Biomarkers for sepsis: past, present and future

Giuseppe Chesi, Natale Vazzana, Claudio Giumelli

Department of Internal Medicine, C. Magati Hospital, Scandiano (RE), Italy

ABSTRACT

Sepsis is a complication of severe infection associated with high mortality and open diagnostic issues. Treatment strategies are currently limited and essentially based on prompt recognition, aggressive supportive care and early antibiotic treatment. In the last years, extensive antibiotic use has led to selection, propagation and maintenance of drug-resistant microorganisms. In this context, several biomarkers have been proposed for early identification, etiological definition, risk stratification and improving antibiotic stewardship in septic patient care. Among these molecules, only a few have been translated into clinical practice. In this review, we provided an updated overview of established and developing biomarkers for sepsis, focusing our attention on their pathophysiological profile, advantages, limitations, and appropriate evidence-based use in the management of septic patients.

Sepsis: definition and diagnosis

Depending on the characteristics of the patient, pathogen profile and setting of development, sepsis could be a rapidly life-threatening condition.[1] A high suspicion index is crucial for appropriate investigations and prompt diagnosis and management. Sepsis is a systemic inflammatory response syndrome (SIRS) caused by infection.[2] Because SIRS is not specific for sepsis and can be caused by several non-infective conditions, extensive research has been performed to identify rapid biomarkers for differentiating sepsis from other causes of SIRS, in order to limit the inappropriate use of broad-spectrum antibiotics and its consequent deleterious effect on bacterial ecosystem.[3]

Early management of severe sepsis and usefulness of biomarkers

In the presence of high clinical suspicion of sepsis,

especially in the presence of criteria of severe disease or septic shock, early supportive therapy - including aggressive fluid resuscitation to sustain adequate perfusion and prevent organ dysfunction - and empiric antibiotic therapy should be immediately instituted, tailored on the characteristics of the patient and the clinical setting.[4] In critically ill patients, microbiological tests - preferably obtained before antibiotic administration - are more important than diagnostic biomarkers as they can guide adjustments to initial therapy.[5] In these situations, biomarkers may rather have a role for response monitoring and prognosis.[6] In case of persistent negative results of certain biomarkers, the diagnosis of sepsis should be reconsidered, especially in the absence of significant improvement after appropriate antibiotic treatment. There is also increasing evidence that biomarkers can guide the duration of antibiotic therapy, promoting shorter duration of treatment in some patients.[7] Moreover, in uncertain and non-critical situations such as probable or possible sepsis, the use of biomarkers may have a crucial role for confirming or excluding the diagnosis, thus avoiding the institution of inappropriate antibiotic treatments.[8]

Biomarkers: appropriate use in the specific clinical setting

As for other diagnostic tests, biomarkers of sepsis should be always framed within the specific clinical setting of the patient and ordered only if they may provide additional information. An adequate knowledge of their pathophysiological profile - including the release kinetics, causes of false-positive or -negative results, and serum clearance time - is also required for appropriate and focused clinical use.[9] Without these preconditions, the use of biomarkers could be confounding and misleading. In the present review, we

critically discuss the role of biomarkers for sepsis diagnosis and risk stratification, focusing our attention on advantages, limitations, clinical evidence of cost-effectiveness and future perspective.

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed, Web of Science, the Cochrane Library, and references from relevant articles with the search terms *biomarker*, *sepsis*, *procalcitonin*, *C-reactive protein*, and/or *antibiotic therapy*. Only articles published in English between January 1990, and March 2016, were included. Publications were selected for review based on original research, randomized controlled trials, and meta-analyses and evidence-based reviews.

Searching for biomarkers: *one, no one and one hundred thousand*

In the last 10-years, approximately 170 potential biomarkers for sepsis have been identified and proposed for clinical use, thus underlining the unmet clinical need of practical tools to improve the diagnosis and management of this serious condition.[10] Nevertheless, none of the currently available or experimental biomarkers fulfils all the characteristics required of an ideal biomarker (Table 1). Among these molecules, only a few are currently used in clinical practice. Many other biomarkers are still in exploratory phases and their investigation also provides interesting insights into the pathophysiological cascade of sepsis-related SIRS.[11] In the following section we review the preclinical and clinical development of currently available biomarkers, focusing our attention on their strength and weakness and trying to extrapolate their appropriate use in the clinical setting.

