Markers for Sepsis Diagnosis: What is Useful?

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Sepsis is a leading cause of death and the main cost driver in ICUs. Recent epidemiologic data showed that sepsis is the third frequent cause of death after coronary heart disease and myocardial infarction in Germany [1].

The first clinical signs of sepsis are usually unspecific (e.g., fever or leukocytosis). More specific symptoms, such as arterial hypotension or raised lactate, often indicate progression to organ dysfunction (severe sepsis) associated with an increased mortality rate of 35% to 70%. Early goal-directed therapy can reduce mortality significantly if initiated within 6 hours [2], further highlighting the need for reliable diagnostic tools. The requirements for an ideal sepsis marker are high sensitivity and specificity, easy handling, and low costs. The marker should be able to indicate the stages of the disease and the prognosis of the patient.

The inflammatory reaction, which is a key part of any healing process, consists of humoral, cellular, and molecular pathways. Changes in body temperature, leukocyte count, heart rate, blood pressure, and respiration rate are clinical signs of systemic inflammation. They are neither specific nor sensitive for sepsis and occur also in noninfectious states. Generalized inflammation with organ dysfunction (systemic inflammatory response syndrome) may also result from pancreatitis, major trauma, and burns. The American College of Chest Physicians/Society of Critical Care Medicine consensus criteria [3], which have helped to unify definitions, are not helpful for clinical differentiation at the bedside [4].

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A sepsis marker is useful only if it adds value to the physician’s clinical judgment. Ideally, an infection or sepsis marker should meet the following demands:

1. It should shorten the time to and improve the diagnosis
2. It should facilitate the differentiation between infectious and noninfectious causes of inflammation and its sequelae organ dysfunction or shock
3. It should allow the differentiation between underlying viral and bacterial infections
4. It should reflect the effectiveness of antimicrobial treatment and other measures of source control more accurately than conventional clinical and laboratory signs.

In patients with sepsis, blood cultures are positive in not more than 30% to 40% [5], and also may be found in patients without sepsis. Microbiologic proof of infection is expensive and may be difficult in patients with prior antibiotic treatment [6]. Positive results may indicate colonization or contamination without pathophysiologic relevance. In 35%, sepsis cannot be proved microbiologically despite the presence of clinical signs and suspicion of a focus [6].

This article focuses on the diagnostic usefulness of established and novel biomarkers to identify sepsis early and reliably.

Humoral and cellular host response

Sepsis biomarkers are derived from the complex host response to an infectious stimulus. Among others, bacterial toxins or membrane surface antigens (pathogen-associated molecular patterns) can initiate activation of plasmatic (complement system, coagulation cascade, kallikrein-kinin-system, eicosanoids) and cellular pathways (granulocytes, thrombocytes, macrophages, endothelial cells), releasing different mediators and molecules (cytokines, chemokines, acute-phase proteins).

C-reactive protein

C-reactive protein (CRP) is an acute-phase protein that is released from hepatic cells after stimulation by inflammatory mediators like interleukin (IL)-6 and IL-8 [7]. CRP has both pro- and anti-inflammatory properties. It activates the complement system after binding bacterial polysaccharides or fragments of cell membranes. CRP prevents the adhesion of granulocytes on endothelial cells and the synthesis of superoxides. It stimulates the production of IL-1 receptor antagonists. Some studies positively evaluated high plasma levels of CRP in patients with infection and sepsis [8], whereas results from many other studies failed to show an impact of elevated CRP
levels for the diagnosis of infection and sepsis or the assessment of sepsis severity. Contrary to cytokines and procalcitonin (PCT), plasma levels of CRP reach their peak not before 48 hours (Fig. 1) [9,10]. Circulating CRP increases during minor infections, does not correlate with the severity of host response, and does not differentiate between survivors and nonsurvivors of sepsis [11,12]. CRP plasma levels may remain elevated up to several days even after elimination of the infectious focus [11]. CRP is found in many noninfectious conditions, such as autoimmune and rheumatic disorders [13], acute coronary syndromes [14], and malignant tumors and after surgery [15].

