Reports

BioTechniques

Antibody-mediated soluble CD14 stabilization prevents agitation-induced increases in presepsin levels in blood component specimens

Gota Morino[1], Gaku Takahashi*,[1], Shigenori Kan[1], Yoshihiro Inoue[1], Kotaro Sato[2] & Kamon Shirakawa[3]

*[1]Department of Critical Care, Disaster & General Medicine, School of Medicine, Iwate Medical University, Morioka, Japan; [2]Department of Orthopedic Surgery, School of Medicine, Iwate Medical University, Morioka, Japan; [3]Clinical Development Group of LSI Medience Corporation, Tokyo, Japan; *Author for correspondence: gakut@iwate-med.ac.jp*

BioTechniques 70: 00–00 (March 2021) 10.2144/btn-2020-0136

First draft submitted: 13 September 2020; Accepted for publication: 3 December 2020; Published online: 29 January 2021

ABSTRACT

Presepsin is a 13-kDa N-terminal glycoprotein of CD14. Previously, agitation-induced increases in presepsin levels have been reported; however, the mechanism remains poorly understood. In this study, we aimed to reveal the mechanism of presepsin increase. The agitated plasma or serum was separated using gel exclusion chromatography and analyzed by ELISA. The effect of an anti-CD14 antibody (F1024-1-3) was examined. We observed elevated presepsin levels in the agitated plasma and aggregated soluble CD14 (sCD14). However, treatment with F1024-1-3 before agitation prevented the aggregation and the increase in presepsin levels. Depletion of aggregated sCD14 decreased the presepsin levels. Our findings indicate that agitation induces the aggregation of sCD14 and triggers an increase in presepsin. Anti-CD14 antibody prevents an increases in presepsin.

KEYWORDS:

agitation ● anti-CD14 antibody ● CD14 ● plasma ● presepsin ● sCD14-ST ● sepsis

Presepsin, also known as the soluble CD14 subtype (sCD14-ST), was first discovered in human blood in 2002 [1]. Presepsin is a 13-kDa N-terminal glycoprotein fragment (amino acid sequence: 1 to 64–70) of the lipopolysaccharide (LPS) receptor CD14. In healthy individuals, the serum concentration of soluble CD14 (sCD14) ranges from 2 to 5 μg/ml [2]. Unlike its parent molecule, presepsin does not bind to LPS and is not recognized by anti-CD14 antibodies [3]. Using these distinctions, we developed an ELISA using two anti-presepsin antibodies that recognize the specific epitopes of presepsin in the blood but do not target sCD14 [4].

Sepsis is a clinical condition associated with high mortality, particularly among patients with septic shock who face mortality rates near 30%. Therefore, it is important to diagnose sepsis correctly at an early stage. Yaegashi *et al.* revealed that in a clinical setting, sepsis can be distinguished from systemic inflammatory response syndrome by measuring the presepsin level in the blood [5]. Subsequently, a chemiluminescent enzyme immunoassay (CLEIA) kit was developed for this new and highly promising diagnostic biomarker of sepsis [6], and the diagnostic usefulness of this glycoprotein has been widely studied. Some previous research has also led to the identification of a presepsin cutoff value of 600 pg/ml for sepsis [7].

Numerous biomarkers are used to diagnose diseases, and scientists have aimed to determine the sources (e.g., production mechanisms) of these biomarkers. For example, cytokines are produced by protein synthesis in cells [8], whereas soluble TNF-α is initially synthesized as a membrane-bound, cell-associated 26-kDa protein that is further cleaved to yield the soluble 17-kDa form [9]. Regarding the production of presepsin, Arai *et al.* reported that this component is produced via the cleavage of CD14 by enzymes associated with phagocytosis in monocytes and neutrophils and demonstrated the cleavage of sCD14 by elastase to yield presepsin *in vitro* [10]. In our previous studies of rabbit models of sepsis, we observed that presepsin production required phagocytosis and that the lysosomal enzyme cathepsin D cleaved sCD14 [11]. In contrast, however, some reports have demonstrated the phagocytosis-independent elevation of presepsin. For example, Chenevier-Gobeaux *et al.* reported that presepsin was produced by the THP-1 monocytic cell line in response to LPS stimulation [12]. Furthermore, elevated presepsin levels have been detected in patients with systemic lupus erythematosus, a noninfectious autoimmune disease, suggesting that the underlying mechanism may be related to neutrophil activation [13].

