

Presepsin (sCD14-ST) secretion and kinetics by peripheral blood mononuclear cells and monocytic THP-1 cell line

Cinétique de libération de la présepsine (sCD14-ST) par des cellules monocytaires macrophagiques circulantes et par une lignée monocyttaire

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Abstract. Presepsin could help for early diagnosis of systemic infection. Little is known regarding its kinetics. We studied presepsin concentration after challenge with bacterial agonist lipopolysaccharide (LPS) stimulation in peripheral mononuclear cells (PMNC) collected from 5 healthy volunteers and in a human cell line of monocytic cells (THP1). In PMNC, an exposure to LPS (100 ng/mL) induced an increase of median presepsin levels as early as hour 1 (+31%, p=0.007), concomitantly to IL-6 synthesis. In THP1 cells, presepsin was detected at 1 hour after LPS exposure, and peaked at 3 hours, in THP1 cells. In conclusion, we report here that presepsin, a surrogate marker of the host response to bacteria, increases early in PMNC and in a monocytic cell lineage. Our findings might confirm the potential usefulness of presepsin bedside as an early marker of infectious diseases.

Key words: biomarkers, lipopolysaccharide, presepsin, sepsis

Résumé. La présepsine est proposée comme marqueur biologique d'aide au diagnostic précoce de l'infection systémique. On sait peu de choses de sa cinétique. Dans ce travail, nous avons étudié l'évolution de la concentration de présepsine après stimulation par le lipopolysaccharide (LPS) de cellules périphériques mononucléées (PMNC) provenant de 5 volontaires sains et d'une lignée cellulaire humaine de cellules monocytes (THP1). Une exposition de PMNC au LPS (100 ng/mL) induit une augmentation des concentrations médianes de présepsine dès la première heure (+ 31 %, p = 0,007), de manière concomitante à l'augmentation de l'IL-6. Dans les cellules THP1, la présepsine a également été détectée dès la première heure après exposition au LPS, atteignant un pic à 3 heures. En conclusion, nous rapportons ici que la présepsine, un marqueur potentiel de la réponse de l'hôte aux bactéries, est rapidement sécrétée par des PMNC et dans une lignée cellulaire monocyttaire après exposition au LPS. Nos résultats pourraient confirmer l'utilité potentielle de la présepsine comme un marqueur précoce de pathologie infectieuse chez l'homme.

Mots clés : biomarqueurs, lipopolysaccharide, présepsine, sepsis

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Deciding appropriately to prescribe antimicrobial therapy is a daily challenge in emergency medicine. However results of trials testing procalcitonin in lower respiratory tract

infections has durably modified perception of infectious diseases in this setting. Since then, development of new tools to detect infection and stratify severity has raised enthusiasm in emergency physicians. Presepsin (soluble CD14 subtype or sCD14-ST) is an emerging biomarker that corresponds to the processing of membrane CD14, a

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co-receptor involved in the innate immune response at the membrane of monocyte-macrophage [1]. Healthy subjects are permanently exposed to microorganisms in a balanced manner; therefore, circulating leukocytes release presepsin in blood stream at steady state [1] and changes in physiological conditions alter baseline levels [2]. Presepsin concentrations increase in patients suffering systemic infection, with promising specificity and in a severity-dependent manner [3, 4]. Therefore, it has been suggested that this biomarker could help for early diagnosis of systemic infection. However, little is known regarding kinetics of presepsin; preliminary studies indicate that presepsin induction is very rapid: in a caecal ligation and puncture (CLP) sepsis model, presepsin was detected within 2 hours after procedure [5]. Accordingly, we studied presepsin concentration after bacterial agonist (lipopolysaccharide, LPS) stimulation in peripheral mononuclear cells (PMNC) of healthy subjects, and in a human cell line of monocytic cells (THP1) stably transfected with CD14.

Materials and methods

Blood samples

Peripheral blood samples were collected on heparinated tubes from 5 healthy volunteers (5 women, mean age 32 years).

THP1 cell line

THP1 cells were stably transfected with CD14 (THP1-CD14) in a Dulbecco culture medium with 10% fetal calf serum. Before stimulation, THP1-CD14 were suspended (0.5×10^6 /mL) in serum-free culture medium.

Cell stimulation

THP1 (0.5×10^6 /mL) and blood samples (2 mL per sample in duplicate) were stimulated with detoxified LPS obtained from Sigma Aldrich (St Louis, MO, USA), at the final concentration of 100 ng/mL [6], and incubated at 37°C during 4 hours. Immediately after stimulation (at H0), a 500 μ L-sample was collected and centrifuged. Every hour after stimulation (from H1 to H3 or H4), a 500 μ L-sample was collected and centrifuged. Supernatants and plasmas were collected and stored at -40°C until completion of the experiment. Remaining cells were used to perform flow cytometry staining.

