J R Coll Physicians Edinb 2019; 49: 207–16 | doi: 10.4997/JRCPE.2019.308 **REVIEW**

Biomarkers and newer laboratory investigations in the diagnosis of sepsis

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Abstract

Sepsis is a major cause of death in hospitalised patients accounting for mortality rates as high as 60% and, hence, is called 'a hidden public health disaster'. Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis is not a disease but is a clinical syndrome, where the initial features are nonspecific resulting in delayed diagnosis. Lack of specific laboratory tests to diagnose the syndrome adds to the diagnostic confusion. Failure to identify sepsis in the early stages itself delays effective treatment resulting in high morbidity and mortality. Various biomarkers and newer laboratory tests help to address these issues. However, to date there is no ideal test to diagnose sepsis. The most commonly used markers are C-reactive protein (CRP) and procalcitonin (PCT). There are around 180 biomarkers reported to be useful in sepsis. In addition to CRP and PCT, various emerging laboratory markers, such as like serum amyloid A, soluble triggering receptor expressed on myeloid cell-1, mannan and antimannan antibodies, and interferon γ inducible protein-10 etc., have been reviewed and their clinical usefulness discussed in this paper.

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Introduction

Sepsis is one of the most common causes of death in hospitalised patients and, hence, it is called 'a hidden public health disaster'. Sepsis is associated with mortality rates as high as 60%.[1]

Risk of sepsis is increasing worldwide posing challenges to the medical fraternity. The factors contributing to increased incidence of sepsis include advanced age, performance of more invasive procedures, associated multiple comorbidities and emergence of antibiotic resistance.[2]

Sepsis was initially defined as a clinical condition associated with infection and at least two of the four criteria for systemic inflammatory response syndrome (SIRS), which are: 1) temperature >38°C or <36°C; 2) tachycardia; 3) tachypnea; or, 4) white blood cell count >12,000 or <4,000. Sepsis is not a disease but is a clinical syndrome.[3] Because of inadequate specificity and sensitivity the recent sepsis (Sepsis-3) guidelines recommend avoiding use of SIRS criteria and proposed a new definition: a life-threatening organ dysfunction caused by a dysregulated host response to infection.[4] A new measure, qSOFA [quick sequential (sepsis-related) organ failure assessment], was introduced, incorporating altered mentation, systolic blood pressure of ≤100 mmHg and respiratory rate of ≥22 breathes per minute, which are quick bedside clinical tools for identifying adult patients with signs of infection who are likely to have poor prognosis.[5]

Even though there are various guidelines, international discussions and published algorithms to assist early diagnosis and treatment of sepsis, diagnostic confusion still persists resulting in improper (under or over) treatment. Infectious Diseases Society of America had multiple disagreements with the 2016 Surviving Sepsis Campaign's recommendations published under the auspices of the Society of Critical Care Medicine and the European Society of Intensive Care Medicine.[6]

Early diagnosis and assessment of severity are important essential steps for early comprehensive treatment, thus reducing sepsis-related morbidity and mortality. An ideal biomarker should be validated, inexpensive and widely accessible, and results should be rapidly available. There is no ideal test to diagnose sepsis and various biomarkers are helpful to make a reasonable conclusion in the context of clinical scenario. As per Pierrakos and Vincent,[7] around 180 biomarkers are reported to be useful in sepsis and the figure is likely to increase in the future. However, only a few biomarkers are assessed as appropriate for the diagnosis of sepsis. Lack of proper assay methods and interference with the testing methods limit the use of

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various biomarkers in clinical practice. The most commonly used and widely available markers are C-reactive protein (CRP) and procalcitonin (PCT). Other emerging useful markers are serum amyloid A (SAA), soluble triggering receptor expressed on myeloid cell-1 (sTREM-1), mannan (Mn) and antimannan (A-Mn) antibodies, and interferon gamma inducible protein-10 (IP-10).

Biomarkers can be used in suspected sepsis for 1) identifying or ruling out sepsis; 2) evaluating the severity and assessing the prognosis; and, 3) evaluating patients' response to proper treatment.