In clinical practice, samples of critically ill patients are often sent from the emergency department or medical ward to the central clinical laboratory via pneumatic tubes to save time. In 2015, Ham *et al.* reported an increased presepsin level in a plasma samples subjected to vigorous mixing by an automated hemocytometer [14]. Potentially, therefore, this transport via pneumatic tube may also agitate the samples and thus lead to falsely elevated presepsin levels and incorrect diagnoses of sepsis.

Because serum does not contain cells or active proteinases (due to proteinase inhibitors), the mechanisms underlying the observed increases in presepsin remained unclear, and the role of agitation remains unknown. In this study, we aimed to identify the mechanism by which agitation increases the level of presepsin and to propose a method for preventing this elevation.

Materials & methods

Materials

The protocol for this study was approved by the ethics committee of School of Medicine, Iwate Medical University, Morioka, Japan. All participants provided informed consent before sample collection. The samples were collected from healthy donors and patients undergoing dialysis due to renal failure. All samples were treated gently and stored below −30°C before further analysis.

The anti-CD14 antibody (clone F1024-1-3), anti-presepsin antibodies (clone: F1106-13-3 and polyclonal S68) and recombinant presepsin were a kind gift from Mochida Pharmaceutical Co., Ltd. (Tokyo, Japan). A biotinylated anti-CD14 antibody (clone: 61D3) was purchased from eBioScience (CA, USA). Recombinant His-tagged CD14 (CD14-His) was purchased from Sino Biological (PA, USA). For ELISA assays, TMB was purchased from Nacalai Tesque (Kyoto, Japan), streptavidin-horseradish peroxidase (HRP; RPN-4401) was purchased from Merck (NJ, USA) and 1 M H_2SO_4 was purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan). All other reagents used in our experiments were analytical grade.

Agitation of the specimens

Plasma or serum specimens were agitated for 5 min using a vortex mixer (Genie 2T, Scientific Industries, Inc., NY, USA). The samples were then stored at room temperature before further use.

Size-exclusion chromatography

The plasma or serum samples were filtered through a 0.45-μm pore membrane and subjected to size-exclusion chromatography (SEC). Briefly, approximately 0.2–0.5 ml of each sample was applied to a Sephadex G75 10/300 column (GE Healthcare Japan, Tokyo, Japan) and eluted with 0.05% Tween-20/phosphate-buffered saline (PBS) (pH 7.4) using an AKTA-purifier (GE Healthcare Japan). The eluent was collected in 0.5-ml fractions, and the presepsin concentrations were determined using a specific ELISA.

Determination of presepsin concentrations in the plasma

The concentrations of presepsin in the plasma were determined using PATHFAST-Presepsin (LSI Medience Corporation, Tokyo, Japan), which is a commercially available system used in clinical settings.

Preparation of biotinylated F1106-13-3 antibody

A biotinylated form of the F1106-13-3 antibody was prepared using a biotin-labeling kit-NH_2 (DOJINDO, Kumamoto, Japan) according to the manufacturer's protocol.

Presepsin ELISA

For the ELISA, 96-well microtiter plates (Maxisorp, Thermo Fisher Scientific, Waltham, MA, USA) were coated with a polyclonal antibody specific for presepsin (S68; 2 μg/ml in 0.1 M carbonate buffer [pH 9.6], 50 μl/well) and incubated at 4–8°C for 16 h. After washing the plates three times with 0.05% Tween-20/PBS (pH 7.4), 200 μl of blocking buffer (0.1% bovine plasma albumin [BSA], 0.1% Tween-20 in PBS [pH 7.4]) were added to each well. Subsequently, the wells were treated with 50 μl of samples that had been previously subjected to SEC were added directly to the wells. A presepsin standard curve was also prepared by adding 50-μl aliquots of various dilutions of recombinant presepsin (0.078–5 ng/ml) in 0.1% BSA and 0.05% Tween-20/PBS (pH 7.4) to the wells to prepare the standard curve. After incubating the plate with the samples for 1 h at room temperature, the wells were washed four times with 0.05% Tween-20/PBS (pH 7.4; 300 μl/well) using a plate washer (Auto-Miniwasher AMW-8R; BioTek, Tokyo, Japan). Subsequently, 50 μl of a biotinylated antibody specific for presepsin (F1106-13-3; diluted to 0.2 μg/ml in 0.1% BSA and 0.05% Tween-20/PBS [pH 7.4]) were added to each well, and the plate was incubated for 1 h. After four washing steps, 50 μl of streptavidin-conjugated HRP (1/8000 dilution in with 0.1% Tween-20/PBS [pH 7.4]) were added to each well, followed by a 30-min incubation. The plates were washed four times. Finally, 50 μl of a TMB substrate solution were added to each well, followed by a 20-min incubation at room temperature. The reaction was stopped using a solution of 1 M H_2SO_4 (50 μl/well), and the absorbance in each well at 450/650 nm was measured using an ELISA plate reader (Sunrise; Tecan Japan, Kawasaki, Japan).

