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ORIGINAL ARTICLE

Usefulness of presepsin (sCD14-ST) measurements as a marker for the diagnosis and severity of sepsis that satisfied diagnostic criteria of systemic inflammatory response syndrome

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Abstract CD14 is present in macrophage, monocyte, and granulocyte cells and their cell membranes, and it is said to be responsible for intracellular transduction of endotoxin signals. Its soluble fraction is present in blood and is thought to be produced in association with infections. It is called the soluble CD14-subtype (sCD14-ST), and in the following text it is referred to by its generic name, presepsin. We have previously reported that presepsin is produced in association with infection and that it is specifically expressed in sepsis. In the present study we developed a new rapid diagnostic method by using a chemiluminescent enzyme immunoassay that allowed making automated measurements in a shorter time. The results of using this method to measure presepsin values in different pathological conditions were normal, 294.2 ± 121.4 pg/ml; local infection, 721.0 ± 611.3 pg/ml; systemic inflammatory response syndrome, 333.5 ± 130.6 pg/ml; sepsis, 817.9 ± 572.7 pg/ml; and severe sepsis, 1,992.9 ± 1509.2 pg/ml; the presepsin values were significantly higher in patients with local infection, sepsis, and severe sepsis than in patients who did not have infection as a complication. In a comparative study with other diagnostic markers of sepsis based on ROC curves, the area under the curve (AUC) of presepsin was 0.845, and greater than the AUC of procalcitonin (PCT, 0.652), C-reactive protein (CRP, 0.815), or interleukin 6 (IL-6, 0.672). In addition, a significant correlation was found between the APACHE II scores, an index of disease severity, and the presepsin values, suggesting that presepsin values can serve as a parameter that closely reflects the pathology.

Keywords Sepsis · SIRS · Infection · Soluble CD14-subtype · Presepsin

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Introduction

Severe conditions, such as multiple trauma, pancreatitis, peritonitis, and extensive burns, are the most common conditions handled in the emergency and intensive care area, and early diagnosis of sepsis secondary to these conditions is one of the keys to improving the results of treatment. Sepsis is a systemic inflammatory response syndrome (SIRS) that is caused by an accompanying bacterial infection, and similar manifestations and test results are often observed in patients with noninfectious inflammation, such as the inflammation caused by multiple trauma or extensive burns, making it difficult to diagnose sepsis on the basis of the clinical findings alone. Identifying the causative organism by smears or culture tests is essential to the diagnosis of infection, but these methods are not always useful because of the time needed to obtain results, the low sensitivity of the tests, and the impossibility of excluding contamination. On the other hand, in recent years procalcitonin (PCT), interleukin 6 (IL-6), and tumor necrosis factor (TNF) have been used as biomarkers that increase in sepsis patients, and although these markers closely reflect the pathology of the sepsis, they cannot be described as having high specificity because they rise transiently in patients with highly invasive trauma [1].

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Presepsin is thought to be a fragment of CD14, the lipopolysaccharide-binding protein (LPS–LBP) complex receptor, and it is produced in response to bacterial infections. Although many aspects of its production in vivo are unknown, based on the results of animal experiments, etc., it is thought that phagocytosis in response to bacterial infection may play a major role, and the possibility that the lysosomal enzymes, e.g., aspartic proteases (cathepsin, etc.), are involved in the mechanism of production is suspected based on the results of enzyme inhibition experiments.

Thinking that there was a strong possibility that presepsin was specifically produced in sepsis, we previously conducted studies comparing it with endotoxin, IL-6, PCT, and other diagnostic markers of sepsis, and reported its usefulness [2–5]. The results of a previous study conducted using a conventional specific enzyme-linked immunosorbent assay (ELISA) kit showed that presepsin had high specificity for sepsis and began to be expressed in the early stage, that its level increased in proportion to the severity of the sepsis, and that it had clinical sensitivity and specificity which was superior to that of the other markers [5]. However, because the measurements by this method require pretreatment of the specimen and 5–6 h to obtain the results, speed was a problem in clinical practice, especially in emergency care settings, where early diagnosis is needed.

In the present study, we developed a new chemiluminescent enzyme immunoassay as a rapid diagnostic method for sepsis. We measured presepsin in various pathological conditions and compared its usefulness as a diagnostic marker for sepsis.

