Advances and pitfalls in using laboratory biomarkers for the diagnosis and management of sepsis

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ABSTRACT

Sepsis is a critical patient condition with high mortality rate caused by a complex and inadequate host response to infection. Since early identification and start of antibiotic therapy in the first few hours after sepsis development dramatically improves outcomes, it is of utter importance to offer fast, reliable and specific early laboratory biomarkers to help clinicians in sepsis recognition. On the other hand, the biomarkers should also be helpful in excluding sepsis and/or confirming therapy effectiveness, and thus prevent overprescribing of antibiotics. In this paper, we discuss the significance and relative merits of three currently available protein biomarkers: C-reactive protein, procalcitonin and presepsin. Although useful, none of these biomarkers has been shown to completely fulfill the roles mentioned above.

INTRODUCTION

The 2016 Guidelines of the Surviving Sepsis Campaign (SSC) classify sepsis and septic shock as medical emergencies and therefore treatment and resuscitation must begin immediately (1). Sticking to the shortest possible time frame for diagnosis and treatment is crucial since it often means life or death for the patient. However, it is –especially in the early stages– not easy to be sure if a patient is septic or not. As a consequence, to be on the safe side, antibiotics are often overprescribed, which generates further problems. As sepsis represents one of the major problems in intensive care units, it is essential for clinicians to have fast, accurate and reliable biomarkers that can help them to make a quick diagnosis and appropriately manage or exclude this life-threatening condition. An ideal biomarker should have all of the following characteristics: fast and specific increase in sepsis, rapid decrease after effective therapy, short half-life and fast and widely available and reliable method of determination. None of the current biomarkers exhibits all of these specifications in full, but the best currently used biomarker available both as a point of care test (POCT) and as a part of several major in vitro diagnostics (IVD) manufacturers' portfolio is procalcitonin (PCT) (2). However, since CRP is still uniformly and inevitably used worldwide, its importance in sepsis diagnosis and management will be briefly discussed. The focus of attention will be placed on the new and rapidly advancing biomarker presepsin whose role is still uncertain, but which might represent a step towards earlier and better sepsis recognition by laboratory means (3). Of course, the golden standard for sepsis confirmation remains within the scope of microbiology laboratory, but the time to result even with the recent advancement of Matrix Assisted Laser Description/Ionization - Time of Flight (MALDI TOF) technology is still inferior to the above mentioned surrogate biomarkers.

C-REACTIVE PROTEIN

C-reactive protein (CRP) belongs to the pentraxin family of calcium-dependent, ligand-binding proteins. Human CRP molecule has a discoid shape and consists of five identical nonglycosylated polypeptide subunits, each containing 206 amino acid residues (4). The *CRP* gene is located on the first chromosome (1q21q23).

CRP was isolated from the sera of patients infected with Streptococcus pneumoniae, and was first described by Tillett and Frances in 1930 (5). The CRP, named for its capacity to precipitate the somatic polysaccharide-F of Streptococcus pneumoniae, was the first acute-phase protein to be described and is an exquisitely sensitive marker of systemic inflammation and tissue damage (4).

CRP is synthesized primarily in the liver, and at lower levels in adipocytes as a response to interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). CRP is an acute phase protein. During acute-phase response to infection, inflammation or tissue damage, the concentration of CRP increases several thousand times within 48 hours. Authors Pepys and Hirschfield stated that the median concentration of CRP is 0.8 mg/L in healthy young adult volunteer blood donors, the 90th percentile is 3.0 mg/L, and the 99th percentile is 10 mg/L (1) but, following an acute-phase stimulus, the values may increase from less than 50 µg/L to more than 500 mg/L, that is, 10,000-fold (6).

The function of CRP is manifold. The CRP can bind to specific ligands and activate a complement on the classical pathway and thereby participate in non-specific defences against infection and prevent the development of autoimmune diseases. The main ligand for CRP is phosphocholine which is present in the cells of most pathogens, including bacteria and fungi. Furthermore, it is considered that the CRP interacts with damaged endothelial cells or the apoptotic and necrotic cells.