Inhibition of presepsin increase via anti-CD14 antibody (F1024-1-3) treatment

We examined the influence of the anti-CD14 antibody (F1024-1-3), which binds to the C-terminus of human CD14 (i.e., does not bind to presepsin), on the presepsin levels in plasma [15]. F1024-1-3 was added to plasma or serum samples to final concentrations of 1.28, 12.8 and 128 μg/ml, after which the samples were agitated for 5 min. Subsequently, the presepsin concentration in each sample was measured using the PATHFAST-Presepsin assay and compared with the concentrations in nonagitated samples to assess the effects of agitation. After that, the remaining samples were analyzed by SEC.

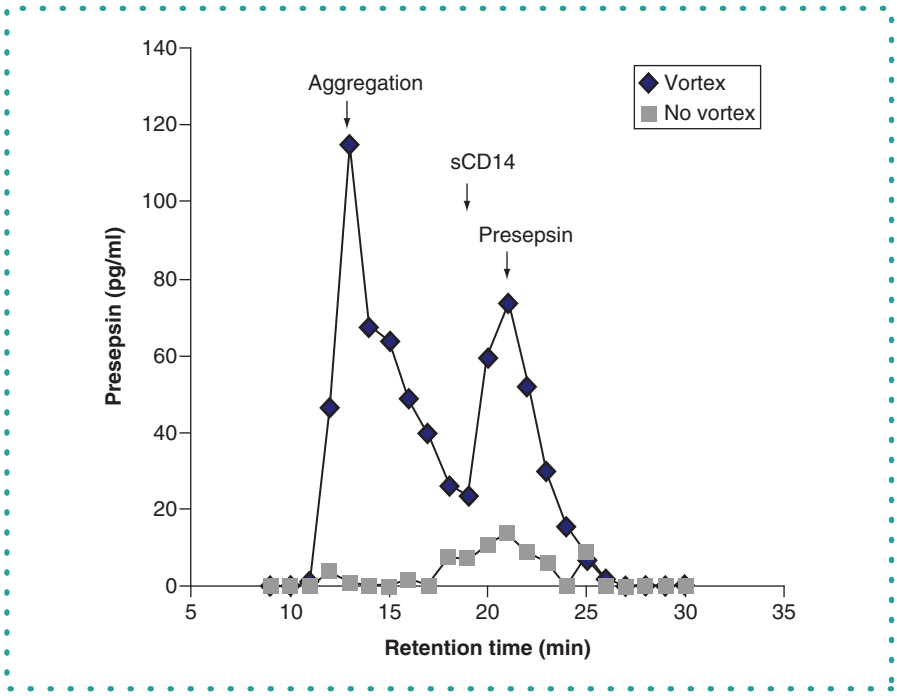


Figure 1. Size exclusion chromatogram of plasma before and after agitation. The presepsin concentration of plasma was determined by PATHFAST-Presepsin. Plasma (before and after vortex) were applied and eluted with PBS containing 0.05% Tween-20 on Sephadex G75 10/300 column and the presepsin concentrations of each fraction were determined. The peak of sCD14 was founded by CD14-ELISA (R&D systems, MN, USA). sCD14: Soluble CD14.

Depletion of aggregated sCD14 by anti-CD14 antibody-coated particles

We also examined the effect of sCD14 depletion by F1024-1-3-coated particles on the concentrations of presepsin in plasma. To prepare the particles, the F1024-1-3 antibody was mixed with protein-A binding particles (MabSpeed RP, Mitsubishi Chemical Corporation, Tokyo Japan) in the presence of 0.2% (w/v) resin in Tris-buffered saline, followed by a 30-min incubation with shaking. After five washes to remove resin, the beads were then suspended in 1 ml of Tris-buffered saline.

Three normal plasma samples were agitated for 5 min. Subsequently, 250 μl of each sample or nonagitated control were mixed with 50 μl of the F1024-1-3-coated particle suspension, and the mixtures were incubated for 30 min. The presepsin concentration in each supernatant was then determined using the PATHFAST Presepsin assay.