Patients and methods

Patients

We obtained informed consent to participation in this study from the patients or their families as well as the approval of the Ethics Committee of Iwate Medical University. The period of the study was the 10 months from August 2009 to June 2010, and it was a prospective cohort study. The subjects were 41 inpatients (25 men and 16 women), 62 ± 19 years old, who had been brought to the Critical Care and Emergency Center of Iwate Medical University and who fulfilled at least two of the diagnostic criteria for SIRS on arrival. Blood specimens were collected a total of six times, i.e., on admission, and 12 and 24 h and 3, 5, and 7 days later, and the presepsin values were measured. The sepsis markers PCT, IL-6, and CRP were also measured for comparison. Each time the blood specimens were collected, a definitive diagnosis was made based on the clinical data by two infection control doctors certified by the Japanese College of Infection Control Doctors.

In addition, 128 healthy subjects (100 men and 28 women) were assessed as controls.

The diagnoses of SIRS, sepsis, and severe sepsis were made according to the seven criteria set forth by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [6].

The Acute Physiology and Chronic Health Evaluation II score (APACHE II score) [7] was used as an index of severity.

Measurement methods

An endotoxin-free syringe containing ethylenediaminetetraacetate (EDTA) was used to collect the blood specimens for the presepsin measurements. After the specimen was collected, (1) a 100-µl volume of whole blood was dispensed into reagent specimen wells with a micropipette. (2) The reagent into which the specimen had been dispensed was set in a cartridge, and the cartridge was placed in the body of the analyzer with a special chip. (3) To 25 µl of specimen, 25 µl specimen dilution solution, 50 µl magnetic latex reagent, and 50 µl labeled antibody reagent were added, and allowed to react at 37°C for 5 min. Washing was then performed three times with 400 µl washing fluid each time. (4) A 100-µl volume of luminescent substrate was added, and luminescence was measured while allowing the enzyme reaction to proceed at 37°C for 1 min. (5) The presepsin concentration in the specimen was measured by comparison with the amount of luminescence of a calibration agent that had been subjected to the same procedure as the sample, and the steps from (3) to (5) were automatically processed with an immunoassay analyzer (PATHFAST; Mitsubishi Chemical Medience Corporation, Japan) [8]. When whole blood is used as the material for measurements with this analyzer, the presepsin value on the analyzer is automatically corrected by entering the hematocrit (Ht). The Ht value is entered individually when measuring the specimen, and the correction is made. Blood plasma can also be used as the material to make measurements with the immunoassay analyzer. Quantitative analysis of PCT was performed with an automated electrochemiluminescence immunoanalyzer (Elecsys reagent, BRAHMS PCT; Roche Diagnostics, Japan). Quantitative analysis of IL-6 was performed with a completely automated immunochemiluminescence system (Siemens Immulite IL-6; Siemens, Germany). Quantitative analysis of CRP was performed by a CRP-latex X2 "Seiken" (Denka Seiken, Japan).

Statistical analysis

The statistical analysis was performed by using Friedman's χ^2 r test and the Wilcoxon t test with Bonferroni correction,

and the data are expressed as mean ± standard deviation. *P* values ≤ 0.05 were considered evidence of a significant difference. In addition, a receiver operating characteristic (ROC) analysis was performed for each of the biomarkers, and their diagnostic performance for sepsis was compared.

Results

The mean presepsin level in the 128 healthy subjects in the control group was 190 pg/ml.

There were 41 patients (25 men and 16 women) who fulfilled at least two of the diagnostic criteria for SIRS on arrival at the hospital, and they were assessed at 192 blood specimen collection points. The background factors of the patients adopted as subjects are shown in Table 1. We made all 192 blood specimen collections at different points; the group of patients who did not fulfill the diagnostic criteria for SIRS and had no signs of infection were assigned to the normal group, whereas the group of patients who were found to have an infection but did not fulfill the diagnostic criteria for SIRS were assigned to the local infection group. In addition, the results of classification into SIRS, sepsis, and severe sepsis based on the definitions proposed by the ACCP/SCCM were normal, 22; local infection, 28; SIRS, 41; sepsis, 87; and severe sepsis, 14. The corresponding presepsin levels were normal, 294.2 ± 121.4 pg/ml; local infection, 721.0 ± 611.3 pg/ml; SIRS, 333.5 ± 130.6 pg/ml; sepsis, 817.9 ± 572.7 pg/ml; and severe sepsis, 1,992.9 ± 1509.2 pg/ml; the patients with local infection or sepsis had significantly higher presepsin levels than the patients who did not have infection as a complication (Fig. 1). In addition, the presepsin levels in SIRS that was not complicated by infection were significantly lower than in sepsis.