CRP is of poor predictive value for the diagnosis of sepsis and its power to assess severity of disease is still unproved. Nevertheless, CRP plays an important role in the guidance of antibiotic therapy in localized infection.

**Cytokines**

Increased plasma levels of cytokines are the primary host response to an inflammatory insult. Cytokines are glycoproteins released by macrophages, monocytes, lymphocytes, and endothelial cells. Their binding to specific receptors leads to defined reactions. Proinflammatory cytokines and anti-inflammatory cytokines may be elevated depending on the stage of sepsis. In clinical routine, however, they play only a minor role, because cytokines have a short half-life of a few minutes and receptor antagonist binding rapidly reduces circulating levels. Cytokines may also be induced by many noninfectious causes, such as surgical treatment or autoimmune disorders.

Tumor necrosis factor-α, IL-1, IL-6, IL-8, and IL-10 are the most important cytokines associated with sepsis. Because tumor necrosis

![Fig. 1. Kinetics of various markers of the inflammatory host response after endotoxin challenge in human volunteers. CRP, C-reactive protein; IL, interleukin; PCT, procalcitonin; TNF, tumor necrosis factor.](image-url)
factor-α plasma levels differ between individuals, they are of minor diagnostic importance [16]. IL-6 and IL-8 are closely related to the severity of the physiologic response to infection and systemic inflammation [16]. IL-6 was shown to be elevated more than 1000-fold in patients with sepsis. Patients with IL-6 values of greater than 1000 pg/mL who were screened for an interventional study with monoclonal antibodies showed a high correlation between circulating levels of IL-6 and severity of organ dysfunction and outcome [17].

In neutropenic patients, IL-6 and IL-8 levels distinguished between patients with proved infection and patients with fever of unclear origin, whereas CRP did not [8]. In neonatal sepsis, combined measurement of IL-6 and IL-8 plasma levels predicted early onset of sepsis with high sensitivity and specificity [18]. IL-8, however, had a low sensitivity in detecting an infected necrosis in patients with necrotic pancreatitis [19]. High levels of IL-6 and IL-8 are also found in patients after major surgery [20], severe trauma [21], autoimmune disorders [22,23], viral infection [24,25], and after graft rejection [26]. IL-6 and IL-8 seem to be of minor diagnostic and prognostic importance in sepsis. No systematic study exists to show whether IL-6 has diagnostic properties in the early stage of sepsis. Nevertheless, IL-6 may be helpful to detect complications in neonatal sepsis.

**Procalcitonin**

PCT is a 13-kd propeptide of calcitonin. In healthy individuals, levels of PCT are below 0.1 ng/mL. In patients with sepsis, PCT levels may increase up to 5000 to 10,000 times with calcitonin still in the normal range [27]. In contrast to the short half-life of calcitonin (10 minutes), the half-life of PCT is approximately 24 hours [9,28].

The physiologic role of PCT and its site of production are not completely understood. Bacterial endotoxins are a major stimulus for PCT induction [28] but gram-positive infections may also induce a PCT release. During severe fungal infections, PCT induction was described in some patients, whereas one case report described a lack of PCT elevation [29]. Apart from bacterial infections, major surgery, severe trauma, or burns may induce an increase of PCT levels. Plasma levels observed under these conditions are not as high, however, as in patients with severe sepsis or septic shock. PCT elevation is recognized already 2 hours after endotoxemia or bacteremia [9,28]. A number of studies confirm PCT as a marker of severe infections and sepsis [30]. Patients with PCT levels below or equal to 0.5 ng/mL are unlikely to have severe sepsis or septic shock, whereas levels above a threshold of 2 ng/mL identify patients at high risk [31,32]. PCT concentrations exceeding 10 ng/mL usually occur in patients with organ failure remote to the site of infection [10,11]. A localized focus of bacterial infection without systemic inflammation often did not show an increase in PCT levels with
the older, less sensitive assays (lower limit of normal 0.3 ng/mL). Recently, an ultrasensitive assay became available using a time-resolved amplified cryptate emission technique [33,34]. Bacterial infection can now be ruled out with a much higher negative predictive value.