The major CRP drawback in sepsis lies in its lack of specificity. Elevated CRP concentrations in the circulation may indicate inflammation and/or tissue damage of any origin, such as bacterial and viral infections, mycoses, allergic complications of infection, various inflammatory reactions, necrosis, trauma, or malignancies (7-13). The particular problem is a common postoperative CRP elevation with values that might easily overlap with the septic ones, particularly in the initial stages when early recognition is of utter importance.

The CRP values are not affected by diurnal variations, food intake and most medications.

Recent evidence suggests that aging has a significant effect on inflammatory response and the immune system of elderly people, which is to be considered when interpreting CRP values (14).

Acute phase proteins such as CRP, PCT, serum amyloid A, IL-6, and hepcidin have been investigated in multiple studies related to neonatal sepsis. CRP is usually used as an indicator of bacterial sepsis in newborns since PCT values may not be easily interpreted in the first few days of life. However, the determination of CRP has a few drawbacks; it is not useful as a marker for the early phase of infection because it can only be detected about 12 hours after the onset of clinical symptoms, it reaches its maximum after 20 to 72 h (15) and does not show satisfactory specificity (16). However, due to its universal availability and fairly straightforward interpretation of values, CRP is still the most common marker used worldwide in all hospitalized patients, including those with high risk of sepsis development.

PROCALCITONIN

PCT is a calcitonin precursor prohormone which consists of 116 amino acids and is normally expressed by neuroendocrine cells of the thyroid gland, lungs and pancreas. In healthy people PCT values are low, less than 0.046 µg/L (95th percentile) (17,18). PCT values in various non-septic infections are usually lower than 0.5 µg/L (18). This fact, i.e. exclusively bacteria-related increase represents one of the crucial PCT advantages when compared to CRP. In severe septic shock PCT can rise up to 1000-fold. The pathomechanism of blood PCT elevation is a reaction to various exogenous and endogenous stimuli, such as inflammatory interleukins, membrane elements, bacteria lipopolysaccharides or peptidoglycans as well as bacterial endotoxins. In sepsis, PCT is synthesized mostly in liver, but also in other parenchymal organs (19).

Increase in PCT level in patient's blood can be detected approximately 2 to 4 hours after the onset of sepsis (19), which represents another major advantage making it both more suitable and specific than CRP in this clinical context. It is important to know that PCT has a plasma half-life of 20-24 hours (20) Therefore, according to current recommendations, the minimum time before testing needs to be repeated (minimum retesting interval) should be 24 hours (21).

PCT can serve as an aid for clinicians in assessing the risk category for their patient of developing sepsis or septic shock, according to the classification (18) in Table 1.

After initial measurement, PCT can be used to monitor progression of the disease and therapy effectiveness, taking into account minimum retesting interval for PCT of 24 hours.

PCT has also proved to be useful in guiding antibiotic therapy. This approach was mainly evaluated in patients with respiratory tract infections; however, it can also be used in critically ill patients with sepsis or severe sepsis of various origins (21,22). In those patients, daily measurement of PCT is indicated and discontinuation of antibiotic therapy should be considered when PCT levels decrease to less than 80% of the peak value or below 0.5 µg/L (23).

Table 1	Risk categories according to PCT values
PCT value (μg/L)	**Risk category**
< 0.5	low risk of systemic bacterial or fungal infection
0.5 - 2.0	high risk of systemic bacterial or fungal infection
2.0 – 10	high risk of sepsis and progression to septic shock
> 10	high risk of septic shock

This approach has been shown to significantly reduce the antibiotics use without compromising the patient outcome, which might prove beneficial towards the goal of minimising the antibiotic usage both for the sake of patients and hospital resources. It is important to note that current recommendations state that measurement of PCT levels can be used to support shortening the duration of antimicrobial therapy in sepsis patients; however, as yet this approach is not based on strong evidence (1). Similar strength of recommendations pertains to the fact that PCT levels can be used to support the discontinuation of empiric antibiotics in patients who initially appeared to have sepsis, but have subsequently been proven to have limited clinical evidence of infection (1).