Diagnosis of sepsis mainly relies on demonstration of presence of organism by blood culture. The time required for the culture to become positive and insensitivity of culture under various situations limits its use as an early diagnostic modality in patients with suspected sepsis.[8] In order to overcome the limitations of culture to diagnose microbial infections alternative molecular-based methods such as enzyme-linked immunosorbent assay kits, flow cytometry, immunoluminometric assays, polymerase chain reaction (PCR) tests, automated microbiological systems and fluorescence in situ hybridisation techniques are used.[7]

Biomarkers in sepsis

A biomarker has been defined as 'an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention'. Doherty et al.[9] defined a biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes [or] pathogenic processes'. The most commonly used biomarkers of sepsis include CRP, PCT, SAA, Mn and A-Mn, and IP-10. There are lot more markers in the pipeline. There is no single ideal biomarker so multiple biomarkers are used in concert to achieve better results.

The clinical relevance of a biomarker depends upon its diagnostic accuracy. A biomarker becomes more relevant as its sensitivity, specificity, positive-predictive value and negative-predictive value increases. Positive and negative likelihood ratios also predict the strength of a biomarker as a diagnostic test.[10]

Why we need biomarkers in sepsis

Sepsis is a critical illness with high mortality, but the initial features are nonspecific and the diagnosis is based on nonspecific physiological criteria (syndromic approach), which results in delayed diagnosis.

Lack of specific laboratory tests to diagnose and high percentage of negative microbiological tests even in patients with sepsis add to the diagnostic confusion. Underestimation of disease severity in the early stage delays effective treatment resulting in high mortality in patients with sepsis. In order to overcome these limitations medical researchers are in continuous search for better laboratory tests and the search for ideal biomarkers still continues.

Pathophysiology of sepsis

Sepsis is a dysregulated host response to infection and involves generalised inflammatory response away from the site of infection or injury. Usually the balance between proinflammatory and anti-inflammatory mediators regulates the inflammatory process. Sepsis occurs when this balance is lost and more proinflammatory mediators are released, causing generalised response exceeding the boundaries of local tissue injury. The multifactorial causes for this response include direct effect of microorganisms and their toxic products, release of large quantities of proinflammatory mediators and compliment activation. Multiple molecules involved in these complex processes are identified and proposed as biomarkers that can be used as indicators of sepsis.[11,12]

Depending upon the pathophysiological stages of sepsis, biomarkers can be grouped into different categories. The classification of laboratory markers based on pathophysiological stages of sepsis is given in Table 1.

Pathophysiological basis of biomarker assay

Sepsis results from exaggerated immune response to infection. Various inflammatory agents and products released during the inflammatory process can be potentially useful biomarkers in sepsis. At the later stage of sepsis patients develop compensatory anti-inflammatory response syndrome (CARS) during which various anti-inflammatory mediators will be useful as biomarkers. Products from the infecting organism will help to identify and plan treatment in sepsis. Patients with sepsis may develop dysfunction of various organs, which are also reflected by various markers.

Proinflammatory cytokines

Proinflammatory cytokines are markers of the hyperinflammatory phase of sepsis. These are tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8 and monocyte chemoattractant protein 1 (MCP-1). They are the important inflammatory cytokines with prognostic value in sepsis.

PCT and CRP as biomarkers of sepsis

PCT and CRP are common proteins synthesised in response to infection/inflammation and are useful as biomarkers in patients with sepsis.

Biomarkers of activated neutrophils and monocytes

Activation of neutrophils and monocytes in sepsis results in increased expression of CD64 and integrin CD11b, which can be potentially useful to diagnose sepsis.

Triggering receptors expressed on myeloid cells-1 (TREM-1), heparin-binding protein (azurocidin) released from

Table 1 Classification of laboratory tests based on the pathophysiological stages of sepsis