A negative control (supernatant or blood sample with solvent -PBS- without LPS) was done: results obtained were not statistically different from H0. Solvent (PBS) without LPS was tested: presepsin concentrations were found below 6 ng/L.

Biomarkers measurement

Presepsin concentrations were measured using a chemiluminescent enzyme immunoassay (PATHFAST™ Presepsin), performed on the PATHFAST point-of-care analyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Measuring range is 20-20,000 ng/L; manufacturer's upper reference limit (URL) is 320 ng/L. Our laboratory CVs for presepsin were <5% at 860 and 2,500 ng/L, during the study period. Furthermore, LPS in concentrations from 1 to 100 ng/mL did not induce significant interference regarding presepsin assay (% of recovery: 86.8 to 103.4%).

Interleukin-6 concentrations were measured using an immunochemiluminescent assay (IL-6) performed on an Elecsys 2010 analyzer (Roche Diagnostic, Meylan, France). Measuring range is from ~1.5 to 5,000 ng/L; manufacturer's URL is 7 ng/L. Our laboratory CVs for IL-6 were <5% at 34 and 225 ng/L, during the study period.

Membrane CD14 expression was measured by flow cytometry on a FC500 flow cytometer (Beckman Coulter, Miami, FL). Briefly, pellets were stained with CD14 PC7 (THP1 cells) or CD14 PC7 and CD64 FITC (blood samples) (Beckman Coulter) according manufacturer's instructions. When necessary, red cells were lysed using Versalyse (Beckman Coulter) according manufacturer's instructions before acquisition. Results were analyzed with Kaluza (Beckman Coulter) software. For THP1 cells, results were expressed as ratio of fluorescence intensity (RFI) for CD14 between stained and unstained cells whereas for patients median of fluorescence intensity were compared. CD64 was used to gate monocytes in patients.

Statistical analysis

Baseline and follow-up characteristics were described by medians (IQR) for continuous variables. We performed Mann-Whitney test for comparison of continuous variables. All tests were two-sided, and p-values <0.05 were considered to denote statistical significance. All statistical analyses were performed using MedCalc software (version 11.5.0.0; MedCalc Software bvba, Mariakerke, Belgium).

Results

Presepsin release, IL-6 synthesis and mCD14 expression after LPS challenge in PMNC from healthy participants

The impact of LPS exposure on presepsin release was tested in healthy donors PMNC (figure 1). Mean presepsin concentration at baseline (H0) was 208 [196-243] ng/L. Exposure to LPS (100 ng/mL) induced a non significant increase of

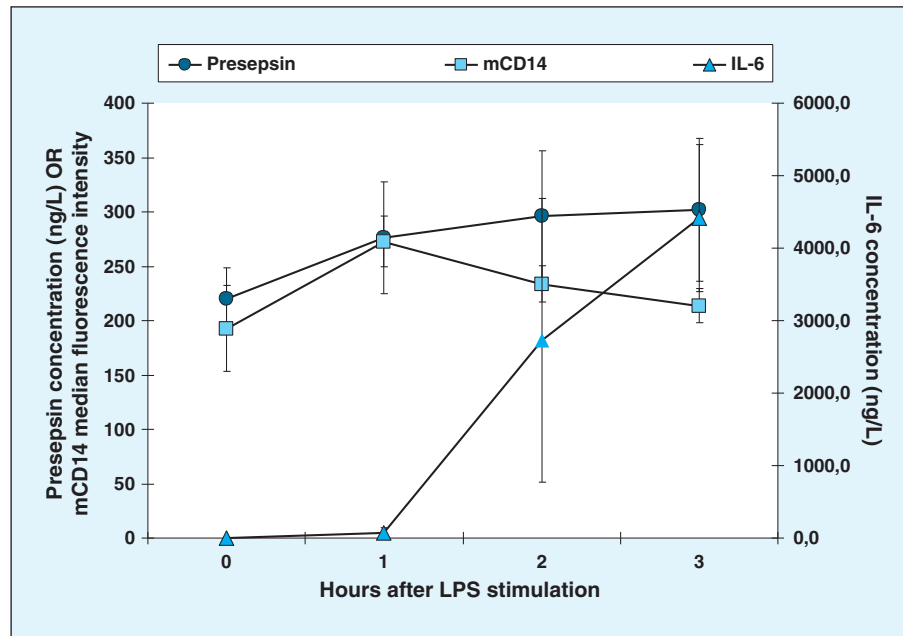


Figure 1. PMNC presepsin release, mCD14 expression and IL-6 synthesis after stimulation with LPS.

presepsin levels at the first hour of stimulation (269 [234-333] ng/L, $p=0.095$ versus H0); presepsin levels raised to 297 [259-348] at 2 hours ($p=0.076$ versus H0), and to 282 [258-310] at 3 hours ($p=0.076$ versus H0).

