PUBLIC HEALTH RESEARCH

Biomarkers Approach in the Diagnosis and Prognosis of Sepsis

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ABSTRACT

Introduction Sepsis is a systematic host response to infection defined by suppression of the immune system and organ failure which can rapidly lead to septic shock and death if not managed properly.

Methods Therefore, the timely diagnosis of sepsis has prompted researchers to assess several number of blood biomarkers. In a complex situation like sepsis where multiple organs and systems are involved, the sensitivity and specificity of blood indicators that can easily and accurately show the severity and prognosis of the disorder are of great importance.

Results C-reactive protein (CRP) and Procalcitonin are the most common biomarkers used in clinical routine to diagnose sepsis and monitor the response to treatment. Other biomarkers including pro-inflammatory and anti-inflammatory cytokines and chemokines, monocyte and lymphocyte, antibodies, and nucleic acid can also be used to assess the disorder. Biomarkers offer utility for disease progress, the prognosis of disorder, risk stratification, and treatment effect rather than diagnosis at the early stage of sepsis.

Conclusions Therefore, although defining molecular properties in septic patients opens up new means to diagnose and manage sepsis in a shorter time compared to conventional methods currently used at hospitals, further clinical evaluation of biomarkers should be performed. In this review, we summarized pathophysiology, pathogeneses, and clinical diagnosis of sepsis. We also provided an overview of the role of candidate biomarkers on diagnosis and prognosis of sepsis.

Keywords Sepsis - Biomarkers - Prognosis - Diagnosis.

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INTRODUCTION

Sepsis is a syndrome that affects around 31 million people annually worldwide and has caused a case fatality rate of 14.7% to 29.9% in the USA between 2004-2009.[1] Sepsis occurs when the body starts to injure itself in the form of tissue and organ failure in response to an infection.[1] Late diagnosis or inappropriate treatment of sepsis, can rapidly induce septic shock, multiple organ failure, and in the worst case death. The latest recommendations by The Third International Consensus (i.e named also Sepsis-3) define sepsis as "life-threatening organ dysfunction caused by a dysregulated host response to infection".[2]

Sepsis can be triggered by bacteria, viruses, fungi, and parasites. The most common etiological pathogens are bacteria including gram-negative bacteria such as *Haemophilus influenzae, Escherichia coli (E.coli), Salmonella* spp., *Neisseria meningitides,* and gram-positive bacteria such as *Streptococcus pneumoniae,* and *Staphylococcus aureus (S. aureus)*.[1] The report published by World Health Organization (WHO, 2017) states that *S. aureus* including methicillin-resistance *S. aureus* (MRSA) is the most common cause of sepsis in hospitalized patients in the European intensive care units.[1] Antimicrobial resistance is one of the factors of failure of the host response to treatment and rapid progression of sepsis to septic shock and death. To the extent that MRSA causes about a 50% higher mortality rate than methicillin-susceptible *S.aureus* (MSSA).[1]

Many efforts have been made to find a golden diagnostic standard, in particular, to reduce diagnostic time and antibiotic resistance. For this purpose, more than 170 blood biomarkers have been studied of which a number are already available for clinical use, such as C-reactive protein and Procalcitonin, however, most have not shown sufficient specificity or sensitivity for routine use in clinical practice.[3] It seems that a single biomarker is not ideal, but many of them are helpful for prognosis and following up on treatment effects. This review summarizes our understanding of sepsis diagnosis over the past decade. We will provide a background of pathogenesis, pathophysiology, and clinical diagnosis of sepsis before focusing on the diagnostic and prognostic potential of the most commonly proposed blood biomarkers for the assessment of sepsis. Much researches have studied the sepsis trend in the specific population. However, since the focus of this study is on sepsis in general population, these cases will not be reviewed in detail and will only be addressed in appropriate cases.

Pathophysiology

Sepsis is a consequence of a complex chain of events including innate and adaptive immune response, complement activation, coagulation cascade, and endothelial vascular system.[4,5]

It is been shown that in gram-negative sepsis, the innate immune system detects the invasive pathogen through the interaction of Pathogen Recognition Receptors (PRRs) with exogenous Pathogen-Associated Molecular Patterns (PAMPs) and endogenous Damage-Associated Molecular Patterns (DAMPs).[3,6,7] So far, four types of PRRs have been identified including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs). Stimulation of TLRs and recognition of PAMPs and DAMPs activate the signaling pathway, transcription of inflammatory genes, and initiation of innate immune responses by production and secretion of cytokines, chemokines, and nitrate oxide (NO).[8-10]