Western blotting analysis of the aggregates

We further performed a Western blotting analysis of the aggregates. Plasma samples were divided into three tubes: tube 1 was not vortexed, tube 2 was vortexed for 5 min and tube 3 was similarly vortexed and subsequently treated with anti-CD14 antibody-coated particles to remove the aggregated sCD14. All samples were then purified using anti-presepsin antibody-bound resin and eluted with 0.1 M glycine-HCl (pH 2.5). The eluates were concentrated by 10-kDa Amicon Ultra filters (Merck) and subjected to SDS-PAGE (4–12% gel, Thermo Fisher Scientific) and transferred to polyvinylidene difluoride membranes. For Western blotting, the membranes were incubated with a biotinylated anti-CD14 antibody (clone 61D3). After washing, the membranes were incubated with streptavidin-conjugated alkaline phosphatase (Promega Corporation, WI, USA). A BCIP-NBT solution (Nacalai Tesque, Kyoto, Japan) was used to visualize the antibody-labeled protein bands.

Results & discussion

Evaluation of presepsin values after vortex mixing and analysis of presepsin by SEC

To evaluate the effects of agitation, normal plasma sample was vortexed for 5 min, and the presepsin concentration was determined before and after vortexing using the PATHFAST Presepsin assay. The presepsin concentration after vortexing increased to 2657 pg/ml, a 15-fold increase over the original concentration of 177 pg/ml. These samples were also subjected to SEC, after which the presepsin concentrations in various fractions were measured by PATHFAST Presepsin. A small presepsin peak was detected in the low-molecular-weight fractions from unmixed plasma samples. In contrast, the presepsin peak was detected in the high-molecular-weight fractions from vortexed samples, suggesting that agitation promoted the formation of aggregates detecting by presepsin assay. In addition, a small peak in small molecular weight which similar retention time of presepsin was increased (Figure 1).

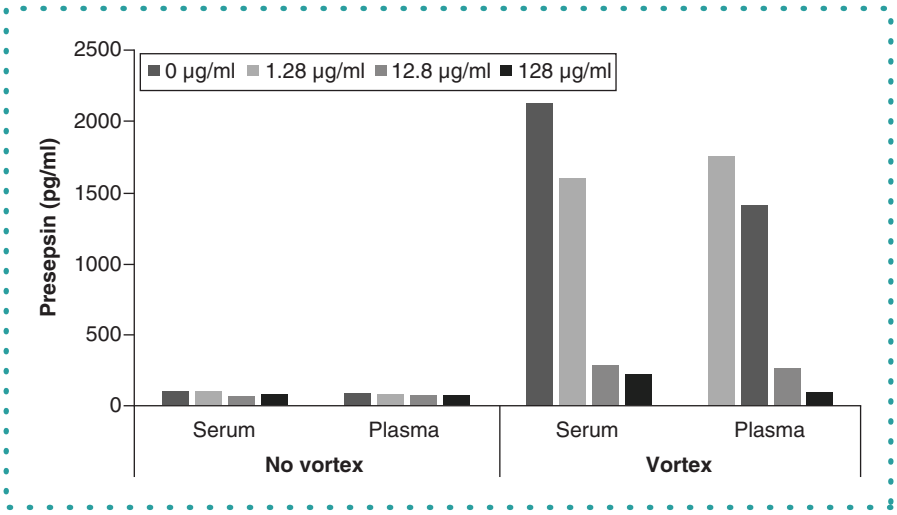


Figure 2. Effect of anti-CD14 antibody (F1024-1-3) upon mixing plasma. The sequential concentrations of F1024-1-3 antibody (0–126 μg/ml) was added to the specimens (serum and plasma) and mixed for 5 min with a vortex mixer. The concentrations of presepsin were then determined by PATHFAST Presepsin.

Treatment with an anti-CD14 antibody inhibits the elevation of presepsin

The increased levels of presepsin in agitated plasma sample suggested that presepsin was produced from sCD14. We hypothesized that agitation reduced the stability of sCD14, leading to denaturation and aggregation. To test this, we added an anti-CD14 antibody (F1024-1-3) to the plasma and evaluated the presepsin levels after mixing. Notably, we observed that this antibody successfully inhibited the increase in presepsin in both vortexed serum and plasma specimens in a dose-dependent manner. A final F1024-1-3 concentration of 128 μg/ml inhibited the elevation of presepsin by 95% (Figure 2).

Analysis of SEC fractions of agitated plasma treated with the anti-CD14 antibody

Plasma samples were treated or not treated with F1024-1-3 (126 μg/ml), mixed vigorously and fractionated by SEC. An ELISA analysis of the fractions revealed a peak level of aggregate in the plasma after agitation, which was not detected in F1024-1-3 antibody-treated samples (Figure 3). In summary, our data demonstrate that treatment with a sufficient amount of F1024-1-3 blocked the formation of sCD14 aggregates.