Laboratory tests	Remarks
Proinflammatory cytokines	
TNF-α, IL-1β, IL-6, IL-8, MCP-1	Elevated level of IL-6 in people with sepsis is associated with increased mortality and also predicts the benefit of treatment.[71] IL-8 helps in the diagnosis of sepsis, whereas MCP-1 helps in the prediction of mortality[72,73]
PCT and CRP	
Biomarkers of complement proteins	
C5a	In severe sepsis C5a levels are very high[74]
Biomarkers of activated neutrophils and monocytes	
CD64, integrin CD11b, TREM-1, HBP (azurocidin), soluble form of RAGE, CD14	TREM-1 has a higher predictive power for poor survival in ED patients than do PCT or CRP.[13] HBP is a good predictor of severe oedema and vascular collapse in patients with severe sepsis.[14] RAGE is able to predict survival in severe sepsis and CAP.[15] CD14 is high in patients with bacterial infection and its levels correlate with the severity of sepsis[16]
Biomarkers related to infectious organisms and their products	
HMGB1, calgranulins and myeloid-related proteins	Released from damaged neutrophils during inflammation. Are high in patients with sepsis[17]
Biomarkers of the immunosuppressive phase of sepsis/anti-inflammatory markers	
Monocyte HLA-DR expression, CTLA-4 in T cells, PD-1 in monocytes and T cells, CD28 in T cells, IL-10, TGF-α	Low levels of HLA-DR expression predict the development of sepsis, poor survival and increased risk of hospital-acquired infection.[19] Increased expression of CTLA-4 and PD-1 are also seen in patients with sepsis. Decreased expression of CD28 is seen in sepsis. IL-10, TGF-α levels are high in sepsis
Biomarkers of organ dysfunction	
Renal function test, liver function test, serum lactate, lactate clearance, angiopoietins, soluble adhesion molecules, endocan, syndecan-1, heparin sulphate	Syndecan-1 and heparin sulphate indicate injury to endothelial glycocalyx, which is the antiadhesive and anticoagulant surface of endothelium

CAP: community-acquired pneumonia; CRP: C-reactive protein; CTLA-4: cytotoxic T lymphocyte-associated antigen-4; ED: emergency department; HBP: heparin-binding protein; HLA-DR: human leucocyte antigen–DR isotype; HMGB1: high-mobility-group box 1; IL: interleukin; MCP-1: monocyte chemoattractant protein-1; PCT: procalcitonin; PD-1: programmed death-1; RAGE: receptor for advanced glycation end products; TGF: transforming growth factor; TNF: tumour necrosis factor; TREM-1: triggering receptors expressed on myeloid cells-1

polymorphonuclear leucocyte (PMN) granules and CD14, which helps the monocyte and macrophage to recognise endotoxin, are useful markers in sepsis.[13–16]

Monocyte activation markers, such as soluble form of the receptor for advanced glycation end products (RAGE), are able to predict survival in severe sepsis and community-acquired pneumonia.[15] Since lung alveolar type 1 cells normally express high RAGE levels, soluble RAGE is found to be high in patients with pulmonary infection even in the absence of sepsis.

Biomarkers of infectious organisms and their products

Blood and other body fluid culture is the gold standard for confirming bacterial infection. Use of automated monitoring systems like VITEK® (bioMérieux, USA), ESP Culture System (Trek Diagnostics Inc., USA), BacT/Alert® (bioMérieux) and BACTEC™ (BD, USA) increases the speed and efficiency of blood culture. Use of molecular diagnostic technologies, such as bacterial DNA fragments by real-time PCR in blood samples, or 16S rRNA fragments of Gram-positive and Gram-negative bacteria and 18s rRNA of *Candida*, might be helpful for early identification of the infectious pathology. Identification of bacteraemia by amplifying specific target nucleic acid sequences using PCR identified double the number of positive specimens compared to conventional blood culture in surgical intensive care unit (ICU) setup in patients with severe sepsis. A high level of endotoxin is a significant risk factor for the development of sepsis. High-mobility-group box 1 (HMGB1), calgranulins and myeloid-related proteins are released from damaged neutrophils during inflammation.[17] Currently, 16S rRNA and 18S rRNA gene sequencing are the best methods to identify microorganisms. Matrix-assisted laser desorption ionisation-time of flight mass spectrometry is a novel method that helps to identify the microorganisms quickly and accurately.[18] Microarrays that help in large-scale screening for simultaneous diagnosis and detection of many

pathogens are also increasingly used for microbial detection. Gene resistance detection testing, loop-mediated isothermal amplification assay and metagenomic assay are other microbial detection methods used in clinical microbiology.[18]

Biomarkers of the immunosuppressive phase of sepsis/ anti-inflammatory markers

With the reorganisation of CARS the role of anti-inflammatory markers in sepsis attracted attention and it was found that monocyte human leucocyte antigen–DR isotype (HLA-DR) expression will improve after initial suppression in survivors of sepsis, usually within 10 days.[19]