A significant correlation was found between the APACHE II scores, which are an index of severity, and the presepsin values (Fig. 2).

The ROC curves for the four sepsis diagnostic markers, including presepsin, in the infection group (local infection group + sepsis group + severe sepsis group) and the non-infection group (normal group + SIRS group) are shown in Fig. 3. The area under the curve (AUC) of presepsin calculated from the ROC curve was 0.845; it was higher than the AUC of PCT (0.652), CRP (0.815), or IL-6 (0.672). When the cutoff value of presepsin was set at 399 pg/ml, its clinical sensitivity was 80.3% and its clinical specificity was 78.5%. The AUCs obtained from the ROC curve of the SIRS patients without infection as a complication and the sepsis patients were presepsin, 0.845; PCT, 0.652; CRP, 0.815; and IL-6, 0.672; the best results were for presepsin (Fig. 4), and when the cutoff value was set at 415 pg/ml, clinical sensitivity was 80.1% and clinical specificity was 81.0%, suggesting usefulness as a diagnostic marker of sepsis.

Table 1 Background of patients and diagnosis of each blood drawing point

Underlying disease	*n*	Diagnosis of each blood drawing point
Appendicitis	4	
Cholecystitis	2	Non-infection
Gastroenteritis	1	$n = 22$
Phlegmonous inflammation	1	
Pancreatitis	2	Systemic inflammatory response syndrome (SIRS)
Urinary infection	4	$n = 41$
Peritonitis	3	
Ischemic enteritis	1	Local infection
Pneumonia	1	$n = 28$
Multiple trauma	8	
Superior mesenteric artery (SMA) thrombosis	1	Sepsis
Burns	4	$n = 87$
Ileus	1	
Perforation of duodenum	2	Severe sepsis
Multiorgan failure (MOF)	3	$n = 14$
Others	3	

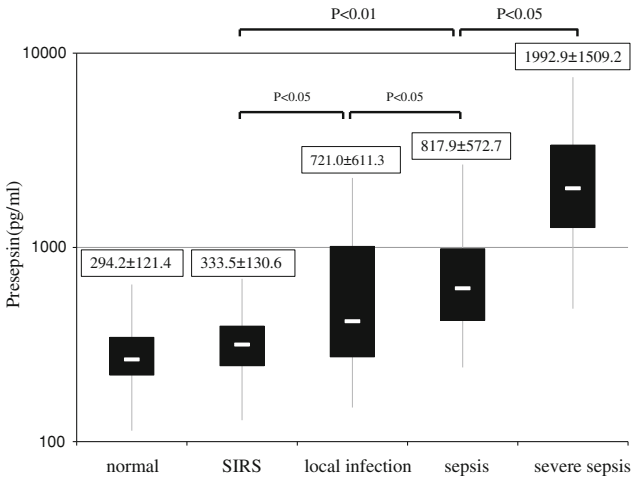

Fig. 1 Comparison of presepsin value in different pathological conditions

Case 1 is described as an example.

The patient was a 51-year-old man who was brought to our hospital with extensive burns that covered 76% of his body. The test data on arrival included an elevated white blood cell (WBC) count of 38,880/μl, and a diagnosis of SIRS was made. Because no elevation of the presepsin value (281 pg/ml) or PCT value (0.98 ng/ml) was observed on arrival, and the blood cultures were negative, no