Analysis of presepsin levels following sCD14 depletion with F1024-1-3-coated particles

Our observation suggested that the agitation-induced increase in the presepsin concentration was related to sCD14. Therefore, we used anti-CD14 antibody-coated particles to deplete aggregated sCD14 from the plasma after agitation. As shown in Figure 4A, although the presepsin concentrations in samples increased after agitation, the addition of the antibody-coated particles removed the aggregated sCD14 and decreased the presepsin levels in the samples. A Western-blotting analysis confirmed that agitation indeed increased the aggregation of sCD14 and that the anti-CD14 antibody-coated particles depleted these aggregates from the samples (Figure 4B).

In this study, we aimed to reveal the mechanism by which agitation might increase the concentration of presepsin in plasma samples. We observed increases in both the presepsin concentration and aggregate formation in agitated plasma samples (Figure 1). Furthermore, specific antibody (F1024-1-3)-mediated depletion of the sCD14 aggregates in the agitated samples reduced presepsin concentrations (Figure 4). These findings strongly suggest that sCD14 aggregation induces presepsin production.

Our SEC data revealed that in addition to the aggregated form, agitated samples also contained presepsin at a similar molecular weight as that detected in nonagitated samples (Figure 1). Previously, Tsai *et al.* reported a physiological level of von Willebrand factor proteolytic activity in the cryosupernatant fraction of normal plasma and described that the exposure of von Willebrand factor to shear stress increases the susceptibility of normal plasma to proteolysis [16]. Moreover, Araki *et al.* demonstrated that the addition of 100 times higher concentration of proteinase inhibitor cocktail, compared with the recommend concentration, before shaking could reduce the presepsin elevation by 96% (unpublished results), suggesting that presepsin will be produced by proteinase-mediated cleavage.

We also demonstrated that treatment with F1024-1-3 inhibited agitation-induced sCD14 aggregation (Figures 2 & 3). Zhang *et al.* demonstrated that protein A almost completely inhibits the agitation-induced aggregation of IgG. These findings suggest that antibodies mediate inhibition by improving conformational stability, which thus reduces aggregation and shields aggregation-prone sites [17]. F1024-1-3 binds to the C-terminus of CD14, which contains the site prone to agitation-induced aggregation, and thus prevents an increase in presepsin. In a previous model study of LPS-induced sepsis, treatment with F1024-1-3 improved the survival rate. In particular, this antibody did not block the binding of LPS to CD14, suggesting that it did not induce a structural change in CD14 [18]. Moreover, other

