels (TGF- β_1) and nitrite/nitrate balance (NOx) were determined, and the correlations between these values and the severity of the sepsis were investigated. Thirty-eight patients were enrolled in this study. While both the serum tumor necrosis factor- α levels (TNF- α) and NOx were significantly higher in the deceased group (patients who eventually died) than in the surviving group, the TGF- β_1 was significantly lower in the deceased group than in the surviving group. There was a significantly positive correlation between the TNF- α and NOx, whereas a significantly negative correlation was determined between the NOx and TGF- β_1 . The NOx/TGF- β_1 ratio was significantly higher in the deceased group than in the surviving group. The severity of the morbid condition in cases of sepsis complicating diffuse peritonitis was closely correlated with the NOx and TGF- β_1 .

Key words : diffuse peritonitis, sepsis, TGF- β_1 , NOx, MODS

Introduction

We have reported previously that several mediators are produced by cytokines and directly or indirectly involved in the development of the hemangioendothelial dysfunction underlying the occurrence of the multiple organ dysfunction syndrome (MODS)^{1~4)}.

It may safely be said that the development of complicating sepsis in cases of diffuse peritonitis significantly influences the severity of the morbid condition and the prognosis of the patients. A number of reports have been published on

the close correlations between the serum endotoxin and cytokine levels and the severity of the morbid condition in cases of sepsis, particularly cases of septic shock, in animals and human beings^{5~11)}.

Furchgott, Moncada et al. and *Ignarro* et al. have reported independently that the characteristics of the endothelium-dependent relaxing factor (EDRF), which has a vasodilatory action and hypotensive effects, closely resemble those of nitric oxide (NO)^{12~14)}.

On the other hand, some reports have shown

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