

SEPSIS BIOMARKERS. PAST, PRESENT AND FUTURE

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Manuscript received: June 2014

Abstract

Sepsis is the immunological response of systemic inflammation to bacterial, fungal or viral infection. An early diagnosis of sepsis is essential for the successful management of this condition; it allows the prompt administration of appropriate antibiotic regimens and avoidance of unnecessary interventions. A sepsis biomarker must be able to differentiate between bacterial and viral infection, to indicate the severity of infection, to guide the therapy and also must be available in a timely and cost-effective manner. Over the time, different biomarkers have been used for diagnosis of sepsis and monitoring of its treatment. Procalcitonin and C reactive protein are probably the most widely used biochemical parameters for diagnosis and management of patients with sepsis, together with lactate. TNF, IL-1 β and IL-6 are cytokines that act as mediators of the immunological response to infection and could be potentially useful as biomarkers of sepsis.

Rezumat

Sepsis-ul este răspunsul imunologic de inflamație sistemică al organismului la infecție, care poate fi bacteriană, fungică sau virală. Un diagnostic precoce este esențial pentru managementul optim al sepsis-ului, deoarece permite administrarea promptă a tratamentului cu antibiotice adecvate și evitarea intervențiilor care nu sunt necesare. Un *biomarker* de sepsis trebuie să fie capabil să diferențieze o infecție bacteriană de una virală, să indice severitatea infecției, să ghideze terapia, să fie disponibil rapid și cost-eficient. De-a lungul timpului au fost utilizați diferiți biomarkeri pentru diagnosticul sepsis-ului și monitorizarea tratamentului. Procalcitonina și proteina C reactivă sunt probabil cei mai folosiți parametri biochimici pentru diagnosticul și *management*-ul pacienților cu sepsis, împreună cu lactat-ul. TNF, IL-1 β și IL-6 sunt citokine care acționează ca mediatori ai răspunsului imunologic la infecție și care ar putea fi utilizate ca biomarkeri de sepsis.

Keywords: sepsis, biomarkers, procalcitonin, lactate

Introduction

Sepsis is the immunological response of systemic inflammation to bacterial, fungal or viral infection. Nowadays sepsis is a growing healthcare problem due to very high costs associated with its treatment and the relatively high mortality rate. In developing countries, sepsis affects up to 30 of every 1000 live births [1, 2] and passes the pressure of high-costs on the healthcare systems. Because the elderlies are at increased risk for sepsis, it is likely that sepsis will become an even greater problem as the population ages. In clinical practice, to establish the diagnosis of sepsis with high sensitivity and specificity may be difficult, due to unspecific manifestations and biochemical similarities to non-infectious systemic inflammatory conditions. An early diagnosis of sepsis is essential for the successful management of this condition; it allows the prompt administration of appropriate antibiotic regimens and the avoidance of unnecessary diagnostic and therapeutic interventions. Furthermore, administration of appropriate antibiotics regimens prevents the

appearance of antibiotic resistance and allows the reduction of bacterial resistance, fungal overgrowth, *Clostridium difficile* infection and all other complications of antibiotherapy, like hepatic or renal dysfunction. In critically ill patients, sepsis is a common problem. A high proportion of critically ill patients have a systemic inflammatory response syndrome (SIRS) which has to be differentiated from sepsis. All these considerations explain the increasing interest and debate regarding the use of biomarkers for the diagnosis of sepsis.

SIRS is the physiologic response to an abnormal insult (sepsis, trauma, burn, pancreatitis etc.). The diagnosis of SIRS can be established if > 2 of the following criteria are met [3]:

- temperature dysregulation: > 38°C or < 36°C;
- tachycardia: heart rate > 90 bpm;
- tachypnea: respiratory rate > 20/min, or pCO₂ < 32 mmHg or the patient requires mechanical ventilation;
- white blood cells dysregulation: > 12000/mm³ or < 4000/mm³ or > 10% bands.

Sepsis is SIRS due to infection, either confirmed or a strong suspicion and altered variables: general, inflammatory, hemodynamic; organ dysfunction and/or tissue perfusion [3]. Severe sepsis is sepsis-induced tissue hypoperfusion or organ dysfunction sepsis with, at least, one acute organ dysfunction (sepsis-induced), evidence of tissue hypoperfusion or hypotension or abnormal serum lactate level or oliguria [3]. Septic shock is sepsis-induced hypotension, despite adequate fluid resuscitation, and requiring vasopressor support [3].