There are a number of noninfectious causes that may result in an increase of PCT levels (Table 1). When PCT is released unspecifically because of major surgery or severe trauma, daily monitoring may be helpful to detect supervening septic complications early [35]. Other unspecific increases of PCT have been reported in neonates, but monitoring was still useful to detect early onset of neonatal sepsis [36]. In prolonged cardiogenic shock, increased PCT levels have been detected together with other signs of systemic inflammation, such as fever, elevated leukocyte count, and cytokines most likely caused by endotoxin translocation after impairment of gastrointestinal perfusion [37,38].

Despite these limitations, PCT can discriminate better between infectious and noninfectious causes of organ dysfunction or shock than other markers

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<th>Table 1</th>
<th>Comparison of various inflammation markers in clinical use</th>
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<td>Specific response to infection</td>
<td>Sensitive for inflammation</td>
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<td>PCT</td>
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<td>CRP</td>
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*Abbreviations:* CRP, C-reactive protein; PCT, Procalcitonin.
and increases the sensibility and specificity of the diagnosis of sepsis when used in addition to clinical criteria.

PCT may also be helpful to differentiate between bacterial and viral infections. Children with bacterial meningitis had significantly higher levels of PCT than those suffering from viral meningitis [24]. PCT could distinguish between infectious and noninfectious causes of the acute respiratory distress syndrome. Interestingly, the extent of hypoxemia did not contribute to the increase of the PCT level [39]. Likewise, PCT could differentiate graft rejection from systemic fungal or bacterial infections in patients after liver, heart, and kidney transplantation [40–42]. PCT was also found to be elevated in septic patients with chemotherapy-induced neutropenia suggesting that white blood cells are not the only source of PCT release [43]. In patients with necrotizing pancreatitis, PCT was a superior predictor of infection of the pancreatic necrosis when compared with CRP and IL-8 with a predictive power almost equal to the gold standard of fine-needle biopsy [19]. Lastly, PCT was able to distinguish between presence and absence of systemic infections in patients with highly active autoimmune disease [13]. This observation results from only a few cases, however, and needs proof of a larger-size study.

In several studies investigating outcome prediction in critically ill patients, PCT proved to be superior to tumor necrosis factor-α, IL-6, and CRP [31,44]. In polytraumatized patients, initially elevated PCT levels indicated a risk for developing septic complications and multiple organ failure [45]. This was found to be similar in patients after abdominal surgery [35]. Patients after cardiac surgery, who showed elevated PCT levels above 5 ng/mL after cardiopulmonary bypass had a higher in-hospital mortality (Fig. 2).

PCT measured by an ultrasensitive assay is increasingly used as a guide for antibiotic therapy. One prospective randomized study in patients presenting with suspected respiratory infection compared antibiotic use guided

![Fig. 2. Procalcitonin (PCT) plasma levels in 691 patients on Day 1 following cardiopulmonary bypass (CPB) in open heart surgery. An increase of PCT > 1 ng/mL was highly predictive of all-cause hospital mortality (in brackets). (Data from Brunkhorst FM, Wegscheider K, Forycki ZF, et al. Procalcitonin for early diagnosis and differentiation of SIRS, sepsis, severe sepsis, and septic shock. Intensive Care Med 2000;26(Suppl 2):S148–52.)](image-url)
by PCT (strongly encouraged if PCT levels were ≥0.5 ng/mL, not encouraged if <0.1 ng/mL) with a control group without PCT guidance. Whereas outcome and ICU length of stay were similar, prescription rate and cost of antibiotics were halved in the PCT group [46]. In the meanwhile, the same authors were able to obtain similar results in patients with community-acquired pneumonia, where they were able to cut the duration of antibiotic therapy in half by PCT guidance [47].

PCT is a marker that correlates very well with the onset of organ dysfunction remote to the site of infection. A differentiation between an infectious or noninfectious etiology of systemic inflammation seems to be possible and may be helpful for therapeutic decision-making at the bedside. Besides enabling earlier diagnosis, PCT can help to monitor the progress of focus control and the efficacy of antibiotic treatment. Mesenterial hypoperfusion leading to translocation of endotoxin may also induce an increase of PCT levels.

