ART & TECHNOLOGY PUBLISHING PTE. LTD.

Biomed Communication

2022 Volume 2, Issue 1

ISSN: 2529-7635

The Sepsis Marker "Presepsin": Biochemistry and Clinical Diagnosis

Kamon Shirakawa*

LSI Medience Corporation, Itabashi, Tokyo 174-8555, Japan

***Corresponding author:** Kamon Shirakawa, shirakwa.kamon@ma.medience.co.jp

Abstract

Presepsin (P-SEP), an approximately 70-amino-acid fragment of CD14, a lipopolysaccharide (LPS) receptor that transmits signals through CD14-MD-2/TLR4 to cells, was first discovered as a sepsis marker in 2002 when high concentrations of a protein with soluble CD14-like immunoreactivity were detected in the blood of sepsis patients. We used an immunoassay to measure the serum concentrations of the new peptide, which was initially named soluble CD14-subtype (sCD14-ST) and later renamed presepsin. Rabbit sepsis models revealed that presepsin is induced by the cecal ligation and punctual (CLP) sepsis model but not by the LPS injection model. *In vitro* experiments using human monocytes and neutrophils suggested that presepsin is produced when bacteria are phagocytosed by immune cells. The first clinical study was initiated by Professor Shigeatsu Endo (Iwate Medical University, Japan). It demonstrated that sepsis patients have higher presepsin levels compared with Systemic Inflammatory Response Syndrome (SIRS) patients, indicating that presepsin could be useful for sepsis diagnosis. This interesting marker is not only helpful for the diagnosis of sepsis but is also indicative of disease severity, as measured by the Sequential Organ Failure Assessment (SOFA) score. More researches are necessary as the understanding of the fundamental aspects of presepsin is still limited. Presepsin is elevated in high-mortality elective cardiac surgery and severe COVID-19 patients, but the mechanism by which presepsin production is increased during severe COVID-19 disease is unknown. In this article, presepsin biochemistry is described, then the application of presepsin in clinical diagnosis is discussed.

Keywords

Presepsin
Sepsis
Severity
CD14
Diagnostic marker

1. Introduction

Sepsis is a global healthcare problem affecting approximately 27 million people annually. Sepsis is defined as life-threatening organ damage caused by an uncontrolled host response to infection and is a major complication in burns, immunocompromised

patients, trauma patients, and hospitalized patients. Early diagnosis and treatment are considered the most effective way to treat sepsis, but blood cultures to detect pathogens have a low positivity rate and take a long time to yield results, so new diagnostic methods are needed.

In recent years, improved blood culture methods and identification methods using mass spectrometry have been put to practical use, and the time required to obtain results has been shortened. However, information on the severity of sepsis and prognosis cannot be obtained. Conventional markers (e.g. C-reactive protein, white blood cell count, interleukins) are also elevated in sepsis but are not sepsis-specific. Procalcitonin (PCT), a precursor of calcitonin produced by thyroid C cells, is produced by cells throughout the body in bacterial infections and is used as a diagnostic marker for sepsis. However, PCT is not an ideal biomarker for sepsis, as it is also susceptible to inflammation and does not rise easily in the early stages of infection.

Presepsin has been identified as the N-terminal fragment of the CD14 molecule on the membrane of monocytes, neutrophils, and macrophages. Soluble CD14 (sCD14) is present in blood and elevated in sepsis, but it is not a diagnostic marker for sepsis as it is also elevated in various diseases. On the other hand, presepsin was found by Endo *et al.* to be specifically elevated in sepsis, leading to its clinical application as a diagnostic marker for sepsis. Subsequent clinical evaluation showed that it was elevated in the early stages of sepsis, less affected by inflammation, and more likely to reflect the patient's condition. Recently, it has also been reported to be elevated in high mortality groups of patients undergoing elective cardiac surgery and in severely ill COVID-19 patients, thus providing new values. However, it is not clear what mechanisms underlie the elevation of presepsin in these diseases.

In this article, the biochemistry of presepsin is first introduced, followed by a discussion of the clinical diagnostic applications of presepsin in light of recent data.