BIOMARKERS OF SEPSIS

To be clinically useful, a sepsis biomarker needs to provide additional information to that already available from history, clinical examination and laboratory investigations. A sepsis biomarker must be able to differentiate between bacterial and viral infection, to indicate the severity of infection, to be able to guide the therapy and also must be available in a timely and cost-effective manner. The ideal sepsis biomarker must have a single, reliable cut-off value, unaffected by acute illness or chronic disease. In clinical practice, biomarkers are needed for diagnosis (to identify patients in whom antimicrobial therapy is likely to be beneficial), prognosis (to provide estimates of patient risk for outcomes), assessing severity of illness (may be useful as a variable in organisation of studies and clinical trials) and monitoring the response to therapy by serial measurements.

Over the time, different biomarkers have been used for diagnosis of sepsis and monitoring of treatment. In the 1980s the initial focus was on the early hyper-inflammatory phase and high-dose corticosteroids were an important component of sepsis treatment [4]. Tumour necrosis factor (TNF), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), three pro-inflammatory cytokines that produce SIRS, as well as C-reactive protein (CRP), were investigated as potential biomarkers. In the 1990s, different studies discovered that the levels of procalcitonin (PCT), a precursor of the hormone calcitonin, were elevated in patients with bacterial infections and procalcitonin started to be used as a potential biomarker [5]. In 2003, elevations of both CRP and PCT were added to the updated definition of sepsis. During the last decade, serum lactate has been used for guiding therapy of severe sepsis and septic shock [6]. No single biomarker of sepsis is ideal, but many of them have proven the utility in identifying critically ill patients who are at high risk and who need to be diagnosed as soon as possible.

TNF, IL-1 β and IL-6 are cytokines that act as mediators of the immunological response to infection and could be potentially useful as biomarkers of sepsis [7]. IL-6 has been extensively

studied for its potential clinical uses, especially in diagnosis and management of autoimmune rheumatic disorders. IL-6 is not specific for sepsis, its role as a biomarker of sepsis may be prognostic, not diagnostic. A number of studies have demonstrated that increased levels of IL-6 in septic patients are associated with increased mortality [8, 9]. Chemokines are another group of cytokines which can be used as biomarkers, due to their role in organizing the adaptive immune system. Chemokine IL-8 was used for the diagnosis of sepsis [10]. Another chemokine, monocyte chemo-attractant protein (MCP-1) was used as predictor for sepsis mortality [11].

Only a few biomarkers have been integrated in clinical practice, PCT and CRP being the exceptions. PCT and CRP are probably the most widely used biochemical parameters for diagnosis and management of patients with sepsis, together with lactate. CRP has been validated as a biomarker of inflammation and infection for a long time. It is part of a group of acute phase reactants. Its role in acute inflammatory processes is not clearly understood. The level of CRP increases much more than other acute phase reactants, during acute inflammation, being used in clinical practice for a long time as an indicator of inflammatory or infectious diseases. More recently, CRP was used as a biomarker of inflammation associated with atherosclerosis and cardiovascular diseases [12]. CRP has a low specificity as a biomarker of sepsis in adult patients, its main clinical use being for screening of the early onset of sepsis in new-borns (during the first 24 hours of life), because it has a very high sensitivity in this context [13]. CRP is used also for monitoring of post-operative sepsis in surgical patients. CRP level rises slowly and peaks 36 h after an endotoxin infection [14].

PCT was described for the first time more than 30 years ago. It is a 116 aminoacid protein with a molecular weight of 14.5 kDa [5]. Nowadays, PCT has become a widespread biomarker for sepsis. PCT is synthesized in the thyroid C cells, being a precursor of the hormone calcitonin. The normal level of PCT in the serum is low, less than, 0.1 ng/mL [5]. When bacterial infections occur, PCT is further produced in extrathyroidal neuroendocrine tissues, as a response to proinflammatory stimulation. PCT is very rapidly secreted in the blood and becomes detectable in the plasma 2 hours after the onset of infection, reaching the peak within 12-24 hours. The highest level of PCT appears in bacteriemic infections; in viral or intracellular bacterial infections, the increase of PCT is negligible. Moreover, bacteriemias with Gram-negative germs cause higher PCT rises than Gram-positive germs [15]. In bacterial infections, the level of PCT rises in the serum 4 hours after the