The most common starting point for gram-negative sepsis is lipopolysaccharide (LPS), the bacterial cell membrane also known as endotoxin. This unique compound of gram-negative bacteria is released by bacterial lysis and consists of three components. The outer domain is called the O-antigen, the chain composition of which varies from strain to strain and has different antigen effects. This layer is recognized by the host antibodies. The middle layer is called the "core", a less diverse oligosaccharide domain, and the third layer is a hydrophobic inner domain known as lipid A (or endotoxin) which is a conserved region.[9] Lipid A; an excellent example of PAMPs, is the immune system activator, which can act directly on the host cell, however, its predominant effect is the activation of macrophages and the formation of a coagulation cascade that is essential for the transformation of infection into sepsis and septic shock.[9,10] After the endotoxin is released from the bacteria, it binds to the "lipopolysaccharide-binding protein". This set activates CD14 and TLR4 receptors on macrophages and releases cytokines mediators. In some cases, gram-positive bacteria, viruses, fungi, and parasites can also trigger sepsis and septic shock by triggering a coagulation cascade, including the release of TNF-α and other cytokines.[10,11] Practically, sepsis involves the complex intervention of pro-inflammatory mediators and anti-inflammatory mediators.[5,6,12]

Pro-inflammatory mediators include TNF-α (Tumor Necrotizing Factor α), IL-1β (Interleukin-1β), IL-6 (Interleukin-6) which are released immediately from activated macrophages, and also IL-8 (Interleukin-8), Platelet-activating factor, Leukotrienes, and Thromboxane A2. The most important anti-inflammatory cytokines are Interleukin-1 Receptor Antagonists (IL-1RA), Interleukin-4, and Interleukin-10. Among pro-inflammatory mediators, TNF-α is the major mediator of the onset of sepsis.[5,9,12] Pro-inflammatory cytokines activate other types of cells such as endothelial cells, lymphocytes, hepatocytes,

neutrophils, and platelets which in turn cause tissue damage, vasodilation, and lung dysfunction.[11]

Other signs and symptoms of sepsis are due to the activation of the complement system, which leads to the production of anaphylatoxins and a severe inflammatory reaction. At this stage, increased levels of C-reactive protein (CRP), inhibition of fibrinolysis, and ultimately Disseminated Intravascular Coagulation (DIC) is diagnosed. DIC is a common circumstance in gram-negative sepsis caused by the Hagman factor and the release of endotoxins, leading to homeostasis imbalance and organ dysfunction.[3,13]

Pathogenesis

Immunomodulation describes a complex network of defensive factors tending to overcome invasive pathogens in the form of collective action. These factors can cause dysregulations, known as 'Systematic Inflammatory Response Syndrome' (SIRS) and 'Multiple Organ Dysfunction Syndrome' (MODS). Patients pass five stages to reach the MODS phase.[7,10,14] (Figure 1)

Local reaction to injury or infection

An infection causes the release of some pro-inflammatory mediators which try to improve the condition through various mechanisms. In opposition, the 'Compensatory Anti-inflammatory Response' is activated to ensure the safety of surrounding areas by reducing the activity of pro-inflammatory cytokine-producing cells.[14] Some of these pro- and anti-inflammatory mediators are listed in Table 1.[9]

Table 1 Pro- and anti-inflammatory mediators in sepsis cascade

Pro-inflammatory mediator	Anti-inflammatory mediator
TNF-α	Interleukin 1(ra),4,10,13
Interleukin 1β, 2, 6, 8, 15	Interleukin 1 receptor II
Interferon-γ	Epinephrine
Thromboxane	Soluble TNF-α receptor
CD14	Transforming growth factor β (TGF-β)

Primary systematic response

Pro-inflammatory and anti-inflammatory mediators appear in the bloodstream respectively. The presence of pro-inflammatory mediators in the bloodstream is part of the normal response to infection indicating a lack of control over the invasion. Therefore, these mediators need to provide the condition for the involvement of neutrophils, lymphocytes B and T cells, platelet, and coagulation factors, which in turn leads to more activity of compensatory anti-inflammatory response cascade through down-regulation of pro-inflammatory mediators. Clinical symptoms and organ dysfunction are very rare at this phase.[11]

Widespread Systematic Inflammation

Loss of regulation of the inflammatory response leads to a broad response characterized by clinical findings of SIRS. Functional disorders of organs and their eventual failure occur unless the homeostasis mechanisms used in the early stages bring the condition closer to baseline.[9,15]