2. Discovery of presepsin

CD14 is a glycoprotein present on the plasma membrane of monocytes, neutrophils, and macrophages, which regulate innate immunity, and it is a receptor for lipopolysaccharides (LPS), which are represented by endotoxins on the membrane surface of bacteria. LPS binds to TLR4/MD-2 on the plasma membrane via CD14 and activates cells. In addition, sCD14 is present in blood at concentrations of several μg/mL and is involved in the activation of vascular endothelial cells, which lack CD14 on the plasma membrane. Thus, CD14 is part of the mechanism to recognize bacterial infection and is an important molecule in biological defense. In a search for markers associated with CD14 that are specifically elevated in sepsis, soluble CD14 with a small molecular weight (sCD14-ST) was found in the blood of septic patients. sCD14-ST was subsequently identified as a pre-sepsis-protein, meaning "protein that increases before sepsis", that is presepsin.

3. Structure of presepsin

Presepsin is a glycoprotein with a molecular weight of approximately 13 kDa, consisting of approximately 70 amino acids in the N-terminal peptide of CD14. Presepsin is a novel protein that is distinct from CD14 because it does not bind to LPS and is not recognized by the anti-CD14 antibodies, 3C10 and MEM18. The currently used standard is a polypeptide with a sequence of 1–64 amino acids produced in COS-1 cells as a recombinant (**Figures 1** and **2**).

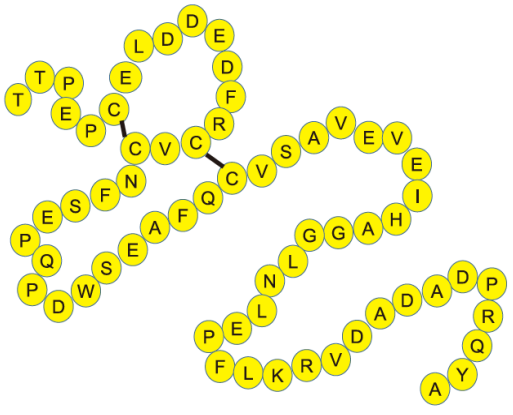


Figure 1. Schematic diagram of the amino acid sequence of presepsin. As the C-terminus has not been determined, the 1–64 amino acid sequence used as a standard is shown.

項目	プレセプシン	sCD14
アミノ酸数	約70アミノ酸	356アミノ酸
分子量	13 kDa	56 kDa
産生臓器	単球、顆粒球	単球、好中球、肝臓
産生刺激	細菌感染、真菌感染	細菌感染, サイトカイン, LPSなど
LPS結合能	なし	あり
抗CD14抗体	結合せず	結合
抗プレセプシン抗体	結合	結合せず
健常人血中濃度	50〜150 pg/mL	2〜5 μg/mL

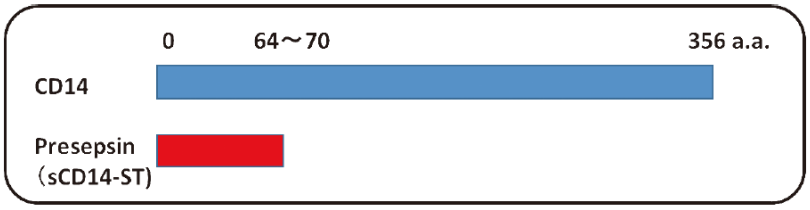
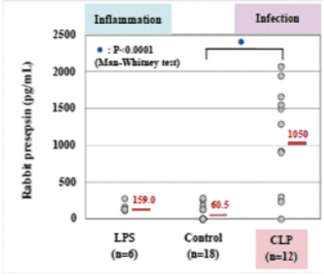


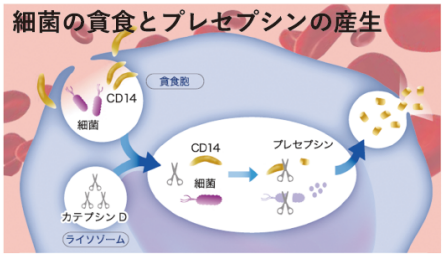
Figure 2. Comparison of presepsin and sCD14

LPSモデルとCLPモデルによるプレセプシンの産生

	LPSモデル	CLPモデル
刺激剤	LPS(エンドトキシン)	腸内細菌
モデル	炎症	感染
菌血症	No	Yes
体温	上昇	上昇
白血球数	上昇	上昇
サイトカイン	上昇	上昇

CLP(cecal ligation and puncture：盲腸結紮穿刺)





細菌が血中の顆粒球に貪食された時にCD14も取り込まれ、顆粒球内で酵素消化される際にプレセプシンがCD14より産生され、血中に放出される

Figure 3. Rabbit sepsis model and production of presepsin. Source: Naitoh K, Shirakawa K, Hirose J, et al., 2010, SEPSIS; adapted from Poster: P-19.