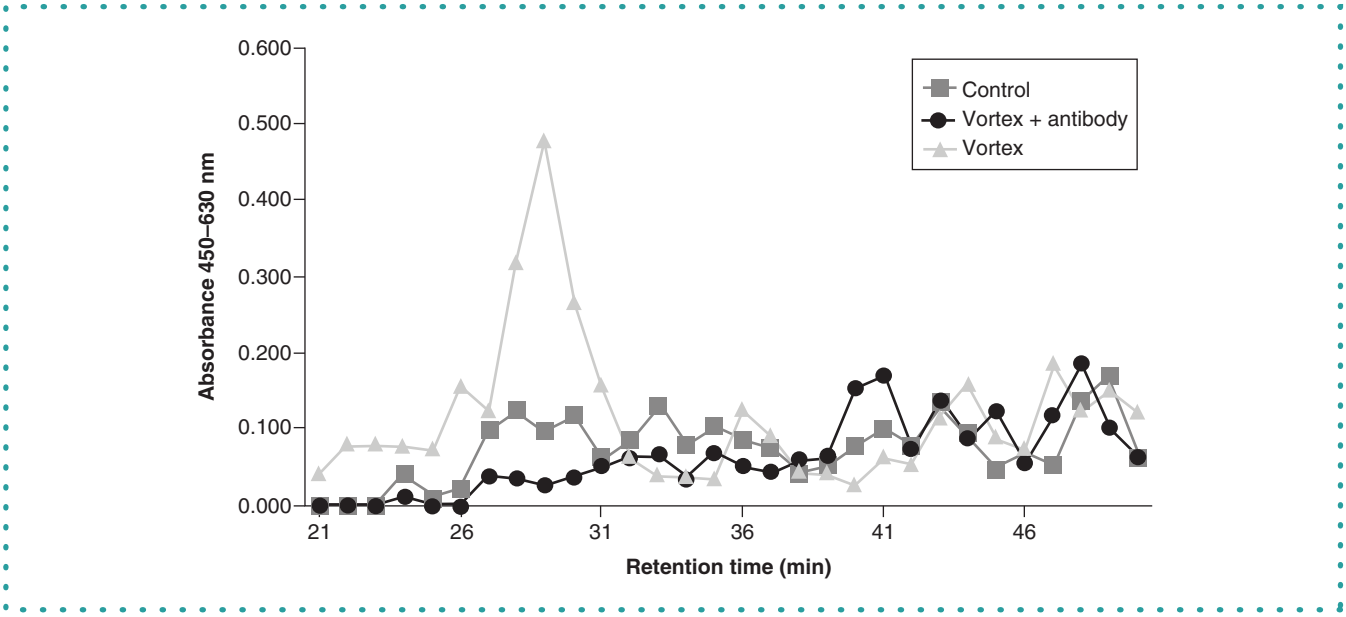


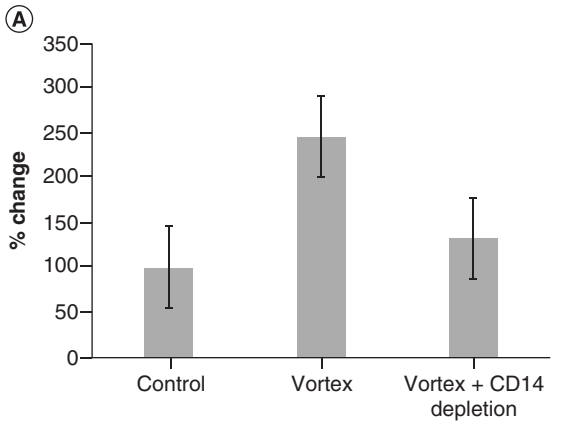
Figure 3. Size exclusion chromatogram of plasma containing anti-CD14 antibody (F1024-1-3). Plasma was added to the F1024-1-3 antibody (126 μg/ml) and then mixed for 5 min. The control (no vortex) and samples (vortex without antibody and vortex with antibody) were fractionated by SEC, and the presepsin were determined by presepsin ELISA.

anti-CD14 antibodies (3C10 or MEM18) that target the N-terminus of the LPS-binding region do not bind to presepsin [4]. Therefore, we speculated that the epitopes of presepsin are not exposed in native CD14.

The recombinant sCD14 (S286C) replaced the amino acid of serine to cysteine at 286 position and the length was 307 amino acids, which is shorter than native sCD14 (356 amino acids) were using in the first-generation presepsin ELISA kit. The cross-reactivity of this recombinant sCD14 showed 1/200 cross-reactivity to the presepsin assay comparing with presepsin standard [5]. According to a PATHFAST Presepsin assay, the native sCD14 containing in a specimen have no significant cross-reactivity (<0.001%) on the presepsin determination at the concentration of 7.75 μg/ml [4]. These results suggested that the length of sCD14 or the amino acid substitution may contribute to the cross reactivity of sCD14. A study by Kim *et al.* indicated that the monomeric subunit of sCD14 contains 13 β strands. sCD14 forms a dimer in solution and the dimerization is mediated by residues in β13 and in the loop between β12 and β13 [19]. These data demonstrate that the C-terminal amino acid sequences play an important role to maintained the sCD14 structure. The shorter or substituted sCD14 may change the structure to increase the cross-reactivity of sCD14. Our and other studies suggest that sCD14 undergoes structural and conformational changes in response to agitation, leading to the exposure of presepsin epitopes, which exist between amino acids 1 and 64. We therefore propose an agitation-mediated pathway to explain the observed elevation of presepsin (Figure 5). Specifically, vigorous vortexing induces the denaturation and aggregation of sCD14, which exposes the target epitopes of anti-presepsin antibodies. The resulting cross-reaction suggests an increased level of presepsin in the sample. Moreover, denatured sCD14 can be more easily digested by proteinases to produce presepsin. Therefore, treatment with an anti-CD14 antibody (F1024-1-3) stabilized sCD14 and prevented a subsequent increase in the presepsin concentration.

This study has several limitations that warrant consideration. First, we used a small sample size, especially in the SEC analysis. Second, we did not consider the effect of presepsin concentration in plasma, particularly in sepsis patients. Third, we could not examine the conformational change of sCD14 before and after agitation. Fourth, we could not explain the stabilization mechanism of anti-CD14 antibody. Furthermore, one study of dialysis patients revealed high presepsin levels even in the absence of infection [20], and Nagata *et al.* reported that hemodialysis patients had elevated blood presepsin concentrations of 1160 pg/ml [21]. In these patients, sCD14 aggregation may occur in response to physical stress caused by tubing pumps and contact with dialysis membranes. Moreover, Korabecna *et al.* reported that dialysis activates neutrophils, further suggesting a role for neutrophilic activity in presepsin formation [22]. Therefore, more studies are need for examine the elevation of presepsin.

