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Literature Review

## BACTERIA CAUSED SEPSIS BIOMARKERS

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### ABSTRACT

Sepsis is a clinical condition of patients with serious infections that show a systemic inflammatory response, with or without a positive blood culture. sepsis is one of the most frequent causes of death in patients in intensive care units. We are at urgent need for biomarkers and reliable measurements that can be applied to risk stratification of septic patients and that would easily identify those patients at the highest risk of a poor outcome. Such markers would be of fundamental importance to decision making for early intervention therapy. Pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukins-1,-6,-8 (IL-1, IL-6, IL-8) are postulated to play a major role in the pathogenesis of the syndrome. C-reactive protein (CRP) and procalcitonin (PCT) are among a few biomarkers that incorporated into clinical practice although their precise role in the pathophysiology of sepsis and organ dysfunction still unclear.

**Key words:** Sepsis, biomarker, inflammatory, C-reactive protein (CRP), procalcitonin (PCT)

### ABSTRAK

*Sepsis adalah suatu kondisi klinis penderita dengan infeksi serius yang memperlihatkan respons inflamasi sistemik, dengan atau tanpa kultur darah yang positif. Sepsis merupakan salah satu penyebab kematian yang paling sering pada penderita di unit perawatan intensif. Kita berada pada kebutuhan mendesak akan biomarker dan pengukuran yang handal yang dapat diaplikasikan untuk risiko stratifikasi pada pasien septik dan akan diidentifikasi dengan mudah pada pasien dengan risiko tertinggi pada hasil. Penanda akan menjadi kepentingan mendasar dalam pengambilan keputusan untuk therapy. Pro-inflammatory sitokin seperti nekrosis tumor faktor- $\alpha$  (TNF- $\alpha$ ), interleukins-1,-6,-8 (IL-1, IL-6, IL-8) merupakan acuan dasar yang berperan penting dalam sindrom patogenesis. C-reactive protein (CRP) dan procalcitonin (PCT) adalah beberapa biomarker yang dimasukkan ke dalam kegiatan klinis meskipun mereka berperan patifisiologi sepsis dan disfungsi organ masih tak jelas.*

**Kata kunci:** Sepsis, biomarker, inflamasi, C-reactive protein (CRP), procalcitonin (PCT)

### INTRODUCTION

Sepsis is a clinical condition of patients with serious infections that show a systemic inflammatory response, with or without a positive blood culture.<sup>1</sup> The diagnosis of sepsis was referring to the consensus criteria in 1991. These criteria are inviting a lot of dissatisfaction, so that a better approach might be to the stratification system. The new system is based on the PIRO characterize sepsis predisposition, basic infection, response and organ dysfunction. However this system works well, is very important to identify biomarkers of response profiles that can identify patients at risk of developing into an organ dysfunction.<sup>2</sup>

Sepsis is one of the most frequent causes of death in patients in intensive care units. In America, there are approximately 700,000 patients each year and 210,000 of them died. Despite new therapies that support and more potent antibiotics, sepsis remains often causes death in 30–70% of patients with severe sepsis and significantly lowers the quality of life for patients who survived.<sup>2</sup>

Generally, sepsis is a spectrum disorder that is caused by infection by bacteria, viruses, fungi or parasites or toxic products. The spectrum of disorders of sepsis is the result of microbial invasion of the bloodstream or intoxication with early signs of circulatory compromise include tachycardia, tachypnea, peripheral vasodilation and fever

(or hypothermia) to circulatory collapse with multiple organ dysfunction and death.

Several different bioactive molecules have been proposed as a biomarker to assess the degree of patients with sepsis. Among them are bacterial products such as endotoxin and bacterial DNA, acute phase proteins (protein C, procalcitonin), coagulation factors (fibrin degradation products, anti-thrombin III, D-dimer), cellular processes (apoptosis), hormones (cortisol, ACTH) and cytokines (TNF- $\alpha$ , IL-1, IL-6, IL-8, IL-10). Unfortunately, only a few biomarkers that can be used in clinical practice.<sup>2</sup> In this literature review will discuss some of the biomarkers are often used in studies.