Biomarkers of organ dysfunction

Deranged renal and liver function tests indicate renal and hepatic dysfunction. Serum lactate is one of the common markers used to assess organ dysfunction. Since liver plays an important role in lactate clearance its level can be elevated in patients with liver dysfunction as a part of organ failure in sepsis. Systemic inflammation induces increased anaerobic glycolysis resulting in elevation of blood lactate level in sepsis. Mitochondrial dysfunction associated with sepsis also contributes to increased lactate level. Serum lactate level >2 mmol/l is regarded as a sensitive marker of septic shock. Decreasing or normalised lactate levels indicate recovery from septic shock.[20] Serum lactate >4 mmol/l with a systolic blood pressure of at least 90 mmHg is defined as 'cryptic shock'.[21] In critically ill patients the mortality risk rises with increase in serum lactate levels. In one study serum lactate level >4.0 mmol/l was associated with a 27% mortality rate compared to those with lactate level between 2.5 and 4.0 mmol/l where the mortality rate was 7%.[22] Low lactate clearance is useful as a predictor of mortality in patients with sepsis.[23] Serial measurement of serum lactate will help to assess the disease progression in patients with sepsis.[23] At least 10% lactate clearance at 2 hours of initiation of resuscitation is a positive sign of response to resuscitation in patients with severe sepsis.[24]

Endothelial dysfunction in sepsis contributes to organ dysfunction and markers of endothelial activation, such as angiopoietins, soluble adhesion molecules and endocan, were found to be high in patients with sepsis.

Involvement of coagulation system also adversely affects the clinical course of sepsis. Presence of disseminated intravascular coagulation (DIC) increases the risk of mortality in patients with sepsis.

Microparticles are vesicles shed from the cell surface by blebbing. Microparticles release their contents that have an important role in systemic inflammation and DIC in patients with sepsis.[25]

Clinically relevant biomarkers in sepsis

There are large numbers of biomarkers in the pipeline. The following are clinically relevant biomarkers that have been found to be useful in patients with sepsis (Table 2).

CRP

CRP is an acute phase protein that belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins, produced by liver in response to inflammation or tissue damage. Various cytokines induce its production. However, IL-6 is its prototypical stimulus. Serum levels of CRP increase 1,000 fold during inflammation. CRP level begins to increase within 4–6 hours after the stimulus and doubles every 8 hours. Its half life is 19 hours and the peak level attained at 36–50 hours. CRP is widely used as a marker of infection and sepsis. Various studies have shown the CRP cut-off value to diagnose infection is between 5 and 10 mg/dl.[26] The role of CRP as a diagnostic predictor was analysed in various studies and one study showed that CRP had a sensitivity of 84.3%, specificity of 46.15%, positive-predictive value of 84% and negative-predictive value of 42.8%.[27] However, measurement of CRP after the onset of sepsis failed to predict the chance of survival in comparison with PCT and IL-6.[28] The major drawback of CRP as a sepsis biomarker is its low specificity. CRP has high negative-predictive value to exclude sepsis, if measured in the early course of illness.[29] CRP levels >100 mg/l are usually due to bacterial infection, whereas levels <100 mg/l are due to fungal infection, thereby helping to differentiate among the two.[30]

CRP levels are also increased in various rheumatologic conditions, inflammatory bowel disease, haematological disease and graft-vs-host disease. CRP is also used to assess the inflammation associated with atherosclerotic cardiovascular disease.

PCT

PCT is a prohormone of calcitonin. In healthy individuals PCT is produced from parafollicular cells of the thyroid and neuroendocrine cells of the lung and intestine. However, in patients with bacterial infection PCT is produced from numerous organs in the body. PCT is classified as a 'hormokine' in view of its relation with both the hormone calcitonin and the inflammatory cascade. Normal reference value of PCT is ≤0.15 ng/ml. Localised infections (without systemic signs) usually show a level between 0.15 and 2.0 ng/ml. PCT level >2.0 ng/ml is usually associated with systemic bacterial infection/sepsis or severe localised bacterial infection. PCT levels increase within 2–4 hours of infection and the maximum level is reached by 6–8 hours and with continued infection or sepsis the elevated level persists.[31] Once infection is controlled PCT levels halve daily. PCT half-life is about 20–24 hours. PCT levels persists as long as the inflammatory process continues and the level correlates with the severity of sepsis. PCT is produced in response to endotoxins or inflammatory mediators released in response to bacterial infection.