In conclusion, we demonstrated that the agitation-induced increase in the presepsin concentration in a sample can be attributed to the aggregation of sCD14. Presumably, this aggregation exposes the presepsin epitope present in sCD14, thus allowing detection by presepsin-specific antibodies. We also revealed that the aggregation of sCD14 could be inhibited by treatment with an anti-CD14 antibody. Our findings suggest that a method could be developed to prevent the agitation-induced increase in presepsin in clinical plasma and warrant further studies to explore this possibility.



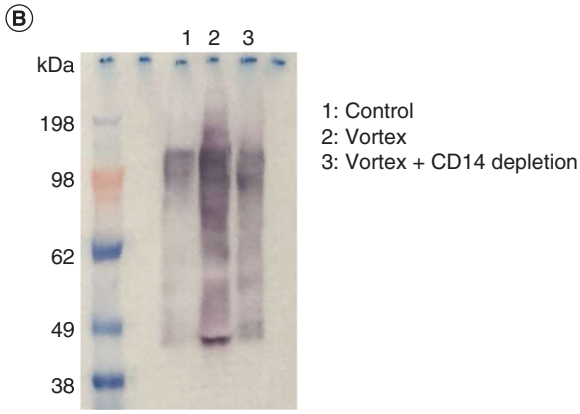


Figure 4. Anti-CD14 antibody (F1024-1-3) absorbed the aggregated soluble CD14 (sCD14) and decreased the presepsin values caused by agitation. (A) Three samples were vortexed for 5 min, then the sCD14 was depleted by anti-CD14 antibody (F1024-1-3). Presepsin values were determined by PATHFAST Presepsin and compared with control and presented by percentage change. **(B)** The samples were analyzed by Western blotting, which was detected by anti-CD14 antibody (61D3).

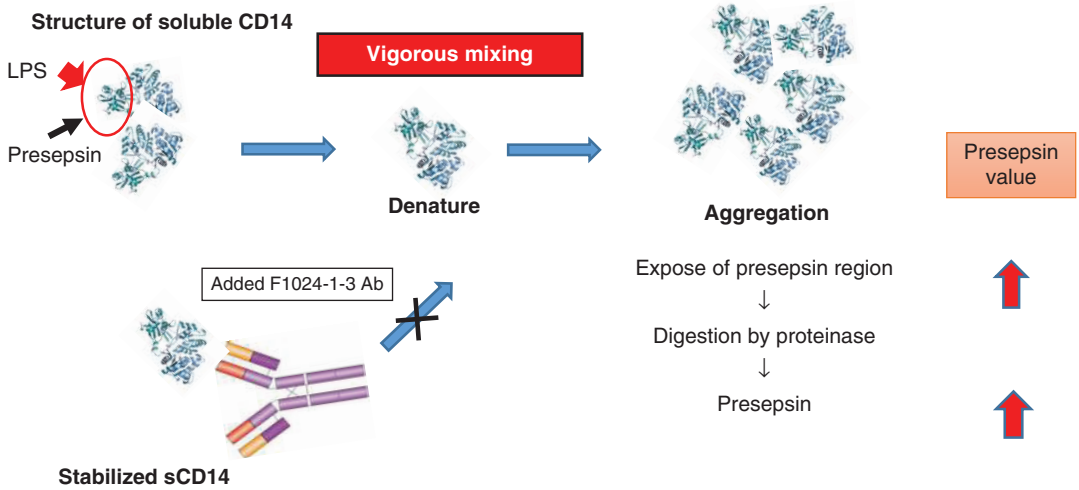


Figure 5. Proposed model for agitate-induced presepsin elevation in plasma. Agitation induces sCD14 denaturation and aggregates sCD14, and thereby epitopes of presepsin will be exposed to sCD14; it may then be digested by proteinase to produce presepsin.
LPS: Lipopolysaccharide; sCD14: Soluble CD14.

Future perspective

If the findings of this study continue to develop, the cause of elevated preceptin in dialysis patients should be clear. In that case, we believe that we can set a useful cutoff value for the diagnosis of sepsis in dialysis patients.

Author contributions

G Morino: project administration, investigation, writing review & editing. G Takahashi: supervision. S Kan: data curation, formal analysis. Y Inoue: funding acquisition. K Sato: visualization. K Shirakawa: methodology, writing of original draft.

Acknowledgments

Some devices and reagents used in this study were provided by LSI Medience Corporation, Tokyo, Japan.