Excessive immunosuppression

Excessive suppression of the immune system by the anti-inflammatory compensatory mechanism causes paralysis of the immune system and the development of 'Compensatory Anti-inflammatory Response Syndrome' (CARS).[9] Some of the cellular and molecular changes that characterize CARS are apoptosis of lymphocyte, production of IL-10 which suppress the production of TNF-α, decrease in the number of monocyte's human leukocyte antigen receptors, reduction of cytokines response of lymphocyte, and expression of inhibitory coreceptors in lymphocytes.[16]

Immunologic dissonance

'Multiple Organ Dysfunction Syndrome' (MODS) also known as 'immunologic dissonance' occurs due to inadequate response of the immunomodulatory system. Organ failure is most likely due to inhibition of the production of pro-inflammatory mediators required by the organ to restore function. It seems that if the balance between pro-inflammatory and anti-inflammatory systems is restored, the organs will regain their function.[7]

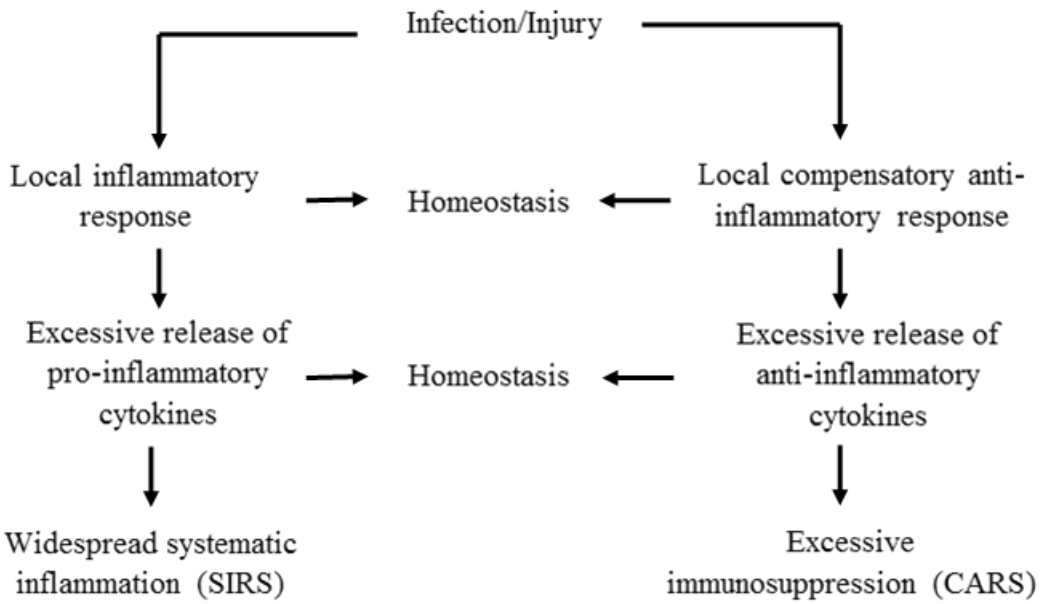

Figure 1 Association between Systematic Inflammatory Response Syndrome (SIRS) and Compensatory Anti-inflammatory Response Syndrome (CARS)

Clinical Diagnosis

The diagnosis of sepsis is based on clinical manifestations, blood biomarkers, and microbiological findings. According to the latest definition of sepsis by The Third International Consensus, also known as sepsis 3, clinical diagnostic criteria vary in hospitalized patients in the intensive care unit (ICU) and non-ICU patients. For hospitalized patients in the ICU, the assessment for evidence of organ dysfunction is based on a scoring system known as Sequential Organ Failure Assessment (SOFA) (Table 2). A rating score of two or more by this system is associated with sepsis. In non-ICU patients suspected of infection, a quick scoring system (qSOFA) is used, which measures the respiratory rate of 22/min or more, systolic blood pressure of 100mmHg or less, and changes in consciousness. The existence of at least 2 of these clinical criteria is sufficient to confirm sepsis.[2]

Table 2 Sequential Organ Failure Assessment (SOFA) score*

Indicator/Score	0	1	2	3	4
PaO2/FIO2,mmHg	≥400	<400	<300	<200 with respiratory support	<100 with respiratory support
Platelets x $10^3/\mu L$	≥150	<150	<100	<50	<20
Bilirubin, mg/dl	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Hypotention	MAP ≥70 mm Hg	MAP <70 mm Hg	Dopamine <5 or dobutamine (any dose) [a]	Dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine ≤0.1 [a]	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1 [a]
GCS score [b]	15	13-14	10-12	6-9	<6
Creatinine, mg/dL	<1.2	1.2-1.9	2.0-3.4	3.5-4.9	>5.0
Urine output, mL/d				<500	<200

*adapted from Singer *et al.* [2]

PaO2, partial pressure of oxygen, FIO2, the fraction of inspired oxygen; MAP, mean arterial pressure

a Catecholamine doses are given as μg/kg body weight/min for at least 1 hour.

b Glasgow Coma Scale scores range from 3-15; a higher score indicates better neurological function.