beginning of systemic infection, and reaches a peak after 8-24 hours, earlier than the CRP level. Numerous studies have compared the usefulness of PCT with the usefulness of CRP for the diagnosis of sepsis. Some studies found that PCT is more sensitive and specific than CRP for the diagnosis of bacterial infections [16]. However, this is still a matter of debate and there is no consensus between the experts regarding the diagnostic specificity and sensitivity. In 2006, a large meta-analysis of 49 studies comparing PCT and CRP for the diagnosis of sepsis was published [17]. The conclusion of this meta-analysis was that the global odds ratio for PCT (14.69) was significantly higher than that for CRP (5.43). In 2010, another meta-analysis of 9 studies comparing PCT and CRP for the diagnosis of late-onset neonatal sepsis was published [18]; four of the analysed studies, that required documentation of infection, have found a higher pooled sensitivity of PCT as compared to CRP (72% *versus* 55%, $p < 0.05$) [18]. Also, pooled specificity and odds ratio were higher for PCT, even without statistical significance. The other 5 studies that did not require evidence of infection have found a higher specificity for PCT, but without statistical significance [18]. A systematic review reported a low diagnostic performance of PCT for identifying sepsis, with a mean sensitivity and specificity of 71% (95% confidence interval 67-76%) and the area under the receiver operator characteristic curve of 0.78 (95% confidence interval 0.73-0.83); an ideal biomarker should offer a value greater than 0.90 [19].

Although PCT is less likely than CRP to be increased in systemic inflammation without sepsis, PCT is not very specific for infection as it was believed. Other clinical conditions which induce transiently increases of PCT in the absence of infection are renal diseases, trauma, surgery, pancreatitis [20]. This is the reason why a definite cut-off value for the diagnosis of sepsis is not established. However, PCT is important for ruling out sepsis due to a high negative predictive value [21]. Most of the studies performed in intensive care patients have searched the value of biomarkers to limit the duration of antibiotic therapy, and only a few have examined its initiation. The initiation of antibiotic therapy in intensive care patients have been assessed in only 2 studies that used a PCT-based algorithm [22, 23]. In a multicentre study published in 2010, the risk reduction of initiating antibiotic therapy using PCT varied between 5% and 13% across centres [22]. In another study published in 2012 there was no difference in the rate of initiation of therapy between the control group and the PCT-based group, where antibiotics were strongly discouraged if PCT was lower than 0.25 ng/mL and strongly recommended if PCT was

higher than 1 ng/mL [22]. Although changes in PCT levels might be useful for the initiation of antibiotic therapy in intensive care patients suspected of intensive care unit (ICU) -acquired infection, currently data are insufficient to make a clear recommendation.

Elevated levels of IL-6 a pro-inflammatory cytokine, leads to increasing production of polymorphonuclear leucocytes (PMNs) in the bone marrow. Circulating PMNs in septic patients are also activated by cytokines, with changes in their appearance. Cluster of differentiation 64 (CD64), a high-affinity receptor for the Fc segment of the immunoglobulin molecule, has been analysed in a number of studies as a biomarker of sepsis. A small study has found that increased CD64 identified sepsis in intensive care patients with a sensitivity similar to PCT, but with a better specificity [24]. A more recent prospective study of CD64, on over 700 infants, was performed in a neonatal intensive care unit [25]. The majority of cases were late-onset sepsis, in this population increased CD64 demonstrating a sensitivity of 75% and a specificity of 77% for the diagnosis of sepsis [25]. It is probable that CD64 will be accepted in the future as a biomarker of sepsis in the neonate population.

Integrin CD11b is also increased in bacterial infections, some authors proposing the use of both CD64 and CD11b for the diagnosis of sepsis. Other neutrophil activation markers have been studied in sepsis, including TREM-1 (triggering receptor expressed on myeloid cells-1); the studies with TREM-1 have failed to demonstrate the ability of TREM-1 to diagnose sepsis. A meta-analysis of 11 studies on the diagnostic utility of TREM-1 found a pooled sensitivity and specificity of 79% (95% CI 65-89%) and 80% (95% CI 0.84-0.89), respectively, concluding that TREM-1 is not appropriate as a single biomarker for the diagnosis of sepsis [26].

Macrophage migration inhibitory factor (MIF) is released from macrophage and T cells. High levels of MIF were detected in patients with sepsis and septic shock [27, 28]. MIF serves as a general marker for systemic inflammation in septic and non-septic critical illness, but does not differentiate between infectious and non-infectious causes [29].