Biomarkers in sepsis: the past

The history of biomarkers in sepsis can be briefly summarized, as there were only few indicators of in-fection and/or SIRS until the end of the 90's. The clinical suspicion of sepsis or other infections was supported only by nonspecific markers such as the increase in erythrocyte sedimentation rate and neutrophil count. Microbiology culture tests were also less accurate and slower than today. Nevertheless, in past microbiological epidemiology there was only a fraction of the current number of virulent and resistant pathogens that have been selected by excessive and often irrational use of antibiotics, especially within hospital and nursing institutes.

Biomarkers in sepsis: the present

The currently available sepsis biomarkers are essentially two - C-reactive protein (CRP) and procalcitonin (PCT). These tests are widely - but not universally - used in the clinical practice for improving sepsis diagnosis and monitoring.[12] Beyond these true biomarkers, several other laboratory tests can be helpful for septic patient management, including blood cell count to evaluate the presence of leukocytosis or leukopenia, and - more important - serum lactate levels as a marker of tissue hypoperfusion.[13] All these markers can be used synergistically with bedside ultrasonographic evaluation of inferior vena cava diameter and collapsibility index in order to support the initial diagnosis and to estimate adequate fluid support and response monitoring.[14]

C-reactive protein

CRP has a long history as it was first detected by Tillet and Francis in 1930 in the serum of patients with pneumococcal pneumonia.[15] It was subsequently recognised as an acute-phase protein and proposed as a clinical marker of inflammation independent of the underlining cause. CRP is a member of the pentraxin family of calcium-dependent ligand-binding plasma proteins. It is synthesized and released by hepatocytes in response to cytokines, particularly interleukin (IL)-6. Its half-life is about 19 h and it is poorly affected by inter-

Table 1. Characteristics of the ideal biomarker for sepsis and relative clinical implications.

Characteristic	Clinical implications
High sensitivity	Low rate of false-negative results
High specificity	Low rate of false-positive results
Early release peak	Early diagnosis
Reflective of disease severity and course	Risk stratification, treatment decisions and response evaluation
Rapid, simple, inexpensive, and standardized assay	Readily available, reproducible and cost-effective
Limited renal or hepatic clearance	Useful in patients with sepsis-related organ dysfunctions

current pathophysiological circumstances. Following its release induced by specific cytokines, CRP itself acts as a direct inflammatory mediator in response of several pathophysiological triggers. In the clinical setting, the major limit of CRP is the lack of specificity, as it is constantly increased in SIRS, independent of the etiology, as well as in other inflammatory conditions such as disreactive or autoimmune diseases and cancer, thus it cannot be used as a diagnostic marker.[16] However, due to its short half-life, it may be helpful to monitor the course of certain disorders, including infections.[17] Some data suggest that CRP may be used for diagnostic purpose in combination with other biomarkers such as PCT.[18] Nevertheless, its positive predictive value for sepsis diagnosis remains limited even if higher threshold levels are chosen.[19] On the contrary, CRP has a high negative predictive value against sepsis, especially if measured few hours after symptoms onset.[20]