In conclusion, PCT has proven to be a helpful biomarker in early diagnosing of sepsis in emergency departments and intensive care units. It has also been recommended for monitoring effectiveness and modulating duration of antibiotic therapy. It is now widely available both as a laboratory and as a point-of-care test, whereas laboratory methods are preferred due to semi-quantitative nature of results on most POCT devices. When ordering PCT, it should be essential that the result provides the clinician with an answer that could not have been resolved by other laboratory tests combined with clinical signs and symptoms, such as complete blood count or CRP measurement. It is important to note that PCT and all other biomarkers can provide only supportive and supplemental data to clinical assessment. Decisions on initiating, altering, or discontinuing antimicrobial therapy should never be made solely on the basis of changes in any biomarker, including PCT (1). The following questions therefore cannot as yet unequivocally be answered exclusively either by PCT or any other available biomarker: Does my patient have sepsis? Is this antibiotic therapy effective for my patient? Can I now safely discontinue antibiotic therapy? PCT seems to be of help in answering those questions in many clinical situations; however, it cannot fulfill this role by itself and should therefore be ordered rationally.

PRESEPSIN

Presepsin is a newly investigated sepsis marker that has been shown to have potential as an early marker of sepsis recognition, for antimicrobial therapy monitoring and as a prognostic marker. Presepsin (sCD14-ST) is a peptide sized 13 kDa that is generated by proteolytic cleavage of soluble forms of CD14 cluster (sCD14). CD14 is a cell surface glycoprotein with molecular mass of 53-55 kDa that is anchored by glycosylphosphatidylinositol (GPI) to cell membrane and represents a membrane form of CD14 (mCD14). The mCD14 as a co-receptor mediates the binding of the bacterial endotoxin,

lipopolysccharides (LPS) and complex LPS-lipopolysaccharide binding protein (LBP) to toll-like receptors (TLRs), causing the activation of inflammatory responses: cell activation, fagocytosis and cytokine production defending host against pathogen (24-26). After the sTLR activation, the mCD14 undergoes the proteolysis, producing two soluble forms (sCD14) of different sizes. The smaller sCD14 is produced by protease cleaving of mCD14 and the bigger one is produced intracellularly and is directly released from the cell in the protease-independent manner (27-29). CD14 is present mostly on monocytes, macrophages, neutrophils, B-lymphocytes and also on chondrocytes, dendritic cells and human epithelial intestinal cells. Hepatocytes can also express CD14, especially during endotoxemia (30). Recent investigation of presepsin kinetics has shown that, when the polymorphonuclear and monocytic cells were exposed to LPS, presepsin could be detected as early as one hour after the exposure, with maximum concentration in the third hour (31). This finding may confirm that presepsin can be a useful marker of host response to bacteria and can be both a specific marker of infection as well as an early indicator, compared to current markers.

Many studies have investigated accuracy of presepsin in diagnosis of system immune response syndrome (SIRS), sepsis and septic shock in different clinical conditions. The systematic reviews and meta-analysis showed that presepsin as a diagnostic marker of sepsis has diagnostic sensitivity and specificity of 0.83 and 0.78, respectively, diagnostic accuracy (expressed as ROC AUC, receiver operating characteristic, area under the curve) of 0.88, and positive and negative likelihood ratios of 3.9 and 0.21, respectively (32).

Multicenter prospective studies have demonstrated a statistically significant difference in presepsin levels between patients with bacterial and non-bacterial infective disease. Presepsin at the cut-off value of 600 ng/L has diagnostic sensitivity and specificity of 87.8% and 81.4%, respectively, which is comparable to PCT at the cut-off of 0.5 μg/L. A study showed that there was no statistically significant difference in presepsin levels between patients with localized and systemic infection, which might preclude its usefulness in sepsis (33).