In patients with sepsis, there are a variety of laboratory tests used to diagnose end-organ dysfunction, some of these being included in physiological scoring systems, like APACHE (acute physiology and chronic health evaluation) or SOFA (sequential organ failure assessment). The most used biomarker for organ dysfunction is serum lactate. The majority of hospitals use lactate as a screening test for sepsis, with a cut-off value of 4.0 mmol/L [30, 31]. However, recent studies have

indicated that an intermediate level of lactate (2.0-4.0 mmol/L) may identify patients with significant risk [32]. Lactate clearance, a parameter used in trauma patients, was also used in septic patients. A low lactate clearance has been demonstrated to correlate with elevations of PCT and IL-6 as predictors of sepsis in trauma patients [33].

Conclusions

In conclusion, there is not one single biomarker nowadays that can be used for the accurate diagnosis of sepsis. The future of sepsis diagnosis seems to be a panel of biomarkers. The best panel of biomarkers for the diagnosis of sepsis probably will include both pro-inflammatory and anti-inflammatory markers. Two studies have analysed the combination of pro-inflammatory and anti-inflammatory markers. One study, published in 2012, simultaneously measured almost 20 different cytokines in approximately 30 patients with severe sepsis [33]. IL-6 and IL-8 (pro-inflammatory) as well as IL-10 and MCP-1 (anti-inflammatory) were increased in patients who died (mortality rate 59%); a combined score was more predictive than one cytokine [34]. Another study assessed the monocyte human leukocyte antigen-DR (HLA-DR) expression compared with IL-6 and IL-10 levels in 100 trauma patients admitted in the intensive care unit, from which 37% developed sepsis [34]. In this study, a combination of a lack of increase in HLA-DR expression and increased IL-6 levels was a powerful predictor for the development of sepsis [35]. The right combination of multiple biomarkers for the diagnosis of sepsis remains to be identified by future studies, in order to guide the appropriate treatment of sepsis and to improve prognosis of these patients.

References

- Cheng B.L., Xie G.H., Yao S.L., Epidemiology of severe sepsis in critically ill surgical patients in ten university hospitals in China. *Crit. Care Med.*, 2007; 35: 2538-2546.
- Sankar M.J., Agarwal R., Deorari A.K., Paul V.K., Sepsis in the newborn. *Indian J. Pediatr.*, 2008; 75(3): 261-266.
- Van Gestel A., Bakker J., Veraart C.P., van Hout B.A., Prevalence and incidence of severe sepsis in Dutch intensive care units. *Critical Care*, 2004; 8: R153-R162.
- Bone R.C., Fisher J.C.Jr., Clemmer T.P., A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. *N. Eng. J. Med.*, 1987; 317: 653-658.
- Karzai W., Oberhoffer M., Meier-Hellmann A., Reinhart K., Procalcitonin: a new indicator of the systemic response to severe infections. *Infection*, 1997; 25: 329-334.
- Rivers E., Nguyen B., Havstad S., Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N. Engl. J. Med.*, 2001; 345: 1368-1377.
- Mohamad M.A., Mitrea N., Nicolae A.C., Constantinescu M.Z., Dragoi C.M., Arsene A.L., Barbu C.G., The dynamics of adiponectin and leptin on metabolic syndrome patients and age matched healthy subjects. *Farmacia*, 2014; 62(3): 532-545.
- Petilla V., Hynninen M., Takkunen O., Predictive value of procalcitonin and interleukin 6 in critically ill patients with suspected sepsis. *Intensive Care Med.*, 2002; 28: 1220-1225.
- Panacek E.A., Marshall J.C., Albertson T.E., Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody Fab'2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. *Crit. Care Med.*, 2004; 32: 2173-2182.
- Stryjewski G.R., Nylen E.S., Bell M.J., Interleukin-6, interleukin-8, and a rapid and sensitive assay for calcitonin precursors for the determination of bacterial sepsis in febrile neutropenic children. *Pediatr. Crit. Care Med.*, 2005; 6: 129-135.
- Bozza F.A., Salluh J.I., Japiassu A.M., Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. *Crit. Care*, 2007; 11: R49.
- Benzaquen L.R., Yu H., Rifai N., High-sensitivity C-reactive protein: an emerging role in cardiovascular risk assessment. *Crit. Rev. Clin. Lab. Sci.*, 2002; 39: 459-497.
- Hofer N., Zacharias E., Muller W., Resch B., An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. *Neonatology*, 2012; 102: 25-36.
- Shehabi Y., Seppelt I., Pro/con debate: is procalcitonin useful for guiding antibiotic decision making in critically ill patients? *Crit. Care*, 2008; 12: 211-216.
- Charles P.E., Ladoire S., Aho S., Serum procalcitonin elevation in critically ill patients at the onset of bacteremia caused by either Gram-negative or Gram-positive bacteria. *BMC Infect. Dis.*, 2008; 8: 38.
- Simon L., Gauvin F., Amre D.K., Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin. Infect. Dis.*, 2004; 39: 206-217.
- Uzzan B., Cohen R., Nicolas P., Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. *Crit. Care Med.*, 2006; 34: 1996-2003.
- Yu Z., Liu J., Sun Q., The accuracy of the procalcitonin test for the diagnosis of neonatal sepsis: a meta-analysis. *Scand. J. Infect. Dis.*, 2010; 42: 723-733.
- Tang B.M., Eslick G.D., Craig J.C., Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and metaanalysis. *Lancet Infect. Dis.*, 2007; 7: 210-217.
- Billeter A., Turina M., Seifert B., Early serum procalcitonin, interleukin-6, and 24-hour lactate clearance: useful indicators of septic infections in