4. Production of presepsin

A rabbit sepsis model was used to clarify the elevation of presepsin in a sepsis-specific manner. First, two sepsis models were created, which are a rabbit sepsis model in which LPS was administered (LPS model) and a cecal ligation and puncture (CLP) model in which peritonitis occurs. Interestingly, presepsin was not elevated in the LPS model, and presepsin production was only observed in the CLP model. In addition, presepsin was elevated earlier than interleukin (IL)-6. Furthermore, the production of presepsin was confirmed when *E. coli* was added to the *in vitro* system prepared using granulocytes, but not when LPS was added. When the phagocytosis inhibitors wortmannin and cytochalasin D were added separately to this system, the production of presepsin was inhibited and it was presumed that the production of presepsin was caused by "cleavage of CD14 by CD14-bearing cells during phagocytosis of the bacteria," as shown in **Figure 3**. Following the rabbit model system, Arai *et al.* investigated the mechanism of presepsin production in an *in vitro* system using monocytes,

neutrophils, and lymphocytes isolated from human blood, and found that presepsin was not produced by cell-activating agents such as LPS, but was produced by the addition of *E. coli*, particularly in monocytes [1].

5. Methods for measuring presepsin

Immunological assays are used to measure presepsin. Kim *et al.* reported that the C-terminal region of CD14 is important for maintaining the N-terminal structure of the LPS-binding region, and Juan *et al.* reported that CD14 shorter than 152 amino acids has no LPS-binding capacity [2,3]. This suggested that short CD14 fragments such as presepsin do not maintain their native conformation and that antibodies that do not recognize the conformation would specifically recognize presepsin. Based on this hypothesis, we attempted to produce antibodies specific to presepsin and completed a presepsin-specific assay using two types of antibodies. Using this assay, it was possible to specifically measure only presepsin without the influence of sCD14 present in the blood. In 2011, a highly sensitive measuring reagent (PATHFAST-Presepsin) using chemiluminescent enzyme immunoassay was developed, enabling the measurement of presepsin in blood in a short time of 17 minutes.

6. Normal values and reference ranges for presepsin

Giavarina *et al.* reported that presepsin levels in 200 healthy subjects aged 18 to 75 years ranged from 55 to 184 pg/mL (90% CI), and there were no differences between men and women [4]. On the other hand, the diagnostic efficiency of sepsis (86.0%) was calculated from the prevalence of positive diagnosis (94.2%) and the disease-free positive diagnosis (68.1%), which was calculated from measurements in 103 patients with sepsis and 47 patients with Systemic Inflammatory Response Syndrome (SIRS) in a Japanese clinical trial. A sepsis cut-off value of 500 pg/mL was adopted.

7. Presepsin stability and hemodynamics

Clinical samples are stable within 4 hours after collection at room temperature for whole blood, 8 hours at 2–8°C for serum, 3 days at 2–8°C for plasma, and 1 year below -20°C. Ham *et al.* reported an increase in presepsin levels when samples were shaken in an automated hemocytometer [5]. In addition, physical stimulation increases the presepsin level, as plasma is vigorously shaken in a vortex. Sakamoto *et al.* reported that presepsin levels did not increase when blood was transported by air shooter in a coagulated state [6]. These results indicate that presepsin is a relatively stable protein but may be affected by sCD14 present in the blood.

Regarding the metabolism of blood presepsin, in a study of recombinant presepsin in dogs, it was estimated that presepsin is rapidly metabolized into urine, with a half-life in blood of approximately 0.5–1 hour. The clinical half-life of presepsin has also been reported to be approximately 4–8 hours. A rabbit sepsis model suggested that presepsin was elevated approximately 2 hours after CLP surgery, suggesting that it is elevated early in the course of infection. Ebisawa *et al.* also reported from an analysis of hematological tumor patients that presepsin rises within 1–18 hours after fever and can diagnose infection earlier than procalcitonin [7].