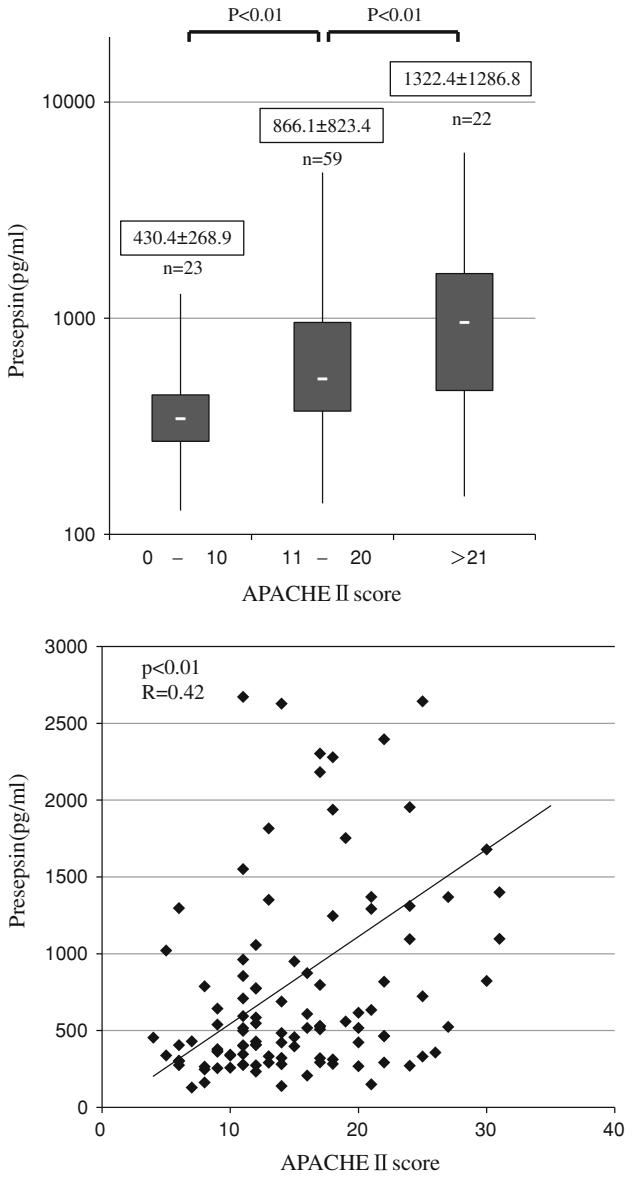

Fig. 2 Correlation of presepsin value and Acute Physiology and Chronic Health Evaluation II (APACHE II) score

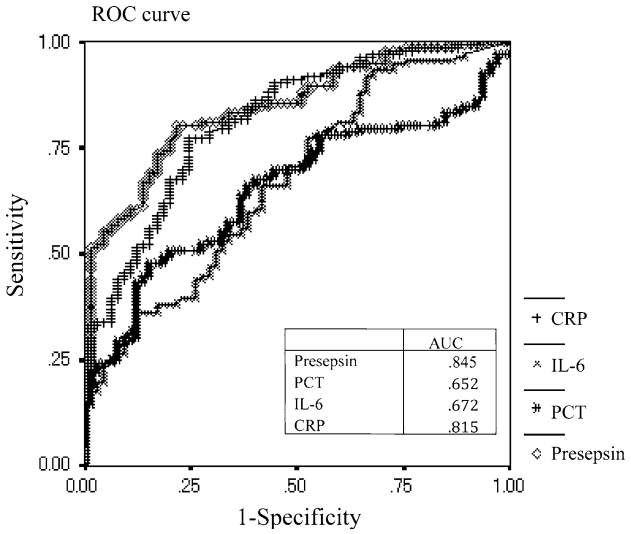

Fig. 3 Receiver operating characteristic (ROC) curves for the four sepsis diagnostic markers, including presepsin, in the infection group (local infection group + sepsis group + severe sepsis group) and the non-infection group [normal group + systemic inflammatory response syndrome (SIRS) group]. *CRP*, C-reactive protein; *IL-6*, interleukin 6; *PCT*, procalcitonin

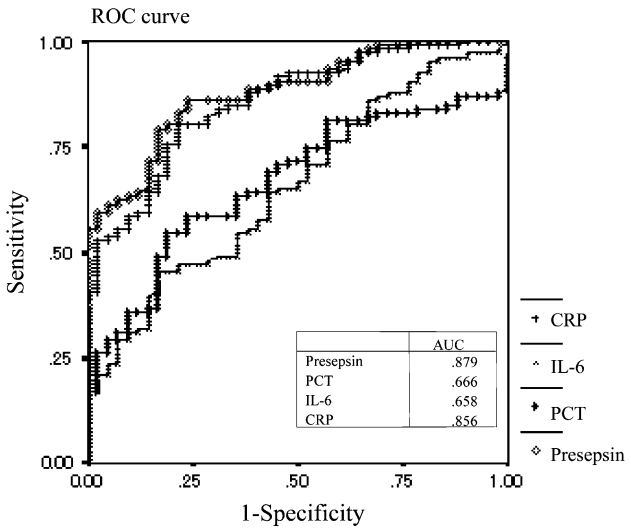

Fig. 4 ROC curves for the four sepsis diagnostic markers, including presepsin, in the SIRS patients without complication by infection and sepsis group (sepsis group + severe sepsis group)

findings suggesting infection were observed at the time. On hospital day 6, staphylococci were isolated by blood culture. A tendency for the presepsin and CRP values to increase was observed starting the same day, and a delayed increase in the PCT value was seen (Fig. 5).