A multicenter randomized clinical trial which investigated the clinical role of presepsin assay for monitoring disease in relation to the development of complications (34) found that higher presepsin levels on the first day were closely associated with higher incidence of subsequent organ failures (SOFA score). It also studied presepsin role in monitoring the host response to antimicrobial therapy and appropriateness of therapy – the trial confirmed that patients with increasing presepsin concentrations during the first 7 days were less likely to have received early appropriate antibiotic therapy. The study also stated prognostic accuracy of presepsin for early and long-term outcomes; early presepsin was higher in non-survivors than in survivors. Patients with lung infection had lower baseline presepsin levels than patients with abdominal and urinary tract infections (34,35).

Another study has shown that presepsin levels are elevated at an early stage of sepsis and increase with its progression. The plasma presepsin levels reached the highest level in septic shock. ROC analysis of presepsin to differentiate SIRS (systemic inflammatory response syndrome) and sepsis revealed that, at the cut-off of 581 ng/L, sensitivity and specificity were 65% and 100%, respectively, AUC was 0.830. When presepsin is combined with the MEDS (Mortality in Emergency Department Sepsis) scoring system, the AUC was significantly higher - 0.95, with the sensitivity and specificity of 85% and 100%, respectively (36). A similar investigation of the diagnostics of sepsis has shown AUC for

presepsin to be 0.82. For predicting severe sepsis in sepsis patients, the AUC was 0.840 and, with the combination of presepsin and MEDS score the AUC was 0.875 (35).

A very important piece of information about presepsin should be emphasized. As presepsin is a small protein (13 kDa) and is filtered by kidneys, its level is strongly dependent on kidney function. The decreased glomerular filtration causes elevated levels of presepsin in circulation and thus, presepsin levels above the cut-off value in patients with renal failure have to be interpreted with caution (37,38). A study showed no statistical difference in presepsin concentration between septic and non septic oliguric patients (stage failure in RIFLE criteria; RIFLE, concensus classification criteria for acute kidney injury: Risk, Injury, Failure, Loss, End-stage kidney disease), which might preclude its use in patients with renal failure (38). In this regard, however, future studies are necessary to confirm this finding. There is also a recent study (39) which deemed presepsin as a valuable biomarker for diagnosis of infection and sepsis; however, its diagnostic accuracy has not shown any superiority compared to PCT. Therefore, the authors question its validity for introduction into clinical practice. Similarly, its added value has been questioned in patients with pyelonephritis (40).

Despite the somewhat controversial findings about its validity, presepsin might be an effective biomarker for the timely diagnosis of sepsis, particularly due to its early elevation. Besides diagnosis, it might prove useful for monitoring therapy effectiveness and it might serve as an aid in prognosis. It is important to note that its measurement can be performed quickly and easily (41) as a POC test; however, as it is still not widely available on common laboratory platforms, its utility might be hampered. Along with, or –as a better scenario for healthcare resources– instead of other diagnostic inflammatory markers, presepsin might therefore get its

place in the septic patient care as a routine laboratory marker used to facilitate current diagnostic strategies, particularly for early recognition of sepsis within the first few hours, which nowadays still represents a diagnostic challenge.

CONCLUSION

So far in clinical practice no single, optimal biochemical marker is available to confirm or exclude the diagnosis of severe infection within the clinically required time frame. Therefore the diagnosis has to include consideration of all important signs of infection. The quest for an ideal sepsis biomarker is still going on. The most reliable one according to current knowledge is still PCT, while the emerging and promising markers such as presepsin still lay in waiting to be unequivocally proven as more useful and effective.

REFERENCES

1. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock. Crit Care Med 2016;45(3):486-552.

2. Hoeboer SH, van der Geest PJ, Nieboer D, et al. The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. Clin Microbiol Infect. 2015;21:474-481.

3. Wu J, Hu L, Zhang H, Wu F, He T. Accuracy of presepsin in sepsis diagnosis: a systematic review and meta-analysis. PloS One 2015;10(7) :e0133057. doi: 10.1371

4. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv Immunol 1983;34:141–212.