8. Clinical applications of presepsin

When using presepsin in clinical practice, the following characteristics of presepsin should be utilized.

8.1. Earlier rise in the diagnosis of sepsis

Endo *et al.* reported that presepsin rises before IL-6, procalcitonin, CRP, and white blood cells [8] in cases of sepsis caused by extensive burns, urinary tract infection, and colon perforation.

8.2. Less susceptible to highly invasive trauma, burns, surgery, etc.

Since IL-6, C-reactive protein (CRP), and PCT are

elevated in the early stages of admission to the hospital for severe burns, it is difficult to distinguish them from infection. Takahashi *et al.* reported that presepsin was elevated before the septic shock, unaffected by inflammation, by observing patients with severe burns over time [9]. Takeuchi *et al.* also showed that presepsin could detect infection on day 5 after surgery in esophagectomy in esophageal cancer, ahead of other markers [10].

8.3. Better reflects the clinical course (severity)

Yu *et al.* compared presepsin and PCT concentrations in survivors and deceased patients with sepsis in 90-day mortality at admission day, 3, 5, 7, and 12 days, and found that presepsin decreased daily in survivors and was maintained or increased in deceased patients, whereas PCT decreased transiently in both survivors and deceased patients, indicating that presepsin reflected the patient's progress well [11]. Fujii *et al.* also reported that mortality was 56% (10/18) in patients whose presepsin levels on the day of ICU admission did not fall by 50% on day 6, whereas mortality was 0% in patients whose levels fell by more than 50% [12].

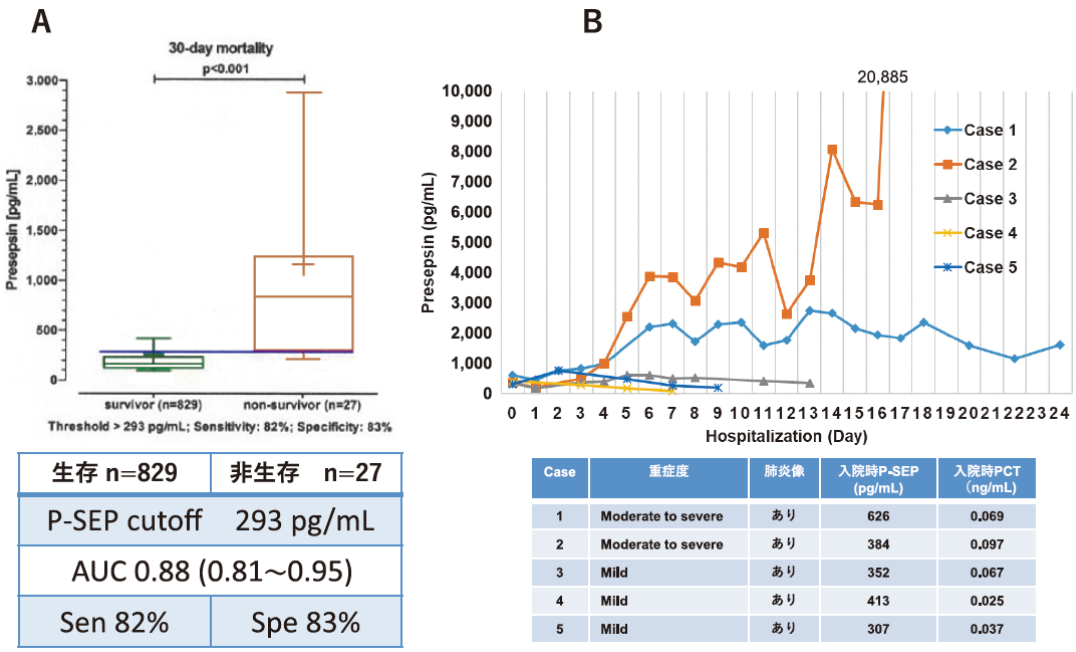
9. Presepsin as a diagnostic marker for sepsis (bacterial)