Procalcitonin

PCT has emerged as the most interesting biomarker for the diagnosis of infective diseases.[21] In recent years an increasing number of studies have investigated the use of PCT as a biomarker of bacterial disease, focusing on its impact on diagnostic performances and management of patients with several infective conditions.[22,23] The great interest on this molecule is due to its high specificity for bacterial infections, even though recent data have identified specific conditions associated with false-positive PCT increases.[24,25] PCT is a 116 amino-acid peptide precursor of the hormone calcitonin and is mainly released by the parafollicular C cells of the thyroid. Under normal circumstances, virtually all released PCT is cleaved into calcitonin by a specific protease, thus very low or absent levels are detectable in serum.[26] PCT is rapidly released by several tissues and inflammatory cells in response to bacterial toxins and bacteria-specific proinflammatory mediators, and its cleavage is simultaneously inhibited; therefore, serum PCT levels may dramatically increase in patients with bacterial infections, thus representing a helpful biomarker for early diagnosis of sepsis in critically ill patients.[27] PCT has a favourable kinetics as diagnostic biomarker, with a rapid rise following its induction, and early peak levels (6-8 h after symptom onset). In addition, PCT shows a rapid decline following treatment or removal of the underlying trigger, with a half-life ranging from 25 to 45 h and little affected by renal function. Based on this profile, PCT use has been extended from a diagnostic purpose to patient monitoring and response evaluation. PCT levels may also have prognostic implication. In fact, elevated PCT concentrations and PCT non-clearance are strongly associated with all-cause mortality in septic patients.[28] It should be noted that a profound knowledge of PCT induction mechanisms and kinetics is required for its appropriate use in the clinical setting. For example, PCT determination should be required only after appropriate time - at least 6-8 h - from symptom onset. Moreover, clinicians should know that PCT peaks are quantitatively related to the degree of bacteremia, thus PCT can be unhelpful in localised or poorly bacteremic infections. Table 2 shows the most common cause of false-positive or false-negative test results. As stated before, patients with severe sepsis or septic shock should receive prompt empirical antibiotic treatment after blood culture has been obtained. Thus, the most appropriate clinical use of PCT is for the diagnosis and management of patients with possible or probable sepsis, particularly in the presence of respiratory tract infections.[29] In fact, in these non-critical situations, the decision to start antibiotic treatment can be delayed until the results of PCT and/or other biomarkers are available. Table 3 reports the PCT cut-

Table 2. Common causes of false-positive and false-negative procalcitonin test results.

False-positive results	False-negative results
Cancer (*e.g.*, medullary thyroid cancer, SCLC)	Early course of infection
Severe SIRS	Previous antibiotic therapy
End-stage renal disease	Immunocompromised host
Massive stress (trauma, burns, surgery, ARDS)	Localized infection or subacute course
Autoimmune disease (*e.g.*, vasculitis, Still disease)	
Prolonged, severe cardiogenic shock or organ perfusion abnormalities	
Acute coronary syndrome	
Malaria and some fungal infections	
Drugs (anti-lymphocyte globulins, alemtuzumab, IL-2)	

SCLC, small-cell lung cancer; SIRS, systemic inflammatory response syndrome; ARDS, acute respiratory distress syndrome; IL, interleukin.

off values for clinical decisions in lower respiratory tract infections and critically ill patients.[29-32] PCT may also be used for response monitoring and decision to discontinue antibiotic treatment. In fact, there is increasing evidence that PCT guidance can reduce the duration of antibiotic treatment for patients with bacterial infection. In the PRORATA trial, which included non-surgical patients in intensive care units, investigators were encouraged to discontinue antibiotics when PCT concentration was less than 80% of the peak concentration or an absolute concentration of less than 0.5 µg/L was reached.[30] A significant reduction in antibiotic treatment duration was obtained, but data on mortality were controversial.[31] Similar results were found in a subsequent meta-analysis of seven studies comprising a total of 1075 patients with severe sepsis or septic shock. More recently, the SAPS trial used the same decisional criterion of PRORATA in the same clinical setting and further confirmed that PCT-guided algorithms can reduce the duration of antibiotic treatment. Moreover, the PCT-guided group had a lower mortality than the standard-of-care group.[32] Although PCT shows a limited positive predictive value outside of the appropriate clinical setting, its negative predictive value is high, especially if persistently low levels are found in repeated measures, and it may help the clinician to consider conditions other than bacterial infections that cause fever or other symptoms. In conclusion, as recently discussed, PCT is the most important biomarker of sepsis currently available in the clinical setting but it should be handled with care and only with a profound knowledge of its kinetics and causes of false-positive and false-negative results.