Biomarkers Used In The Diagnosis Of Sepsis

The application of blood biomarkers provides an opportunity for faster diagnosis and management of the disease. Several biomarkers have been reported with the aim to improve diagnostic and prognostics accuracy and evaluate therapeutic response. Herein, we presented the most commonly suggested biomarkers with great potential for the assessment of the severity of sepsis. A summary of the biomarkers was shown in Table 3.

CRP and PCT

Both proteins are produced in response to infection or inflammation at the early stage of sepsis. C-reactive protein (CRP), the most studied marker, is synthesized by hepatocytes. CRP levels rise within 6 to 8 hours after exposure to pathogens and peak in 36 to 50 hours.[3] The sensitivity and specificity of the CRP test are reported to be 68-92% and 40-67% respectively for diagnosis of sepsis. Simultaneously, it is used for antibiotic therapy assessment as to its level declines by the remission of inflammation in response to antibiotic treatment. Procalcitonin (PCT) is a precursor of the hormone calcitonin secreted from C cells of the thyroid gland which lowers blood calcium levels. PCT could be secreted by various tissues during invasive bacterial infections or sepsis.[3,17] The protein increases significantly within 2 to 6 hours and peaked at 6 to 24 hours during sepsis.[3] PCT level in healthy individuals is <0.5ng/ml, whereas quantification of PCT level >2ng/ml on the first day of ICU admission is highly related to septic shock. The PCT levels are also used as a guide for antibiotic treatment. Current guidelines specify the withdrawal of antibiotic therapy when the serum level is <0.25ng/ml.[3] Hence, PCT is a biomarker for the diagnosis of bacterial sepsis and the assessment of antibiotic treatment. Therefore, CRP and PCT contribute to the prognosis and monitoring of treatment progression in sepsis.[3,17,18]

TNF-α and IL-6

The interaction of PRR and PAMP stimulates pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β which eventually widespread in the bloodstream and causes fever. Blood levels of cytokines also increase in other conditions, such as trauma, surgery, and autoimmune disease. Therefore, the use of pro-inflammatory cytokines has no diagnostic value due to non-specificity. However, TNF-α, IL-6 levels are known to be associated with organ damage and mortality, thus they contribute to the prediction of prognosis.[3,17] IL-6 increases 4 to 6 hours after endotoxemia and decreases 24 to 48 hours later, while TNF-α has a short biological half-life of 17 minutes. Consequently, IL-6 is more feasible in assessing the prognosis of the inflammation due to its stable plasma kinetics.[3,17,18]

Lipopolysaccharide binding protein (LPB)

LPB is a 58-kilo-dalton protein secreted by the liver that begins signaling after forming a complex with LPS and binding to CD14 on the surface of monocytes and macrophages [19]. This protein has a dual function, at low concentrations, it intensifies the effect of LPS while at very high concentrations the protein reduces or inhibits the effect of the endotoxin.[17,19,20] Under physiological conditions, its concentration is 5 to15 ng/ml, and a sharp increase in serum levels of this protein is useful for the diagnosis of sepsis, and can even be used as a predictor of severity and prognosis.[17,19]

Interleukin-27(IL-27)

Increased levels of immunosuppressive cytokine IL-27 have been observed in the plasma of many septic patients.[21,22] IL-27 has presented the highest diagnostic power among biomarkers with 92% specificity for bacterial infection in critically ill pediatric septic patients.[23-25]

Blood lactate level

Lactate serum levels reflect tissue hypoperfusion and anaerobic metabolism in sepsis and septic shock. In hypoxic conditions, an increase in lactate levels is seen due to increased glycolysis and limited tissue oxygenation. This, in turn, indicates a defect in liver lactate clearance and the mortality rate of hospital-acquired sepsis. Lactate level serial monitoring can be used as a marker for predicting mortality rate and risk classification.[17,20,24,26] Clinically, the serum lactate level greater than 2mmol/L (>18mg.dL) in the absence of hypovolemia characterizes septic shock.[2]

Angiopoietin (Ang)