Endotoxin (lipopolysaccharide) and endotoxin activity essay

Endotoxin is an essential structure of the outer cell membrane of gram-negative bacteria. The Limulus amoebocyte lysate assay showed methodologic problems with variations of more than 50%, low specificity because of differences in the endotoxin structure in several gram-negative bacteria, and interactions with plasma proteins and antibiotics leading to differing results in clinical studies. Endotoxin measurement did not play a role in the clinical setting [48]. Recently, however, a highly sensitive ex vivo biologic assay has been cleared by the Food and Drug Administration for use in the United States. It measures the zymosan-antibody and anti-endotoxin antibody–elicited respiratory burst in a kinetic luminometric assay. Clinical studies confirmed a high negative predictive value for the diagnosis of gram-negative infection and sepsis [49] with a sensitivity of 85.3% and a specificity of 44% for the diagnosis of gram-negative infection [50]. The Endotoxin Activity Essay may be a promising marker to exclude gram-negative infection, but it is not useful to describe the state of the host response and does not correlate with either SOFA or APACHE II scores.

Surface and soluble triggering receptor expressed on myeloid cells-1

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently identified cell surface molecule, a member of the immunoglobulin superfamily, which is expressed on human neutrophils and monocytes and triggers secretions of proinflammatory mediators, such as IL-8, tumor necrosis factor-α, and IL-1β [51]. Human TREM-1 is strongly expressed in acute inflammatory lesions caused by bacteria and fungi but not in lesions derived
from noninfectious inflammatory diseases, such as psoriasis, ulcerative colitis, or immune complex vasculitis [52]. In experimental endotoxemia, TREM-1 was shown to be up-regulated on monocytes during human endotoxemia [53] and circulating concentrations were markedly elevated in a murine sepsis model and in patients with sepsis [54,55]. Moreover, blockade of TREM-1 signaling protected mice from lethal effects of lipopolysaccharide-induced septic shock [52]. The power of sTREM-1 at admission to predict outcome was significant but less than for PCT and it did not correlate with the severity of the disease [56]. Measurement of sTREM-1 in bronchoalveolar fluid had a high sensitivity of 98% and specificity of 90% for the diagnosis of infectious pneumonia in mechanically ventilated patients [57]. These findings merit further clinical studies to investigate the potential role of sTREM-1 as a marker of bacterial and fungal infection.

**Adrenomedullin**

Adrenomedullin (AM) is a 52–amino acid peptide identified in 1993 with strong and sustained hypotensive effects [58]. It belongs to a superfamily of peptides including calcitonin gene-related peptide, calcitonin, and amylin, which are structurally related peptides with N-terminal 6– to 7–amino acid ring structures linked by a disulfide bridge and with amidated C-termini [59]. AM is expressed in a large variety of tissues in addition to the adrenal gland for which it was originally named [60]. From a growing number of studies it is becoming evident that AM is able to act as an autocrine, paracrine, or endocrine mediator in many biologically significant mechanisms, such as the endothelial regulation of blood pressure [61] or protection against organ damage in sepsis or hypoxia [62] and the control of blood volume through the regulation of thirst [63,64] and potent antimicrobial properties against gram-positive and gram-negative bacteria [65]. AM may modulate the complement cascade through interaction with its binding protein complement factor H [66].

Increased values of circulating AM have been reported in patients with systemic inflammatory response syndrome [67], and in patients with sepsis [68,69] and septic shock [67,70]. Application of AM antibodies could prevent the hyperdynamic phase of septic shock in a rat sepsis model [71] and transgenic mice overexpressing adrenomedullin showed less severe organ damage and better outcome after lipopolysaccharide-induced septic shock than wild-type mice [72]. It was postulated that AM may play a pivotal role in the development of the hyperdynamic state of septic shock [71]. Plasma AM levels were found to correlate with severity scores and predicted outcome in septic shock [67].