Biomarkers: the future

At present, PCT and PCR are the only markers of sepsis routinely used in the clinical practice in Italy and in most of the developed countries. A major limit of these biomarkers is their relatively low positive predictive value and specificity. Research has then focused on novel tests with increased specificity, and a number of novel molecules have been identified and proposed for clinical use. However, only a few have the kinetic profile, specificity and diagnostic performances required for translation to the clinical setting. In the following section, we briefly review the characteristics of these molecules.

Cytokine biomarkers

As cytokines are key players in the inflammatory response, they are candidate biomarkers for sepsis. Among these, tumor necrosis factor (TNF)-α, IL-1β, and IL-6 have been tested for potential clinical use. These molecules show an early increase after inflammatory stimulation, rapid clearance and extremely high sensitivity. However, their specificity is too low to be

Table 3. Procalcitonin cut-off levels for clinical decisions.

Serum PCT level	Interpretation*
Suspected lower respiratory tract infection	
PCT <0.1 ng/mL	Very low likelihood for bacterial infection, antibiotic therapy strongly discouraged
PCT 0.1-0.25 ng/mL	Low likelihood for bacterial infection, antibiotic therapy discouraged
PCT 0.25-0.5 ng/mL	Intermediate likelihood for bacterial infection, antibiotic therapy should be considered, based on the stability of the patient's clinical condition
PCT ≥0.5 ng/mL	High likelihood for bacterial infection, antibiotic therapy encouraged
Suspected sepsis in critically ill patient	
PCT <0.25 ng/mL	Very low likelihood for bacterial infection, antibiotic therapy strongly discouraged
PCT 0.25-0.5 ng/mL	Low likelihood for bacterial infection, antibiotic therapy discouraged
PCT 0.5-1 ng/mL	Intermediate likelihood for bacterial infection, antibiotic therapy should be considered, based on the stability of the patient's clinical condition
PCT ≥1 ng/mL	High likelihood for bacterial infection, antibiotic therapy strongly encouraged
Response evaluation and duration of antibiotic treatment	
PCT decrease ≥90% of its peak value or PCT ≤0.25 ng/mL	Antibiotic strongly encouraged
PCT decrease ≥80% of its peak value or PCT ≤0.5 ng/mL	Antibiotic cessation encouraged
PCT decrease <80%	Antibiotic cessation discouraged
PCT stable or increased	Uncontrolled infection, consider changing or expanding antibiotic coverage or further diagnostic evaluation; increased risk of mortality

PCT, procalcitonin. *Excludes situations requiring immediate antibiotic treatment (*e.g.*, septic shock, purulent meningitis). *Data from ref.*[29-32]

useful as diagnostic tool. Levels of some cytokines were correlated with disease severity and development of organ dysfunction, making them potentially useful prognosis predictors. Nevertheless, their use is currently limited to research purpose and a translation to the clinical setting is unlikely in short term.

Cell surface markers and soluble receptors

Although still experimental and far from clinical application, this group comprises some of the most promising molecules that fulfil almost completely the characteristics required for an ideal biomarker. The biomarkers proposed and preliminarily tested include CD64, soluble triggering receptor expressed on myeloid cells (sTREM)-1, and soluble urokinase-type plasminogen activator receptor (suPAR).

CD64 is a membrane glycoprotein with increased expression on neutrophils in subjects with bacterial infections. CD64 has a relatively high specificity and a strong correlation was found between CD64 expression, positive blood culture and disease severity. Based on a pooled analysis of 13 studies, the sensitivity and specificity for CD64 expression on neutrophils and bacterial infection is 79% and 91%, respectively.[33] However, the methodological quality of these studies is relatively low and further investigations are needed.

sTREM-1 is a soluble form of TREM-1, a glycopeptide receptor upregulated on the surface of myeloid cells after bacterial infections. Based on available data, its sensitivity and specificity is comparable to currently used biomarkers, thus a translation in the clinical setting seems unlikely within the next few years.[34]

uPAR is a surface signalling receptor expressed on most leukocytes. Its soluble form (suPAR) is found in plasma and other biological fluids after the cleavage of the membrane receptor triggered inflammatory processes. Its performances as diagnostic biomarkers are limited, with less specificity and positive predictive value than currently used molecules.[35] However, circulating suPAR levels are significantly related to the severity of the inflammatory response and have superior prognostic power over other commonly used biological markers in sepsis as well as in other inflammatory conditions.[36]