PCT levels correlate with the severity of infection and help to discriminate patients with infectious and noninfectious systemic inflammation, differentiate bacterial from viral and fungal infections and help to identify bacterial superinfection in patients with viral infection. Studies showed that higher PCT on the day of admission to ICU is associated with

Table 2 Biomarkers and laboratory tests useful in the management of sepsis

Biomarker	Pathophysiology	Current status in clinical practice
CRP	Acute phase reactant produced by liver in response to inflammation or tissue damage	Commonly used. Low specificity as sepsis biomarker
PCT	Prohormone of calcitonin. Produced in response to infection	Commonly used. Correlates with severity of infection. Helps to discriminate infectious and noninfectious systemic inflammation. Useful as diagnostic test and as prognostic marker as is a marker of severity of sepsis and associated mortality. Helps to assess the response to treatment
ChT activity	Secreted by activated macrophages	Needs evaluation
Presepsin	Produced in response to bacterial infection	Reflects the severity of infection. Promising biomarker for early diagnosis and potentially superior to PCT for predicting prognosis in patients with sepsis[45]
IL-27	Produced by antigen-presenting cells in response to microbial products and inflammatory stimulus	High specificity and positive-predictive value for bacterial infection in critically ill paediatric patients[46]
Hepcidin	Produced from the liver. Synthesis is induced by IL-6, which is produced in response to inflammation	Reliable marker of both early and late-onset neonatal sepsis[47]
SAA	An apolipoprotein. Major acute-phase protein	Equivalent to or more sensitive than CRP
MIF	Produced in response to microbial products, hypoxia and proliferative signals	Increased in septic shock. Helps to differentiate survivors from nonsurvivors[49]
LBP	Acute phase reactants	Not an impressive marker[50]
Pentraxin 3	Binds to specific pattern of fungi, bacteria and virus inducing phagocytosis. Secreted by various cells, such as leucocytes and endothelial cells	Correlates with sepsis severity and sepsis-associated coagulation/fibrinolytic dysfunction.[75,76] In patients with febrile neutropenia after chemotherapy high pentraxin 3 values predict the development of septic shock and bacteraemia
Cytokines IL-6, IL-8, TNF-α and IL-1 receptor antagonist	Immune-modulating agent that is produced from nucleated cells	Correlates with sepsis severity and outcome
HMGB1	Released by activated macrophage	Not a predictor of in-hospital mortality in patients with sepsis
Proteins C	Coagulation biomarkers	Low levels in neutropenic patients predict severe sepsis and septic shock
BPW analysis	Based on activated partial thromboplastin time	For diagnosis of sepsis
sTREM-1	Member of immunoglobulin super family. Expression upregulated in presence of bacteria or fungi	Superior to CRP and PCT as an indicator of sepsis.[57] Indicates the severity of sepsis
suPAR	Expressed in various cells. Takes part in various immunological functions	Less useful in sepsis
Midregional pro-adrenomedullin	Potent vasodilating and bactericidal agent	Good predictor of severity and outcome of CAP[59]
Polymorphonuclear CD64 index	Upregulation of CD64 expression is an early immune response to bacterial infection	Early detection of sepsis in neonates[60]

Table 2 Continued

Biomarker	Pathophysiology	Current status in clinical practice
Mean neutrophil volume	Volume, conductivity and scatter parameters of neutrophils are a useful tool to identify bacterial infection	Very accurate and sensitive method compared to the manual method of identifying bacterial infection[61]
Mannan and antimannan antibodies	Mannan is present in the cell wall of invasive fungal organisms	Diagnostic marker of sepsis that is due to fungal infection[62]
IP-10	Proinflammatory cytokine	Useful as a biomarker for diagnosing viral infections[63]

BPW: biphasic waveform; CAP: community-acquired pneumonia; ChT: plasma chitotriosidase triode; CRP: C-reactive protein; HMGB1: high-mobility-group box 1; IL: interleukin; IP-10: interferon-gamma inducible protein-10; LBP: lipopolysaccharide-binding protein; MIF: macrophage migration inhibitory factor; PCT: procalcitonin; SAA: serum amyloid A; sTREM-1: soluble triggering receptor expressed on myeloid cell-1; suPAR: soluble form of urokinase type plasminogen activator; TNF: tumour necrosis factor