5. Tillett WS, Francis TJr. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J Exp Med 1930;52:561-571.

6. Pepys MB, Hirschfeld GM. C-reactive protein: a critical update. J Clin Invest 2003; 111(12):1805–12.

7. Sasaki K, Fujita I, Hamasaki Y, Miyazaki S. Differentiating between bacterial and viral infection by measuring both C-reactive protein and 2'-5'-oligoadenylate synthetase as inflammatory markers. J Infect Chemother 2002;8(1):76-80.

8. Marková M, Brodská H, Malíčková K, Válková V, Cetkovský P, Kolář M, Haluzík M. Substantially elevated C-reactive protein (CRP), together with low levels of procalcitonin (PCT), contributes to diagnosis of fungal infection in immunocompromised patients. Support Care Cancer 2013;21(10):2733-42.

9. Galez D, Dodig S, Raos M, NogaloB. CRP in children with asthma and allergic rhinitis. Biochem Med 2006;16(2):163-169.

10. Lippi G, Favaloro EJ, Montagnana M, Franchini M. C-reactive protein and venous thromboembolism: causal or casual association? Clin Chem Lab Med 2010;48(12):1693-701.

11. Barauskas G, Svagzdys S, Maleckas A. C-reactive protein in early prediction of pancreatic necrosis. Medicina (Kaunas) 2004;40(2):135-40.

12. Neumaier M, Metak G, Scherer MA. C-reactive protein as a parameter of surgical trauma: CRP response after different types of surgery in 349 hip fractures. Acta Orthop 2006;77(5):788-90.

13. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit Rev Clin Lab Sci 2011;48(4):155-70.

14. Ticinesi A, Lauretani F, Nouvenne A, Porro E, Fanelli G, Maggio M, Meschi T. C-reactive protein (CRP) measurement in geriatric patients hospitalized for acute infection. Eur J Intern Med 2017;37:7–12.

15. Gabay C, Kushner I. A cute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54.

16. Ng PC, Lam HS. Diagnostic markers for neonatal sepsis. Curr Opin Pediatr 2006;18:125–31.

17. Rimac V. Procalcitonin - potential, limitations and availability. Signa Vitae 2015;10(suppl 1): 84-86.

18. Meisner M. Update on procalcitonin measurements. Ann Lab Med 2014;34(4):263-273.

19. Procalcitonin new findings relating to synthesis, biochemistry and function of procalcitonin in infection and sepsis diagnosis. Available at: http://www.brahms.de Accessed: 3 April 2017.

20. Sager R, Kutz A, Mueller B, Schuetzcorresponding P. Procalcitonin-guided diagnosis and antibiotic stewardship revisited. BMC Medicine 2017,15:15.

21. The Royal College of Pathologists, The Association for Clinical Biochemistry and Laboratory Medicine, The Institute of Biomedical Science. National minimum retesting intervals in pathology: A final report detailing consensus recommendations for minimum retesting

intervals for use in pathology. Available at: www.rcpath.org Accessed: 3 April 2017.

22. Hausfater P, Garric S, Ben Ayed S, Rosenheim M, Bernard M, Riou B. Usefulness of procalcitonin as a marker of systemic infection in emergency department patients: a prospective study. Clin Infect Dis 2002;34(7):895-901.

23. Schuetz P, Albrich W, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. BMC Medicine 2011,9:107.

24. Bufler P, Stiegler G, Schuchmann M, Hess S, Krüger C, Stelter F, Eckerskorn C, Schütt C, Engelmann H. Soluble lipopolysaccharide receptor (CD14) is released via two different mechanisms from human monocytes and CD14 transfectants. Eur J Immunol 1995;25(2):604-10.

25. Zanoni I, Granucci F. Role of CD14 in host protection against infections and in metabolism regulation. Front Cell Infect Microbiol 2013;3:32.

26. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology [Internet]. 7th ed. Philadelphia. Elsevier Saunders, eBooks. Chapter 4 – Innate Immunity.[cited 2017April09];p.62-63.

27. Labeta MO, Durieux JJ, Fernandez N, Herrmann R, Ferrara P. Release from a human monocyte-like cell line of two different soluble forms of the lipopolysaccharide receptor, CD14. Eur J Immunol 1993;23(9):2144-51.