By the meanwhile, IL-6 was almost undetectable at H0 (1.5 [1.5-1.7] ng/L), increased significantly at H1 (42.3 [33.0-140] ng/L, $p=0.008$) and H2 (4,240 [1,815-5,000], $p=0.008$ versus H0), and peaked at H3 (5,000 [4,377-5,000] ng/L, $p=0.007$ versus H0).

Under LPS stimulation, the mCD14 expression significantly increased at the first hour ($p=0.010$), reaching a peak that decreased at 2 and 3 hours ($p=0.010$ and $p=0.004$, respectively, versus H1 peak).

Individual kinetics of presepsin and mCD14

Individual kinetics of presepsin release and mCD14 expression from PMNC are represented in *figure 2*. Median increase in presepsin release is observed as early as H1 (+31%, $p=0.007$ in comparison to H0), and stays high at H2 (+39%, $p=0.007$ vs H0) and H3 (+35%, $p=0.007$ vs H0) (*figure 2A*). By the meanwhile, median mCD14 expression significantly increased at the first hour (+43%, $p=0.007$ vs H0), reaching a peak that decreased at 2 and 3 hours (+26 and +9%, respectively, versus H0) (*figure 2B*).

Presepsin and mCD14 kinetics after LPS challenge in THP1-CD14 cell line

At baseline (H0), presepsin was not detected in the culture medium. After exposure to LPS (100 ng/mL), presepsin

was detected at 60 minutes and peaked at 3 hours, before observing a decrease at 4 hours (*figure 3*).

We simultaneously observed that CD14 expression at the cell membrane (mCD14) continuously decreased after stimulation of THP1-CD14 by LPS.

Discussion

Our results demonstrate an early presepsin release in both human peripheral mononuclear cells and human cell line of monocytic cells.

CD14 is a co-receptor anchored at the external membrane of myeloid cells. It cooperates with Toll-like receptor (TLR)-4 and LPS-binding protein (LBP) to bind LPS and engage innate immune response [7]. After binding of LPS, membrane CD14 (mCD14) expression decreases by mechanisms of shedding [8] and internalization [9]; our results obtained on THP1 cells are in accordance with these cellular mechanisms, as we observed a continuous decrease after LPS challenge. However, mechanism might be slightly different in PMNC, if considering our results. mCD14 is known to be a pattern-recognition receptor (PPR) that serve as sentinel of the immune system [10].

In addition, a soluble form of CD14 is released and can be detected in peripheral blood flow [11, 12]. It has been reported that circulating CD14 measurements increased during Gram-negative infection. Moreover, concentrations of soluble CD14 have been associated with severity of sepsis in neonates [13] and adults [14]. Presepsin is an

emerging biomarker for early diagnosis of infection that directly depends on CD14 and LBP engagement [1]. Indeed, our results suggest that bacterial infections lead to early changes in circulating levels of presepsin and in mCD14 expression. Therefore, presepsin elegantly supports the paradigm that surrogates of innate antimicrobial defense could help in early diagnosis of sepsis. In their preliminary in vivo studies (CLP in rabbit), Naitoh [5] revealed that presepsin was detected within 2 hours after operation. Furthermore, phagocytosis inhibitors curbed the production of presepsin. This suggests that the secretion mechanism of presepsin depends on phagocytosis. CLP is a model of polymicrobial infection with massive Gram-negative bacteria from the gut. Therefore, the increase of presepsin is forecasted when using LPS challenge. Here we demonstrated that presepsin release is induced by LPS from human PMNC as early as the one of IL-6, which is

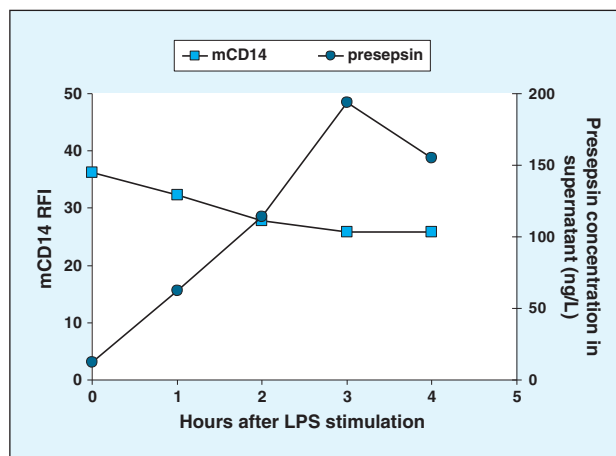


Figure 3. THP1 presepsin release and mCD14 expression after cell stimulation with LPS. RFI, ratio of fluorescence intensity.