Financial & competing interests disclosure

This study was supported in part by the research funding from Department of Critical Care, Disaster and General Medicine, School of Medicine, Iwate Medical University, Morioka, Japan. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was approved by the ethics committee of School of Medicine, Iwate Medical University, Morioka, Japan. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

1. Shirakawa K. A novel biomarker for sepsis diagnosis -Discovery of presepsin and the production mechanism-. *Animus* 99, 27–32 (2019).
2. Grunwald U, Krüger C, Westermann J *et al*. An enzyme-linked immunosorbent assay for the quantification of solubilized CD14 in biological fluids. *J. Immunol. Methods* 155, 225–232 (1992).
3. Furusako S, Shirakawa K, Hirose J. Novel soluble CD14 antigen. US20060068445A1 (30 March 2006).
4. Shirakawa K, Naitoh K, Hirose J *et al*. Presepsin (sCD14-ST): development and evaluation of one-step ELISA with a new standard that is similar to the form of presepsin in septic patients. *Clin. Chem. Lab. Med.* 49, 937–939 (2011).
5. Yaegashi Y, Shirakawa K, Sato N *et al.* Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. *J. Infect. Chemother.* 11, 234–238 (2005).
6. Okamura Y, Yokoi H. Development of a point-of-care assay system for measurement of presepsin (sCD14-ST). *Clin. Chim. Acta.* 412, 2157–2161 (2011).
7. Endo S, Suzuki Y, Takahashi G *et al.* Usefulness of presepsin in the diagnosis of sepsis in a multicenter prospective study. *J. Infect. Chemother.* 18, 891–897 (2012).
8. Khabar KSA. Post-transcriptional control of cytokine gene expression in health and disease. *J. Interferon. Cytokine Res.* 34, 215–219 (2014).
9. Mohan MJ, Seaton T, Mitchell J *et al.* The tumor necrosis factor-alpha converting enzyme (TACE): a unique metalloproteinase with highly defined substrate selectivity. *Biochemistry* 41, 9462–9469 (2002).
10. Arai Y, Mizugishi K, Nonomura K *et al*. Phagocytosis by human monocytes is required for the secretion of presepsin. *J. Infect. Chemother.* 21, 564–569 (2015).
11. Shirakawa K, Naitoh K, Hirose J *et al.* The new sepsis marker, sCD14-ST, induction mechanism in the rabbit sepsis models. *Crit. Care* 14(Suppl. 2), P19 (2010).
12. Chenevier-Gobeaux C, Bardet V, Poupet H *et al*. Presepsin (sCD14-ST) secretion and kinetics by peripheral blood mononuclear cells and monocytic THP-1 cell line. *Ann. Biol. Clin. (Paris)* 74, 93–97 (2016).
13. Tanimura S, Fujieda Y, Kono M *et al.* Clinical significance of plasma presepsin levels in patients with systemic lupus erythematosus. *Mod. Rheumatol.* 28, 865–871 (2018).
14. Ham JY, Song KE. Impact of specimen mixing methods on presepsin point-of-care test results using whole blood. *Clin. Chem. Lab. Med.* 54, e151–e154 (2016).
15. Furusako S, Shirakawa K, Mori S. Anti-CD14 monoclonal antibody having effect of inhibiting CD14/TLR binding. US7264967B2 (4 September 2007).
16. Tsai HM, Sussman II, Nagel RL. Shear stress enhances the proteolysis of von Willebrand factor in normal plasma. *Blood* 83, 2171–2179 (1994).
17. Zhang J, Topp EM. Protein G, protein A and protein-A-derived peptides inhibit the agitation induced aggregation of IgG. *Mol. Pharm.* 9, 622–628 (2012).
18. Nakamura M, Takeuchi T, Shirakawa K, Furusako S. Anti-human CD14 monoclonal antibody improves survival following sepsis induced by endotoxin, but not following polymicrobial infection. *Eur. J. Pharmacol.* 806, 18–24 (2017).
19. Kim JI, Lee CJ, Jin MS *et al.* Crystal structure of CD14 and its implications for lipopolysaccharide signaling. *J. Biol. Chem.* 280(12), 11347–11351 (2005).
20. Shiota J, Ohura N, Higashikawa S *et al.* Presepsin as a predictor of critical colonization in CLI hemodialysis patients. *Wound Repair. Regen.* 24, 189–194 (2016).
21. Nagata T, Yasuda Y, Ando M *et al.* Clinical impact of kidney function on presepsin levels. *PLoS One* 10, e0129159 (2015).
22. Korabecna M, Tesar V. NETosis Provides the link between activation of neutrophils on hemodialysis membrane and comorbidities in dialyzed patients. *Inflamm. Res.* 66, 369–378 (2017).