increased risk of progression to severe sepsis and septic shock.[32] In an observational study it was found that sepsis diagnosis based on PCT level is more reliable than clinical diagnosis.[33] In critically ill patients the high maximum PCT level and a PCT increase for 1 day are independent predictors of 90-day mortality.[34] PCT values help in deciding about the need for antibiotics and duration of antibiotic therapy.[35] It was also found that use of PCT helps in reduction of antibiotic prescription between 11% and 74%, and reduction of days of antibiotic therapy by between 13% and 55%.[36] In patients admitted to ICU use of PCT-based algorithm resulted in the reduction of antibiotic therapy for the initial infection by 2 days and the total duration of antibiotic therapy by 4 days.[37] In patients with community-acquired pneumonia it is a useful marker to guide antibiotic therapy. In patients with sepsis a 30% decrease in PCT levels between days 2 and 3 is an indirect indicator of effective antibiotic therapy and associated with better survival than CRP levels. Serial measurement of PCT in sepsis can guide antibiotic therapy and helps in therapeutic decision-making.[38] Analysis of cost effectiveness of the use of PCT-based algorithm shows that it reduces the length of hospitalisation, antibiotic therapy and number of blood cultures.[39] The MOSES study showed that inability to reduce PCT levels by more than 80% between baseline and day 4 is a significant independent predictor of increased 28-day all-cause mortality.[40] PCT kinetics have prognostic implications in patients with sepsis.[41] PCT level-based treatment results in reduction in the duration of therapy and mortality in critically ill patients.[42]

Studies have shown that PCT can be used as a diagnostic test and as a prognostic marker, to assess the response to treatment, as well as a marker of severity of sepsis and associated mortality.

Various studies have shown that PCT is superior to CRP in the identification and assessment of severity of sepsis.[43] PCT has been found to be more sensitive and specific for bacterial infection than CRP. However, the superiority is not clearly demonstrated in patients with sepsis.[44] PCT is useful in predicting the results of blood culture in patients with critical illness. PCT fails to differentiate sepsis from SIRS; however, low levels of PCT are helpful in ruling out sepsis because of their high negative-predictive value. PCT levels rise earlier than those of CRP helping in early anticipation of diagnosis of sepsis 24–48 hours before the CRP levels would.

PCT can be falsely negative in people with localised infection, infection with atypical bacteria and in patients on steroids. PCT levels can be increased in patients with severe trauma, having surgery or after cardiac arrest.

Plasma chitotriosidase triode activity

Chitotriosidase triode is synthesised and secreted by specifically activated macrophages and belongs to the mammalian chitinase family. Chitotriosidase triode is increased in various disorders in which macrophages are activated, such as Gaucher's disease, atherosclerosis, malaria and haematological disorders.

Presepsin

Presepsin is a 13 kDa protein present in CD14. CD14 is the receptor for lipopolysaccharide–lipopolysaccharide-binding protein (LPS–LBP) complex. In the presence of infectious agents CD14 activates toll-like receptor-4, leading to proinflammatory cascades resulting in the shedding of LPS–LBP–CD14 complex, and plasma protease then generates a soluble CD14 subtype called presepsin. Its production is induced by bacterial phagocytosis and it is the body's response to bacterial infection.[45]

IL-27

IL-27 levels are useful for identifying bacterial infection in critically ill paediatric patients.[46] Their overall predictive power improves when used in combination with PCT.

Hepcidin

Hepcidin is a peptide hormone produced by the liver that has an important role in iron metabolism. Hepcidin interferes with microorganisms' access to iron. It is a reliable marker of both early and late-onset neonatal sepsis.[47]

SAA

SAA is an apolipoprotein whose levels increase 1,000 times by 8–24 hours after the onset of infection.

Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF) is a pleotropic immune regulatory cytokine that promotes the migration and

recruitment of leucocytes into the site of inflammation and infection. MIF is produced from immune cells (monocyte/macrophage, B and T cells) as well as endocrine, endothelial and epithelial cells.[48] When used in combination with other biomarkers MIF has greater value. MIF is a regulator of innate immunity, which is increased in septic shock, and helps to differentiate survivors from nonsurvivors.[49] High levels of MIF and PCT in patients with severe burns is associated with a lethal outcome.

LPS-binding protein

LPS-binding protein is an acute phase reactant. It binds to LPSs of Gram-negative bacteria to form LPS–LBP complex, which in turn binds to CD14 and toll-like receptors to induce signal transduction, leading to the activation of the mitogen-activated protein kinase and nuclear factor κB pathway.[50]

Pentraxin 3

Pentraxins are a super family of proteins that act as pattern recognition receptors and are involved in the acute immunological response. There are two types: 1) classic 'short' pentraxin, which includes serum amyloid P component and CRP; and, 2) long pentraxin 3 that binds to specific patterns of fungi, bacteria and viruses inducing phagocytosis. However, pentraxin 3 is also found to be elevated in noninfectious inflammatory conditions limiting its role.