### Pathophysiology of Sepsis

The body's defense mechanism against bacterial infection is influenced by the structure and bacterial pathogenicity. Depending on the structure of the cell walls, the microbes are classified in the class of Gram-positive bacteria, Gram-negative, and *spirochaeta mycobacteria*. There are several general overview of the immune response to microbes, namely: Defense against microbial-mediated effector mechanisms of innate immunity and acquired immunity, non-specific immune response against microbes play an important role in determining the specific immune response that will take place, The immune system is able to specialize and respond differently to the types of microbes, Survival and microbial pathogenicity is strongly influenced by the ability of microbes to evade the host immune system, tissue damage and disease as a consequence of infection is generally caused by the host response to microbes and their products.<sup>3</sup>

Innate defense system of the body is the first line of defense against infection and can be activated when pathogen via natural defense barrier. The body's defense system includes the humoral elements (the alternative pathway and mannan-binding lectin of the complement system, acute phase proteins and cytokines) and cellular elements (monocytes, macrophages, neutrophils and dendritic cells natural killer cells).<sup>4</sup>

Detection of invading microorganisms mediated by receptors expressed on the surface of innate immune cells. These receptors can recognize structures that are usually found in microbial pathogens.<sup>4</sup>

Lipopolysaccharide (LPS) bacteria are the main targets of immune recognition. Macromolecules is only found in the outer lipid bilayer that surrounds the walls of Gram-negative bacteria. There are two proteins that recognize humoral LPS is LPS-binding protein and soluble CD14.<sup>5</sup>

Parslow, 2001. CD14/LPS complex then interacts with toll-like receptor-4 (TLR4). TLR4 activation causes the transcription of a number of inflammatory genes and the immune response through the mediation mechanism of nuclear factor- $\alpha$ B (NF- $\alpha$ B).<sup>4</sup>

Gram-positive organisms can also cause sepsis least through two mechanisms: through the production of

exotoxins that act as superantigens and through the cell wall components that stimulate the immune cells. Superantigens are molecules that are bound to MHC class II molecules on antigen presenting cell and T cell receptor V $\beta$  chain to produce large amounts of proinflammatory cytokines. Staphylococcus enterotoxin, toxic shock syndrome toxin-1 and *streptococcal* pyrogenic exotoxin are examples of bacterial superantigens. Toll-like receptor 2 (TLR2) mediates cellular responses to kill Gram-positive bacteria and the structure of the cell wall (peptidoglycan, lipoproteins, lipoteichoic acid and phenol-soluble modulin).<sup>4</sup>

Innate immune defense is another important group of serum proteins called complement pathway. Complement can be activated via three routes, all via the C3 complement activation: the classical pathway, the alternative pathway and the lectin pathway.<sup>5</sup>

With the exception of C3, almost all soluble mediators of innate immunity found in small amounts in normal conditions. This concentration can be increased to 1000 times during a serious infection, which is part of the protective reaction called the acute phase response. In these circumstances, the liver increases the synthesis of more than 30 different serum proteins, called acute phase proteins. Some of them are complement factors C3 and B, MBL (mannan-binding lectin), LBP (LPS-binding protein), C-reactive protein and amyloid P protein and other coagulation factors such as fibrinogen include, granulocyte colony-stimulating factor anti-oxidants and serum protein-binding metal. Acute phase response occurs when hepatocytes associated with cytokines, especially interleukin-6 (IL-6), interleukin-1 (IL-1) or tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are released locally or into the bloodstream by other cells.<sup>5</sup>

Excessive stimulation by proinflammatory cytokines or other mediators may cause systemic damage and endothelial cell dysfunction. Endothelial cell activation leads to increased expression of nitric oxide synthase which causes nitric oxide and intra-cell adhesion molecules excessive, stimulates neutrophil chemotaxis and the interaction of endothelial cells.<sup>6</sup>

Bone et al., 1997. Split pathophysiology of sepsis into 5 stages:<sup>1,7</sup>

**Stage 1:** Enforcement infection. When the infectious organisms will begin to proliferate produced inflammatory molecules such as leukotrien, complement components, cytokines and antigen-antibody complexes, attract neutrophils to areas of infection, followed by monocytes. Stacking leukocytes at sites of inflammation is facilitated by IL-8, endothelial cell selectins and cellular adhesion molecules. Leukocytes recognize and phagocytize bacteria and fungi teroponisasi. This process is due to local release of cytokines from macrophages (monocyte tissue). Proinflammatory cytokines and other mediators including TNF- $\alpha$ , IL-1, IL-2, IL-6, interferon- $\gamma$ , platelet-activating factor (PAF) and others. The release of these mediators will be balanced by compensating anti-inflammatory response

of IL-4, IL-10, IL-11, IL-13, soluble TNF receptor- $\alpha$ , IL-1ra, TGF- $\beta$  and other substances.

**Stage 2:** early systemic response. In a state of severe infection, proinflammatory cytokines would result in systemic symptoms. The emergence of clinical symptoms shows microenvironment unable to control the infection. Proinflammatory cytokines in this process is TNF- $\alpha$ , IL-1, IL-6 and interferon- $\gamma$ . The reaction of the body heat produced by the release of IL-1 that reach the hypothalamus. Prostaglandin E2 may also be produced locally in the hypothalamus and increases the set point temperature.