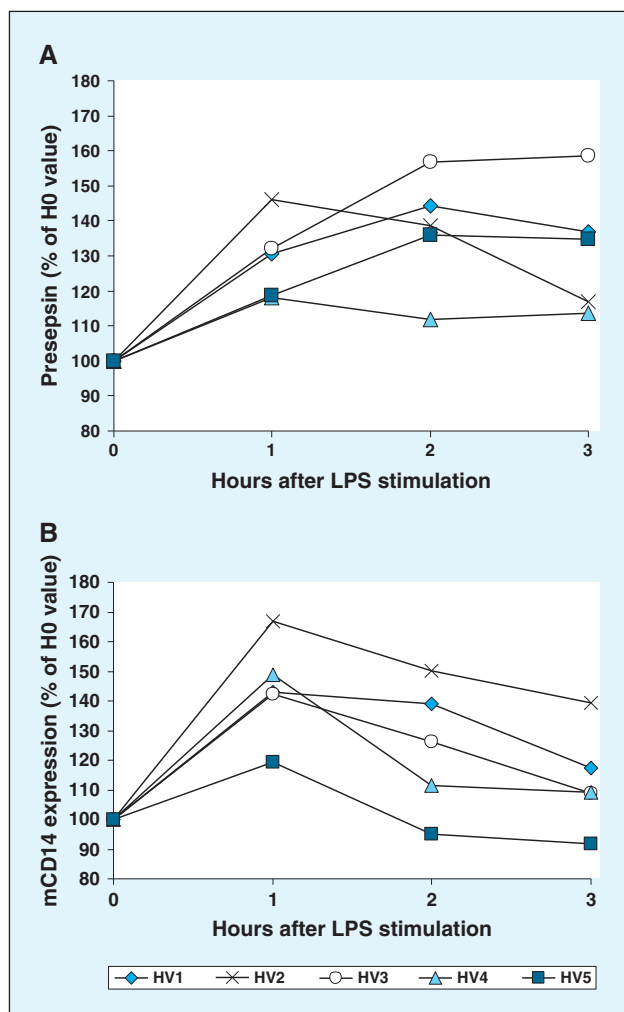


Figure 2. Individual kinetics of circulating presepsin (A) and mCD14 expression (B) for each healthy volunteer (HV). In this analysis, each patient is its own comparator.

to day the earliest sepsis biomarker, before procalcitonin or CRP [15]. In our model, the inter-variability of basal presepsin secretion is high, and the elevation of IL-6 at 1 hour is greater than that of presepsin. Considering our cell model, we could not compare presepsin kinetics to that of procalcitonin or CRP. However, a preliminary evaluation of presepsin in serial left-over plasma samples from 2 neonates with confirmed neonatal bacterial infection indicated that (1) presepsin concentrations observed at birth are elevated, in accordance with that observed in the literature [12], (2) presepsin concentrations continue to increase 2 hours later and are culminant at 6 - 12 hours, (3) presepsin remained high at 18 hours. In comparison, both neonates had CRP values at birth below previous decisional threshold, and one neonate had PCT value at birth below decisional threshold [16].

In adult patients with high probability of Gram-negative bacterial infection (pyelonephritis and gut-related infectious complication), presepsin was increased as compared to patients developing non septic SIRS [3]. On the other hand, CD14 is a co-receptor for several microbial agonists and chances exist that various bacteria may lead to presepsin release. Results from a recent clinical study report a significant elevation of presepsin in patients experiencing local and systemic infections [4].

Limitations

We acknowledge that sequential measurements of presepsin levels in a culture medium may differ from kinetics in a complex organism. As an example, we previously observed that circulating presepsin concentrations increased with renal impairment [2]. Other mechanisms may also contribute to clearance of presepsin as plasmatic esterases and

binding to circulating proteins or cells. We also acknowledge that live bacterial stimulation might provide a better understanding of presepsin release, and should be performed in a new study.

Conclusion

Here we report that presepsin, a surrogate marker of the host response to bacteria, increases early and after challenge with bacterial LPS in both PMNC from healthy subjects and monocytic cell lineage. Our findings might confirm the potential usefulness of presepsin bedside as an early marker of infectious diseases.

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