28. Chenevier-Gobeaux C, Borderie D, Weiss N, Mallet-Coste T, Claessens Y-E. Presepsin (sCD14-ST), an innate immune response marker in sepsis. Clin Chim Acta 2015;23:450:97-103.

29. Le-Barillec K, Si-Tahar M, Balloy V, Chignard M. Proteolysis of monocyte CD14 by human leukocyte elastase inhibits lipopolysaccharide-mediated cell activation. J Clin Invest 1999;103:1039-1046.

30. Liu S, Khemlani LS, Shapiro RA, Johnson ML, Liu K, Geller DA, et al. Expression of CD14 by hepatocytes: up-regulation by cytokines during endotoxemia. Infect Immun 1998 Nov;66(11):5089-5098.

31. Chenevier-Gobeaux C, Bardet V, Poupet H, Poyart C, Borderie D, Claessens Y-E. Presepsin (sCD14-ST) secretion and kinetics by peripheral blood mononuclear cells and monocytic THP-1 cell line. Ann Biol Clin 2016;74(1):93-7.

32. Zhang J, Hu Z-D, Song J, Shao J. Diagnostic value of presepsin for sepsis: a systematic review and meta-analysis. Medicine 2015;94(47):e2158.

33. Endo S, Suzuki Y, Takahashi G, Shozushima T, Ishikura H, Murai A, Nishida T, Irie Y, Miura M, Iguchi H, Fukui Y, Tanaka K, Nojima T, Okamura Y. Usefulness of presepsin in the diagnosis of sepsis in a multicenter prospective study. J Infect Chemother 2012;18(6):891-7.

34. Masson S, Caironi P, Spanuth E, Thomae R, Panigada M, Sangiorgi G, Fumagalli R, Mauri T, Isgrò S, Fanizza C, Romero M, Tognoni G, Latini R, Gattinoni L; ALBIOS Study Investigators. Presepsin (soluble CD14 subtype) and procalcitonin levels for mortality prediction in sepsis: data from the Albumin Italian Outcome Sepsis trial. Criti Care 2014;18(1): R6.

35. Liu B, Chen Y-X, Yin Q, Zhao Y-Z, Li C-S. Diagnostic value and prognostic evaluation of Presepsin for sepsis in an emergency department. Criti Care 2013;17(5):R244.

36. Carpio R, Zapata J, Spanuth E, Hess G. Utility of presepsin (sCD14-ST) as a diagnostic and prognostic marker of sepsis in the emergency department. Clin Chim Acta 2015;450:169-175.

37. Kotera A, Sagishima K, Tashiro T, Niimori D, Kamohara H, Kinoshita Y. A validation of presepsin levels in kidney dysfunction patients: four case reports. J Intensive Care 2014;2(1):63.

38. Nakamura Y, Ishikura H, Nishida T, Kawano Y, Yuge R, Ichiki, R, Murai A. Usefulness of presepsin in the diagnosis of sepsis in patients with or without acute kidney injury. BMC Anesthesiol 2014;14(1):88.

39. de Guadiana Romualdo DG, Torrella PE, Acebes SR, Oton MD, Sanchez RJ, et al. Diagnostic accuracy of presepsin (sCD14-ST) as a biomarker of infection and sepsis in the emergency department. Clin Chim Acta 2017;464:6-11.

40. Claessens YE, Trabattoni E, Grabar S, Quinquis L, Der Sahakian G, Anselmo M, Schmidt J, de la Coussaye JE, Plaisance P, Casalino E, Potel G, Lecomte F, Borderie D, Chenevier-Gobeaux C. Plasmatic presepsin (sCD14-ST) concentrations in acute pyelonephritis in adult patients. Clin Chim Acta 2017;464:182-188.

41. Okamura Y, Yokoi H. Development of a point-of-care assay system for measurement of presepsin (sCD14-ST). Clin Chim Acta 2011;412(23):2157-2161.