Ang2 is an endothelial-derived vascularized growth factor that facilitates endothelial destruction, vascular leakage, and inflammation; in contrast, Ang1 supports endothelial cells and vascular stability.[27,28] Endothelial dysfunction is associated with the severity of sepsis and MODS. The Ang2/Ang1 ratios have shown a clinically valuable marker for risk classification in septic patients.[3,27]

D-dimer

D-dimer is a product of fibrin degradation due to fibrinolysis and is associated with thrombosis and DIC. Recent studies have shown that this factor has significant predictive value for the presence of bacteremia in patients with sepsis and the adverse outcomes of organ dysfunction and death.[17,19,29,30]

HMGB1 and MIF

Two inflammatory mediators, HMGB1 and MIF, are late-stage proteins in acute infection.[8,17,31-33] HMGB1 is a nuclear and cytoplasm protein released from active monocytes and necrotic tissues during infection and inflammation.[32] MIF is a pro-

inflammatory cytokine released from macrophages and lymphocytes.[33] The high circulating concentration of MIF and HMGB1 in sepsis and septic shock are used as prognostic markers of sepsis.[3,17]

CD64

The CD-64 is a leukocyte antigen and IgG receptor on macrophages and monocytes. It also presents in low concentrations on inactive neutrophils. CD64 increases upon bacterial infection in premature neonates and adults hours after the activation of the innate immune system, therefore CD64 reflects the early stages of infection and prognosis of the disease.[3,17] The sensitivity and specificity of CD64 stand 75% and 77% respectively in neonate sepsis.[17]

CD14

The CD14 is a surface protein in monocytes and macrophages assisting TLRs in detecting PAMPs. The release of LPS-binding protein (LBP)-CD14 complex into the bloodstream, forms a soluble version of CD14 (sCD14). During inflammation, sCD14 cleaves into a subtype known as sCD14-ST or presepsin. This marker elevates within 2 hours after the onset of infection and peaks within 3 hours which is earlier than IL-6 and PCT. This factor is considered as a primary predictive biomarker for the diagnosis and prognosis of sepsis.[3,17]

sTREM-1

Soluble Triggering Receptor Expressed on Myeloid Cells-1 (sTREM-1) is a kind of receptor located on the surface of polymorphonuclear cells and mature monocytes. sTREM-1 is upregulated after binding of TRL2 and TRL4 with bacterial endotoxin which in turn increases the amount of TNF-α, IL-8, and IL-1β meanwhile decreases the expression of the anti-inflammatory cytokine IL-10. Its diagnostics sensitivity and specificity are 79% and 80% respectively.[3,17,18]

miRNA

miRNAs are involved in the regulation of various cellular processes. Altered circulating miRNA can serve as a high diagnostic and prognostic biomarker in sepsis, particularly level of circulating miR-223 has shown significant reduction in septic patients in comparison to healthy controls.[34,35]

Plasma cell-free DNA (cf-DNA)

Mitochondrial DNA (mtDNA) has a sensitivity and specificity of 88% and 94% respectively in the diagnosis of sepsis. mtDNA has been presented as a unique biomarker for predicting death in septic patients as a 1.0ng/mL increase in plasma level of mtDNA corresponds to a 0.7% increase in mortality.[3]

Table 3 Summary of biomarkers and their role of diagnosis and prognosis in sepsis

No	Biomarker	Diagnosis	Prognosis
1	CRP	•	
2	PCT	•	
3	TNF-α		•
4	IL-6		•
5	LPS binding protein	•	•
6	IL-27	•	
7	Blood lactate level	•	•
8	Ang2		•
9	D-dimer	•	•
10	HMGB1		•
11	MIF		•
12	CD64	•	•
13	sCD14-ST	•	•
14	sTREM-1	•	
15	miR-223	•	•
16	mtDNA	•	•

CONCLUSION

Despite significant advances in understanding the pathophysiology and molecular properties of sepsis, timely diagnosis and proper treatment of the disease remains a challenge for physicians to reduce the mortality of sepsis. Extensive research has been done to develop biomarkers to facilitate the diagnosis of sepsis. These indicators have presented diagnostics and prognostics value, as well as advantages in risk stratification and following up the progress of the disease and the effectiveness of treatment. Few biomarkers are applicable at clinical diagnosis, each with different sensitivity and specificity to sepsis diagnosis, therefore, further clinical evaluation should be performed to overcome the lack of sepsis-specific biomarkers.

Compliance With Ethical Standards
The author is responsible for the content and writing of this article. The author confirms that this article's content has no conflict of interest.

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