Discussion

Early diagnosis and early treatment are essential to improving the results of treatment of infections, and, in particular, more accurate diagnosis has become important because treatment policy, e.g., drug selection or blood purification method, changes depending on the causative organism. Moreover, methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant bacteria, such as multidrug-resistant *Pseudomonas aeruginosa* and vancomycin-resistant enterococcus (VRE), have become a problem in recent years, and it is no exaggeration to say that patient vital prognosis depends on infection control. Detection of the microbe by microbiological tests, such as blood cultures, is essential to a definitive diagnosis of infection, but because of low detection sensitivity, the problem of contamination, and the fact that cultures

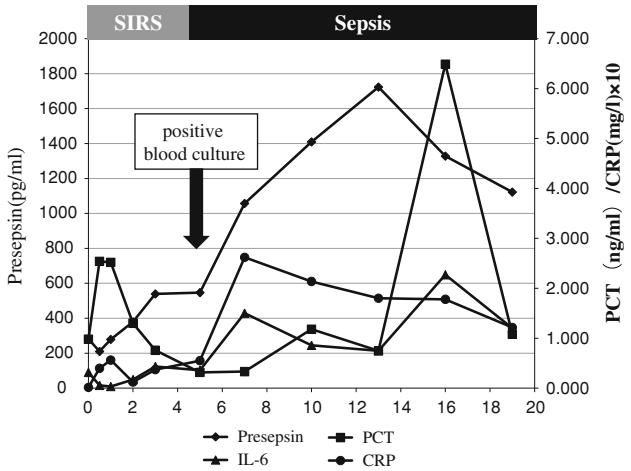

Fig. 5 Clinical course of each septic marker in burn patient

themselves are not performed as often as in Western countries, they cannot necessarily be said to be convenient and practical test methods. On the other hand, up until now the biological markers and immunological markers of sepsis have not been very effective, and in recent years novel markers have been developed and are being widely adopted in clinical settings.

At our institution we have been measuring and monitoring various immunological markers in infections, especially in the sepsis area. In a clinical study of PCT we obtained better results than with CRP, TNF-α, IL-6, etc., as a method of diagnosing bacterial infection or evaluating severity, and we established a new diagnostic method for sepsis in Japan. The physiological roles and sites of production of PCT have not been fully elucidated, but it has been demonstrated that it is usually produced by the C cells of the thyroid gland during sepsis. On the other hand, PCT also increases in highly invasive conditions in the absence of complication by infection, and a comprehensive evaluation that included the clinical manifestations is necessary in multiple trauma and burn patients. A similar tendency was observed in the present study.

Presepsin is a novel marker for the diagnosis of sepsis, and the results of a previous study in which an ELISA kit was used showed a specific increase in sepsis in the early stage that also correlated well with severity. However, because the assay method requires pretreatment of the specimen and the measurements take some time (about 5–6 h), as well as the fact that only retrospective tests were possible, it has not always been an effective assay kit in the emergency and intensive care area, where speed is required. Moreover, when we also developed a convenient diagnostic kit based on the immunochromatography principle with values of 2+ or more considered positive, clinical sensitivity was 73% and clinical specificity 84%, and its effectiveness was 80%. In the present study we developed a new rapid measurement method for whole blood that uses a chemiluminescence enzyme immunoassay. Because measurements in whole blood are possible with this method and no pretreatment of the specimen is necessary, measurements can be made in a very short time. We used the immunoassay analyzer to assess the usefulness of presepsin values in various pathological conditions.