Other biomarkers

Proadrenomedullin (proADM) is the mid-regional fragment derived from the cleavage of ADM, a 52-amino-acid peptide produced by the adrenal medulla. proADM is implicated in the pathogenesis of hypotension associated with severe sepsis. proADM showed an elevated power to predict localized bacterial infection and differentiate sepsis from SIRS in patients with hematologic malignancies.[37] Even though these data should be confirmed in other clinical setting, proADM appears to be a promising biomarker for sepsis diagnosis that may complement the performance of other markers.

Mirco-RNAs (miRNAs) are a recently discovered class of small, non-coding RNAs that regulate protein levels post-transcriptionally.[38] miRNAs are remarkably stable in the circulation and have been proposed as diagnostic biomarkers of numerous human conditions such as cancer and cardiovascular disease. Moreover, recent studies showed promising results supporting the role of single miRNA and/or multiple miRNAs panels (miRNA signatures) for diagnosis and prognosis of sepsis.[39,40]

Other biomarkers have several limitations that could limit their development and/or translation into the clinical setting. Among the molecules, the following should be cited: LBP (lipopolysaccharide binding protein), HMGB-1 (high mobility group box 1 protein), MIF (macrophage migration inhibitor factor), and angiopoietin.

Combinations of biomarkers

Recent studies showed that the limitations of single biomarker could be overcome through an integrated combination of clinical variables and laboratory markers, which appear more likely to be able to guide diagnosis or treatment, or assist in prognostication. Several models and scoring systems have been proposed, but the selection of variables that should be included in such scores remains a critical challenge. The *infection probability score* - which includes six different variables routinely available in septic patients such as temperature, heart rate, respiratory rate, white blood cell count, C-reactive protein, and Sequential Organ Failure Assessment score - is the most validated, simple and universally accessible,[41] even though its use should be limited to critical patients. More recently, a bioscore combining neutrophil CD64, PCT and sTREM-1 showed interesting diagnostic performances.[42] Similar results were obtained through a composite score of PCT, proADM, and TNF-α.[43] In addition, a high negative predictive value for sepsis was found by combining α-2 macroglobulin and PCT.[44] Though interesting, solid data supporting the routine application of these multimarker approaches are lacking. Therefore, it remains crucial that clinicians treating patients with sepsis have detailed knowledge of the characteristics, advantages and limitations of locally available biomarkers, as well as of their integrated use within appropriate decisional algorithms, flow-charts or scoring systems in order to improve the diagnostic performances, therapeutic appropriateness and patients' outcomes.

Conclusions

In the last years, the increased number of high-risk patients as well as the selection and propagation of multidrug-resistant organisms has raised new challenges in the management of sepsis. Advancing age increases the prevalence of frail patients with chronic conditions linked to increased risk of sepsis, which sometimes can be severe and difficult to diagnose. As for other diseases, the approach to patients with suspected sepsis should start from a thorough clinical assessment of disease probability and severity, in order to identify subjects who need aggressive resuscitation and early antibiotic treatment. Along with assessment of organ (dys)function, initial evaluation and risk stratification should include the measurement of inferior vena cava collapsibility index and serum lactate levels. Beside this, extensive researches have been carried out in order to identify biomarkers useful for diagnosis, definition of severity, management, and follow-up of sepsis. To date, although with some limitations, the most validated and clinically informative biomarker is PCT, preferably used in combination with C-reactive protein. These molecules provide useful information for diagnosis and prognosis in several clinical conditions, including patients with mild disease (probable or possible sepsis) as well as those with severe sepsis or septic shock. In addition, solid data support the use of PCT guidance to safely reduce and optimize the duration antibiotic therapy. Many other biomarkers are being investigated and tested in clinical studies. Nevertheless, further studies are needed before their use could be translated into the clinical setting.

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