An ideal biomarker should have high diagnostic accuracy for early and rapid diagnosis. Presepsin has excellent diagnostic accuracy for various infectious diseases, including sepsis. In sepsis, presepsin rises early due to phagocytosis of pathogens and it has been shown to correlate the degree of microbial invasion with severity. Presepsin can also be used to predict the outcome of sepsis. Klouche *et al.* conducted a study on patients with community-acquired pneumonia and reported that serum presepsin levels distinguish sepsis from non-infectious respiratory failure [13]. Bamba *et al.* found that presepsin was also elevated in deep-seated mycosis and that it correlated well with the Sequential Organ Failure Assessment (SOFA) score [14]. Apiratwarakul *et al.* reported that presepsin was elevated in deep fungal infections and viral infections (influenza and dengue), with a median of 2,904 (1,334–4,474) pg/mL for bacterial infection (n = 22) and 204 (164–245) pg/mL for viral infection (n = 66) [15]. Thus, presepsin is elevated in bacterial and fungal infections, but not in common viral infections.

Tambo *et al.* evaluated the ability of presepsin and PCT as an early diagnosis of sepsis (as defined by Sepsis-3) in patients with obstructive acute pyelonephritis. They analyzed 61 patients, which were divided into two groups: those with sepsis (11 patients, 18%) and those without. Presepsin and PCT levels in septic patients were 1,080 (696–1,550) pg/mL (median, IQR) and 31.57 (1.83–134.40) ng/mL, respectively, while in non-septic patients were 387 (313–558) pg/mL and 0.54 (0.14–4.86) ng/mL. The values for septic patients were significantly higher (P < 0.001) than those for non-septic patients. In a multivariate analysis, we also reported that a cut-off value of 515 pg/mL of presepsin (odds ratio = 13.13, P = 0.044) was an independent predictor of sepsis [16].

10. Preceptin as a severity factor and predictor of prognosis

Bomberg *et al.* measured preoperative presepsin, PCT, NT-proBNP, and cystatin C levels in 856 patients undergoing elective cardiac surgery and investigated mortality at 30 days, 6 months, and 2 years after surgery. The results showed that mortality due to complications at 30 days (27 patients, 3.15%) was higher in patients with higher preoperative presepsin levels (cut-off value 293 pg/mL, sensitivity 82%, specificity 83%, AUC 0.88). Based on these results, they reported that elevated preoperative presepsin levels were an independent prognostic factor for risk stratification analysis in cardiac surgery patients, as shown in **Figure 4A** [17]. Fukada *et al.* measured



Case	重症度	肺炎像	入院時P-SEP (pg/mL)	入院時PCT (ng/mL)
1	Moderate to severe	あり	626	0.069
2	Moderate to severe	あり	384	0.097
3	Mild	あり	352	0.067
4	Mild	あり	413	0.025
5	Mild	あり	307	0.037

Figure 4. The severity of illness, prognosis prediction, and presepsin concentration. (A) Risk assessment by presepsin level before cardiac surgery (30-day mortality comparison) [17]; (B) Severity of COVID-19 and presepsin concentration [18].

presepsin concentrations over time from the onset in COVID-19 patients (5 patients). The results showed that the presepsin concentration increased in the two patients with severe disease and rose to 20,885 pg/mL in the patient who died. In contrast, in the three mild cases, the presepsin concentration temporarily exceeded 500 pg/mL, but subsequently decreased, as presented in **Figure 4B** [18].

These results indicate that presepsin may be useful not only as a predictor of severity in patients with sepsis but also as a predictor of severity in patients undergoing standby cardiac surgery and COVID-19.

11. Conclusion

Presepsin is a unique biomarker with a wide range of applications in healthcare compared to conventional sepsis markers. It may be useful in diagnosing infections, diagnosing patient severity, and predicting prognosis. However, assessing presepsin alone may not be sufficient. Therefore, in actual clinical practice, it is important to combine various biomarkers for a comprehensive diagnosis. Presepsin is a diagnostic marker for sepsis and a potential risk factor for predicting severity and prognosis. In the future, it is hoped that the mechanism of its production will be clarified and the significance of its measurement established.