We made all the blood collection points in this study at different points, and they were classified into normal, local infection, SIRS, sepsis, and severe sepsis. The results of a comparison of presepsin values according to the diagnosis at each of the times a blood specimen was collected showed that they were significantly lower in the normal group and in SIRS that was not complicated by infection than they were in local infection, sepsis, or severe sepsis, suggesting that presepsin increases specifically in infections. In previous studies there were very wide variations in the values of this marker in infections, and there were even cases in which the value exceeded 20,000 pg/ml, the limit of the measurements, which appears to be a characteristic of this marker. In addition, because the presepsin values were especially high in severe sepsis, and there was also a positive correlation between the APACHE II scores as a severity index and the presepsin values, the presepsin values were inferred to closely reflect the pathology in sepsis. The reference presepsin values according to this measurement method and the cutoff value for diagnosing sepsis have not yet been determined. When we set the presepsin cutoff value for sepsis at 415 pg/ml calculated from the ROC curve, clinical sensitivity was 80.1% and clinical specificity 81.0%, and thus about 400 pg/ml would appear to be valid. Further study is necessary in regard to the cutoff value.

When we divided the patients into an infection group and a non-infection group and plotted the ROC curves of each of the markers to compare presepsin with other markers, the results showed that presepsin was the best, followed by CRP, IL-6, and PCT. Moreover, when the patients were divided into an SIRS group without infection and a sepsis group and ROC curves were plotted in the same manner, the results again showed that presepsin was the best. In a previous study conducted at our institution we reported finding that PCT was a more effective parameter for diagnosing sepsis and monitoring treatment than was CRP or IL-6. The reason the results for PCT were not superior in the present study appears to be that there were 12 cases of severe burns and multiple trauma, etc., that is, of what is referred to as SIRS not complicated by infection, on arrival at the hospital, and they accounted for approximately 30% of the total number of patients. Because the PCT level increases as a reflection of the severity of the body's reaction to the traumatic stimuli in the early stage of trauma in the absence of signs of infection, it does not

necessarily rise in an infection-specific manner; this appears to be why the diagnostic performance of PCT for infection was low in the cases in the present study. On the other hand, the presepsin values of all the patients with SIRS that was not complicated by infection on arrival were below 500 pg/ml, and the values were not high even in the patients whose trauma was very invasive, unless there was complication by infection. In this respect, presepsin can be said to be highly disease specific in comparison with PCT and proinflammatory cytokines such as TNF, IL-6, or IL-8.

In the present study we were able to obtain results similar to those obtained with the conventional ELISA method, and it was possible to diagnose sepsis more rapidly and conveniently by using the immunoassay analyzer. We are currently using the analyzer in a multicenter clinical study and are in the process of conducting a further clinical dynamics analysis in various pathological conditions. Based on the results of the present study it appears that presepsin will soon be widely used as a diagnostic marker of sepsis in clinical settings.

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References

1. Endo S, Sato N, Kasai T, Suzuki Y, Kojika M, Takahashi G, et al. Assessment of serum procalcitonin levels by immunochromatography: its significance as a rapid diagnostic technique for sepsis. Jpn J Crit Care Endotoxemia 2007;11:186–195 (in Japanese).
2. Endo S, Yaegashi Y, Sato N, Suzuki Y, Ogawa M, Kojika M, et al. Comparative study of soluble CD14 and soluble CD14-subtype in sepsis. Med Postgrad. 2006;44:381–5.
3. Endo S, Yaegashi Y, Sato N, Kojika M, Suzuki Y, Shirakawa K, et al. Usefulness of soluble CD14 subtype which as is a new diagnostic marker for sepsis. Jpn J Crit Care Endotoxemia 2005;9:46–950 (in Japanese).
4. Miyata M, Sato N, Takahashi G, Takahashi M, Endo S, Shirakawa K, et al. The utility of the soluble CD-14-subtype for diagnosis of sepsis, the examination of the simple diagnostic kit. J Iwate Med Assoc. 2007;59:325–31.
5. Yaegashi Y, Shirakawa K, Sato N, Suzuki Y, Kojika M, Endo S, et al. Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. J Infect Chemother. 2005;11:234–8.
6. ACCP/SCCM Consensus Conference Committee. Definitions for sepsis and organ failure and guidelines for use of innovate therapies in sepsis. Crit Care Med 1992;20:867–874.
7. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13:818–29.
8. Kurihara T, Yanagida A, Yokoi H, Koyata A, Matsuya T, Ogawa J, et al. Evaluation of cardiac assays on a benchtop chemiluminescent enzyme immunoassay analyzer, PATHFAST. Anal Biochem 2008;375(1):144–146.