Measuring circulating AM has been difficult because of the existence of a binding protein [66], its short half-life (22 minutes) [73], and technical difficulties [74]. Recently, a novel immunometric assay for midregional
pro-AM has been developed [75]. First data with this assay suggest that pro-AM has a high potential for early outcome detection.

### Atrial and brain natriuretic peptides

Natriuretic peptides play an important role in the regulation of fluid volume and are markers of congestive heart failure. They are released by atrial distention. In patients with septic shock, elevated levels of atrial natriuretic peptide were associated with increased mortality by myocardial depression [76]. A recent observational study of critically ill patients admitted to the ICU showed that mid proatrial natriuretic peptide levels were significantly higher in survivors than in nonsurvivors ($P = .001$). ROC curve analysis showed that the area under the curve for proatrial natriuretic peptide for the survival of patients with sepsis was greater than the area under the curve for PCT and CRP, and similar to the area under the curve for the APACHE II score [77]. Mid atrial natriuretic peptide seems to be a potential new marker for outcome in sepsis and merits further clinical evaluation.

Brain natriuretic peptide is secreted by the cardiac ventricle triggered mainly by left ventricular stretch. A recent prospective study showed that patients with sepsis who had elevated levels of NT–pro brain natriuretic peptide $>1400$ pmol/L were 3.9 times more likely to die than patients with sepsis but lower NT–pro brain natriuretic peptide levels. NT–pro brain natriuretic peptide correlated significantly with troponin I, suggesting cardiac dysfunction in patients with sepsis [78].

### Lipopolysaccharide-binding protein

Lipopolysaccharide-binding protein was first described in 1990 [79]. It is a 58-kd class-1 acute-phase protein that mediates the endotoxin-induced activation of monocytes by the CD14-receptor and the production of IL-6. Lipopolysaccharide-binding protein is mainly synthesized by hepatocytes, intestinal, and pulmonary epithelial cells. The normal plasma level for lipopolysaccharide-binding protein ranges from 5 to 15 $\mu$g/mL. In patients with febrile neutropenia, lipopolysaccharide-binding protein at the onset of febrile neutropenia was significantly higher in patients with gram-negative bacteremia than in those with fever of unknown origin and those with gram-positive bacteremia, using a cutoff value of 46.3 mg/L [80]. A few reports described levels up to 30 times of the normal value [81]. The induction time of 36 hours is slow, however, compared with PCT and CRP [82].

### Protein C

Derangement of the coagulation system correlates with increased mortality in severe sepsis and septic shock [83,84]. Protein C (PrC), which plays an important role in the clotting cascade, is activated by interaction with
thrombin-thrombomodulin and exerts antithrombotic, profibrinolytic, and anti-inflammatory activities [85]. PrC activation in sepsis may be impaired by inflammatory cytokines [86] and reduced levels of PrC in septic patients are associated with poor clinical outcome [87,88]. In severe sepsis and septic shock, median values of PrC dropped significantly already 12 to 16 hours before clinical diagnosis [87]. Recently, a large multicenter study confirmed that continuation or worsening of coagulopathy as measured by prothrombin time, antithrombin activity, and D-dimer and PrC levels during the first day of severe sepsis was associated with increased development of new organ failure and 28-day mortality [84]. Administration of recombinant human activated PrC could significantly reduce 28-day mortality in patients with severe sepsis; interestingly, patients with normal levels of activated PrC also benefited from this therapy [83]. These results suggest that reductions in PrC concentrations may play an important role in the development of disseminated intravascular coagulation and organ failure in sepsis. In a rat model of sepsis, early decrease in protein C concentration predicted mortality [89].

Because PrC is secreted in hepatic cells, decreased levels of PrC can also be found in patients with hepatic failure and cancer. Additionally, any kind of coagulation activation (pulmonary embolism, venous thrombosis) causes protein C levels to drop. Because of the relatively short half-life of PrC (10 hours), low concentrations may be expected after any shock-induced hepatic hypoperfusion.