Cytokines

Cytokines are immune-modulating agents that are produced from nucleated cells. In patients with septic shock there are increased levels of both proinflammatory and anti-inflammatory cytokines, and cytokines have been proposed as a biomarker in neonatal and adult sepsis. High and/or increasing levels are associated with poor prognosis; however, studies show that cytokines (IL-6, IL 8) are less useful than PCT and CRP in sepsis.[51] IL-6, IL-8, TNF-α and IL-1 receptor antagonist (IL-1ra) levels show correlation with sepsis severity and outcome. Increased levels of IL-6 and IL-8 in neonates predict early onset sepsis. In children with septic shock serum IL-8 levels within 24 hours of admission had 95% negative-predictive value for mortality;[52] however, IL-8 is a poor marker in adults with septic shock. Low IL-8 is associated with the high negative-predictive value for sepsis.

IL-6 and IL-8 levels are altered in various conditions, such as major surgery, trauma, exacerbation of autoimmune disease, transplant rejection and viral infections. IL-10, which plays a role in CARS, is found to increase in patients with septic shock and help to predict mortality at 28 days.[53] Use of combined cytokine scores using IL-6, IL-8 and IL-10 are a better predictor of mortality than PCT and CRP.

In a study conducted with 17 different cytokines, nine (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, interferon γ, granulocyte-colony stimulating factor and MCP-1) were able to differentiate survivors from nonsurvivors in sepsis, whereas the remaining eight (TNF, IL-5, IL-7, IL-12, IL-13, IL-17, macrophage inflammatory protein-1 and granulocyte-macrophage colony-stimulating factor) cytokines were not different in both groups.[54] Persistently elevated IL-6 is associated with multiple organ failure and death in patients with sepsis.

HMGB1

HMGB1 is a 30 kDa nuclear and cytosolic protein mediating local and systemic inflammation. In comparison to TNF-α and IL-1B it reacts more slowly and, therefore, has been evaluated as a prognostic marker; however, it did not predict in-hospital mortality in patients with sepsis.[55]

Coagulation biomarkers

As a part of sepsis patients develop various haematological problems including DIC.

Biphasic waveform analysis

Biphasic waveform analysis is based on activated partial thromboplastin time and has been found to be useful for the diagnosis of sepsis.[56]

Soluble receptors

sTREM-1

TREM-1 is a member of immunoglobulin super family. Its expression is upregulated in the presence of bacteria or fungi resulting in release of sTREM-1.[57]

Soluble form of urokinase type plasminogen activator

Soluble form of urokinase type plasminogen activator is expressed in various cells, such as neutrophils, lymphocytes, monocytes, macrophages, endothelial cells and tumour cells, which take part in various immunological functions. High levels of the soluble form of urokinase type plasminogen activator (suPAR) are associated with increased mortality in patients with HIV, tuberculosis, malaria and Crimean-Congo haemorrhagic fever.[58]

Midregional pro-adrenomedullin

Serum adrenomedullin (ADM) is a potent vasodilating and bactericidal agent that is found to be elevated in sepsis. ADM is rapidly broken down in the circulation, therefore, the midregional fragment of pro-ADM, is measured.[59]

Polymorphonuclear CD64 index

Circulating PMN cells bind to endothelial cells and express CD64 during an inflammatory response. CD64 is a high affinity FC receptor for IgG and upregulation of CD64 expression is an early immune response to bacterial infection.[60] Other surface markers, such as CD14, CD18, CD25 and CD28, help to predict mortality at 28 days.

Mean neutrophil volume

Sepsis is known to produce numerical and morphological changes in leucocytes. The volume, conductivity and scatter parameters of neutrophils are useful tools even in the absence of elevated white cell count to identify bacterial infection. The change in the morphology and in the number of these cells, which is reflected in the volume, conductivity and scatter parameters of leucocytes, proves to be a more accurate and sensitive method than the manual method.[61]

Biomarkers for fungal infection

Mn and A-Mn antibodies

Mn is present in the cell wall of invasive fungal organisms. In patients with invasive fungal infection, such as candidiasis or aspergillosis, Mn and A-Mn antibody levels are elevated making it as a useful diagnostic marker of sepsis due to fungal infection. A study showed that a combination of Mn and A-Mn provides better results than either marker alone.[62] β-D-glucan test, which is highly sensitive and specific for invasive mycosis, is usually performed along with Mn and A-Mn antibody testing.