Disclosure statement

The author declares no conflict of interest.

References

[1] Arai Y, Mizugishi K, Nonomura K, et al., 2015, Phagocytosis by Human Monocytes is Required for the Secretion of Presepsin. J Infect Chemother, 2015(21): 564–569.

[2] Kim JI, Lee CJ, Jin MS, et al., 2005, Crystal Structure of CD14 and Its Implications for Lipopolysaccharide Signaling. J Biol Chem, 2005(280): 11347–11351.

[3] Juan TS, Kelley MJ, Johnson DA, et al., 1995, Soluble CD14 Truncated at Amino Acid 152 Binds Lipopolysaccharide (LPS) and Enables Cellular Response to LPS. Biol Chem, 1995(270): 1382–1387.

[4] Giavarina D, Carta M, 2015, Determination of the Reference Interval for Presepsin, an Early Marker for Sepsis. Biochem Med (Zagreb), 2015(25): 64–68.

[5] Ham JY, Song KE, 2016, Impact of Specimen Mixing Methods on Presepsin Point-Of-Care Test Results using Whole Blood. Clin Chem Lab Med, 2106(54): e151–154.

[6] Sakamoto D, Oyamada T, Miyagi H, et al., Effect of Physical Shock from Air Shooter® Transport on Presepsin Values. Medical Testing and Automation (in submission).

[7] Ebisawa K, Koya J, Nakazaki K, et al., 2018, Usefulness of Presepsin for Early Detection of Infections in Patients with Haematologic Disorders. Clin Chim Acta, 2018(486): 374–380.

[8] Endo S, Azushima T, Takahashi M, et al., 2016, Characteristics and Usefulness of the Sepsis Biomarker "Presepsin." Infectious Diseases, 2016(46): 10–15.

[9] Takahashi M, Endo S, Shigekazu Endo, Tatsuyori Azushima, et al., 2016, "Preceptin": What We Have Learned Through Cases. Department of Emergency and Disaster Medicine, Iwate Medical University School of Medicine, 10.

[10] Takeuchi M, Yokose T, Kawakubo H, et al., 2020, The Perioperative Presepsin as an Accurate Diagnostic Marker of Postoperative Infectious Complications. After Esophagectomy: A Prospective Cohort Study. Esophagus, 2020(17): 399–407. http://doi.org/10.1007/s10388-020-00736-7

[11] Yu H, Qi Z, Hang C, et al., 2017, Evaluating the Value of Dynamic Procalcitonin and Presepsin Measurements for Patients with Severe Sepsis. Am J Emerg Med, 2017(35): 835–841.

[12] Fujii E, Fujino K, Eguchi Y, 2019, An Evaluation of Clinical Inflammatory and Coagulation Markers in Patients with Sepsis: A Pilot Study. Acute Med Surg, 2019(6): 158–164.

[13] Klouche K, Cristol JP, Devin J, et al., 2016, Diagnostic and Prognostic Value of Soluble CD14 Subtype (Presepsin) for Sepsis and Community-Acquired Pneumonia in ICU Patients. Ann Intensive Care, 2016(6): 59.

[14] Bamba Y, Moro H, Aoki N, et al., 2018, Increased Presepsin Levels are Associated with the Severity of Fungal Bloodstream Infections. PLoS One, 2018(13): e0206089.

[15] Apiratwarakul K, Srimookda N, Phungoen P, et al., 2020, Presepsin Levels in Emergency Patients with Bacterial and Viral Infection. Open Access Maced J Med Sci, 2020(8): 20–23.

[16] Tambo M, Taguchi S, Nakamura Y, et al., 2020, Presepsin and Procalcitonin as Predictors of Sepsis Based on the New Sepsis-3 Definitions. BMC Urol, 2020(20): 23.

[17] Bomberg H, Klingele M, Wagenpfeil S, et al., 2017, Presepsin (sCD14-ST) is a Novel Marker for Risk Stratification in Cardiac Surgery Patients. Anesthesiology, 2017(126): 631–642.

[18] Fukada A, Nakabefu N, Matsuoka M, et al., 2020, Usefulness of Presepsin in COVID-19. Infectious Diseases Society of Japan COVID-19 Case Report Collection.