**Endocan**

Endocan or endothelial cell-specific molecule-1 is a soluble 50-kd dermatan sulfate proteoglycan that is secreted by the vascular endothelial cells of lung and kidney in response to proinflammatory cytokines [90]. In a prospective observational pilot study, patients with sepsis showed on average fourfold higher endocan levels at admission to ICU than healthy subjects or patients with systemic inflammatory response syndrome. Endocan levels were higher in patients with septic shock than in patients with severe sepsis or sepsis and higher levels at admission revealed higher levels for nonsurvivors (10-day mortality, $P < .01$) [91]. Endocan may be a key player in the regulation of major processes, such as cell adhesion, in inflammatory disorders, and tumor progression [92]. Endothelial injury is pivotal to the development of organ failure and shock in sepsis, and an endothelial marker, such as endocan, may reflect on the stages and severity of the disease. This marker is far from being used, however, in daily clinical practice.

**Complement 3a**

Complement 3a is a proinflammatory mediator, derived from the $\alpha$-chain of C3 after activation of the classical and alternative pathway of the
complement cascade. The assay for complement 3a by column chromatography is complicated and expensive. It is known that systematic inflammation leads to 40-fold elevated levels of complement 3a [93]; however, large sample size studies in critically ill patients have not been undertaken. A recently published study showed plasma concentration of complement 3a in 22 septic patients was significantly higher than in 11 patients with systemic inflammatory response syndrome [94]. The different stages of sepsis severity, however, were not assessed in this study. The meaning of this parameter regarding the diagnosis of sepsis remains unclear.

Neopterin

Neopterin is a substance of low molecular weight released from human monocytes after immune stimulation mainly by the macrophage-activating factor interferon and is biosynthetically derived from guanosine triphosphate. The function of neopterin is probably associated with the cytotoxic reactivity of activated macrophages [95,96]. Because elevated plasma levels are found in all infectious and noninfectious inflammations and in malignant diseases, the specificity of neopterin as a marker of systemic infection is limited [97]. In a prospective study in multiple trauma patients, PCT but not neopterin levels were predictive for multiorgan failure by multivariate analysis [98]. Another disadvantage is the long time of induction (>24 hours) and the accumulation in patients with renal failure.

HLA-DR

HLA-DR is a surface antigen expressed on monocytes. Sepsis and severe infections suppress the HLA-DR expression. This status of immune paralysis characterizes a high-risk patient population. The degree of suppression of the monocytic HLA-DR expression correlates with the severity and the outcome of sepsis [99,100]. This occurs only in a subgroup of septic patients, however, and may also be found in patients after major surgery [101,102]. Apart from some value for outcome prediction, HLA-DR measurement does not play a role in the diagnosis of sepsis. Furthermore, the method is complicated and the results are hard to repeat.

Summary

Requirements for the clinical use of a sepsis marker demand that the test results are able significantly to alter clinical decision making at the bedside. To date, only a few markers are able to fulfill such requirements. This is the case for IL-6 and IL-8, which are used to some degree in pediatrics and neonatology. PCT has gained growing clinical acceptance during the last years for the reasons given later. In the meanwhile, it has been approved by the
Food and Drug Administration as a tool for risk assessment in critically ill patients for progression to severe sepsis and septic shock.

A number of studies indicate that sensitivity and specificity of PCT for severe sepsis is superior to that of CRP, IL-6, IL-8, and conventional parameters like leukocyte count and body temperature. PCT rises 2 to 4 hours after a septic insult, later than proinflammatory cytokines like IL-6 and IL-8 but considerably earlier than CRP. To date, it is the only sepsis marker that is helpful in the differentiation of infectious and noninfectious causes of organ dysfunction and septic shock. Several studies indicate that PCT levels are considerably higher in bacterial than in viral infections. A number of studies demonstrate that the course of PCT serum levels reflects the success of infection or sepsis treatment rapidly, closely, and better than CRP levels. Recent studies moreover suggest that PCT may guide antibiotic therapy in patients with suspected respiratory tract infection and community-acquired pneumonia.

Whether the use of PCT in critically ill patients proves to be cost effective by reducing costs for antibiotics and improving morbidity and mortality in the ICU setting needs further clinical investigation.

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