Biomarkers for viral infections

IP-10

IP-10 is a proinflammatory cytokine that is potentially useful as a biomarker for diagnosing viral infections.[63] IP-10 has also been found to be useful in guiding treatment in patients with hepatitis C infection.

Traditional markers, such as neutrophil count and CRP, are unable to differentiate infection from inflammatory response. The sensitivity and specificity of CRP for the diagnosis of sepsis is 0.75 and 0.67, respectively, whereas for PCT they are 0.77 and 0.79 and for sTREM-1 they are 0.79 and 0.8, respectively.[64–66] Differentiating infectious causes from noninfectious causes of inflammation still remains a challenge.

The receiver–operator characteristic (ROC) curve for various biomarkers helps to assess their discriminative power. The shape and area under the curve (AUC) help to find out the clinical usefulness of a marker. An ideal discriminating biomarker has an AUC of 1, but for a nondiscriminating marker AUC is 0.50. The AUCs for detection of a bacterial cause of inflammation were 0.50 for suPAR, 0.61 for sTREM-1, 0.63 for MIF, 0.72 for PCT, 0.74 for neutrophil count, 0.81 for CRP, 0.84 for the composite three-marker (neutrophil count, CRP, PCT) test, and 0.88 for the composite six-marker test.[67] Another meta-analysis showed that AUC for the seven biomarkers, PCT, CRP, IL-6, soluble triggering receptor expressed on myeloid cells-1, presepsin, LBP and CD64 were 0.85, 0.77, 0.79, 0.85, 0.88, 0.71 and 0.96, respectively.[68]

Infections are the common cause of clinical deterioration in patients with systemic lupus erythematous and antineutrophil cytoplasmic antibody-associated vasculitis. However, clinical deterioration has to be differentiated from disease flare because immunosuppressives are the mainstay of treatment in active disease. If the clinical deterioration was due to infection, inadvertent treatment with immunosuppressive agents results in further deterioration of the patient. So differentiation of infective causes from disease flare is important. However, the commonly used laboratory tests are of limited use in these aspects. It was shown that CD64 expression on neutrophils helps to differentiate bacterial infection from the flare of autoimmune diseases with a sensitivity and specificity of 85% and 84%, respectively, whereas PCT differentiates with a sensitivity and specificity of 75% and 85%, respectively.[69] sTREM-1 levels are not found to be useful in this aspect.[69]

Panel of biomarkers

To date there is no single ideal biomarker for the diagnosis of sepsis hence a combination of biomarkers are used to provide better results. Combinations of multiple markers are expected to increase the sensitivity and specificity in diagnosis and prognosis of patients with sepsis. 'Bioscore', which consists of sTREM-1, PCT and PMN CD64 index, has been found to be more useful.[70] The probability of diagnosis of sepsis increases with an increase in biomarker positivity, with 3.8% when the bioscore is 0 (i.e. all the markers are below threshold) to 100% when the bioscore is 3 (i.e. all the three markers are above threshold).[70] A combination of PCT and biphasic waveform also increases the specificity for the diagnosis of sepsis compared to either marker alone. Measurement of another combination of biomarkers, such as suPAR, sTREM-1 and MIF along with CRP, PCT and neutrophil count, was also found to be more useful than individual agents in patients with SIRS to detect community-acquired bacterial infections with an AUC (ROC-AUC) of 0.88.[67]

Conclusion

Sepsis is a critical illness associated with exaggerated systemic inflammatory response to infection resulting in high morbidity and mortality in patients admitted in ICUs. Early diagnosis and prompt action improves the prognosis. Lack of specific clinical features delays the diagnosis. Various biomarkers are available to help the clinician to diagnose, plan the treatment, assess response to treatment and to predict the outcome. Even though many potential biomarkers have been evaluated CRP and PCT still remain the most commonly used and widely available biomarkers in sepsis. However, to date, there is no single ideal biomarker for sepsis and hence a combination of biomarkers are used along with clinical characteristics of the patient to provide early diagnosis and better risk assessment. 'Bioscore', which consists of sTREM-1, PCT, PMN CD-64 index and a combination of biomarkers such as suPAR, sTREM-1 and MIF, along with CRP, PCT and neutrophil count are found to be more useful in this aspect.

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