- severely traumatized patients. *World J. Surg.*, 2009; 33: 558-566.
21. Chan Y.L., Tseng C.P., Tsay P.K., Procalcitonin as a marker of bacterial infection in the emergency department: an observational study. *Crit. Care*, 2004; 8(1): R12-20.
22. Bouadma L., Luyt C.E., Tubach F., Use of procalcitonin to reduce patients exposure to antibiotics in intensive care units (PROBATA trial: a multicentre randomised controlled trial). *Lancet*, 2010; 375: 463-474.
23. Cucu A., Nica M., Ceausu E., Cioran N., Antimicrobial resistance profile in infectious disease hospital intensive care unit. *Farmacia*, 2014; 62(4): 767-776.
24. Cardelli P., Ferraironi M., Amodeo R., Evaluation of neutrophil CD64 expression and procalcitonin as useful markers in early diagnosis of sepsis. *Int. J. Immunopathol. Pharmacol.*, 2008; 21: 43-49.
25. Streimish I., Bizzarro M., Northrup V., Neutrophil CD64 as a diagnostic marker in neonatal sepsis. *Pediatr. Infect. Dis. J.*, 2012; 31: 777-781.
26. Wu Y., Wang F., Fan X., Accuracy of plasma sTREM-1 for sepsis diagnosis in systemic inflammatory patients: a systematic review and meta-analysis. *Crit. Care*, 2012; 16: R229.
27. Calandra T., Bernhagen J., Mitchell R.A., The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J. Exp. Med.*, 1994; 179: 1895-1902.
28. Calandra T., Macrophage migration inhibitory factor and host innate immune responses to microbes. *Scand. J. Infect. Dis.*, 2003; 35: 573-576.
29. Lehmann L.E., Novender U., Schroeder S., Plasma levels of macrophage migration inhibitory factor are elevated in patients with severe sepsis. *Intensive Care Med.*, 2001; 27: 1412-1415.
30. Trzeciak S., Chansky M.E., Dellinger P.R., Operationalizing the use of serum lactate measurement for identifying high risk of death in a clinical practice algorithm for suspected severe sepsis. *Acad. Emerg. Med.*, 2006; 13: 150-151.
31. Drumheller B., Goyal M., Pines J., Elevated point-of-care lactate at triage is predictive of admission among sepsis patients presenting to the emergency department. *Ann. Emerg. Med.*, 2007; 50: S21-S22.
32. Mikkelsen M.E., Miltiades A.N., Gaieski D.F., Serum lactate is associated with mortality in severe sepsis independent of organ failure or shock. *Crit. Care Med.*, 2009; 37: 1670-1677.
33. Billeter A., Turina M., Seifert B., Early serum procalcitonin, interleukin-6, and 24-hour lactate clearance: useful indicators of septic infections in severely traumatized patients. *World J. Surg.*, 2009; 33: 558-566.
34. Andaluz-Ojeda D., Bobillo F., Iglesias V., A combined score of pro- and anti-inflammatory interleukins improves mortality prediction in severe sepsis. *Cytokine*, 2012; 57: 332-336.
35. Gouel-Cheron A., Allaouchiche B., Guignant C., AzuRea Group. Early interleukin-6 and slope of monocyte human leukocyte antigen-DR: a powerful association to predict the development of sepsis after major trauma. *PLoS ONE*, 2012; 7: e33095.