**Stage 3:** systemic response. Endothelial cell dysfunction is a cause of pathophysiological changes at this stage. As a result of the activity of TNF- $\alpha$ , IL-1 and other cytokines, endothelial cell phenotype shift toward prothrombotic stage. Inflammatory cells and platelets move towards endothelial injury. Disturbances in endothelial cell physiology will affect the ability of the endothelium to regulate blood flow. Consequently there is an increase in microvascular permeability, fluid transudation, organ dysfunction and shock.

**Stage 4:** The reaction of anti-inflammatory compensation. Normally, a cascade of proinflammatory mediators followed by a counter-regulatory cytokine that rapidly regulate the secretion of proinflammatory cytokines and clinical manifestations of sepsis. This regulatory cytokines in principle is IL-4, IL-10, transforming growth factor- $\beta$  (TGF- $\beta$ ) and other anti-inflammatory molecules.

**Stage 5:** The failure of the immune system. This is the final stage, which is seen in some patients. This stage is characterized by the inability of monocytes to respond physiologically, increasing the risk of developing an infection, organ failure and death.

### Biomarker Detection

Biomarkers are any characteristic that can be objectively measured and evaluated as an indicator of biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention.

Measurement of existing biomarkers using ELISA method, measured by immunoluminometric procalcitonin assay is similar in principle to the ELISA.<sup>8</sup>

Protein molecules associated with sepsis is very broad, including cytokines, chemokines, adhesion mediator, soluble receptors and acute phase proteins. Protein biomarker research is currently focused primarily on procalcitonin and interleukin some magic bullet as diagnostic biomarkers for infection. The standard method for assessing the use of this diagnostic is Characteristic Receiver Operator Curve (ROC) by determining the cut point (cut-off) in order to obtain true-positive diagnoses (sensitivity) and false-positive diagnosis.<sup>8</sup>

### Tumor necrosis factor- $\alpha$ (TNF $\alpha$ )

TNF- $\alpha$  is a 17-kd polypeptide is expressed in the form bound to the membrane and form secretion. Activated

macrophages and monocytes, T cells and NK synthesize TNF- $\alpha$ . TNF- $\alpha$  is secreted bound to the cell surface receptors: type I (55 to kd) or type II (75-kd). Stimulation of type I receptor causes activation of NF- $\kappa$ B, induction of IL-6, the expression of tissue factor (TF), regulate thrombomodulin (TM) and TM increases catabolism, activation of fibrinolysis, regulation of endothelial cells, induction of nitric acid synthase, neutrophil activation and biological effects other. Receptor type II facilitates TNF- $\alpha$  binding to type I receptors and signal transduction.<sup>1</sup>

TNF- $\alpha$  is an early factor in the activation of the body's response and a series of cytokines released during infection, where the concentration is increased 24 times (828 ng/L) compared to the concentration before infection at 2 h after LPS interacts with endotoxin *in vivo* during the study. However, the use of TNF- $\alpha$  as a diagnostic tool is not good, in terms of differentiating inflammation and infection. Analysis of the ROC curve shows the sensitivity and specificity were weak. Difficulty TNF- $\alpha$  as a diagnostic tool of sepsis due to an increase in the concentration of bacteria associated with rapid and short half-life of about 17 minutes.<sup>8</sup>

### Interleukin-1 (IL-1)

Other proinflammatory cytokines associated with sepsis is the IL-1 which include IL-1 $\alpha$  IL-1 $\beta$  and IL-1 receptor antagonist (IL-1ra) in which excessive amounts of IL-1 $\beta$  during sepsis.<sup>1,8</sup>

IL-1 $\beta$  interests to provide diagnostic disagreement, between the increase and decrease, so does the same thing has been reported in neonates. Instead concentration of IL-1ra showed a consistent increase in patients with sepsis with a concentration of 2–31 mg/L (concentration in normal individuals is not detected). ROC analysis showed sensitivity of 93% and a specificity of 92% at the time of the diagnosis. But it should be noted that high concentrations have also been reported in patients who underwent thoraco-abdominal aneurysm repair.<sup>8</sup>

IL-1 is the best along with IL-8 in terms of predicting the output. This means that the predictive value of these cytokines is better than the prototype clinical prognostic scores were used in the intensive care unit, the Acute Physiology and Chronic Health Evaluation Score (APACHE II).<sup>2</sup>

### Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a glycoprotein 21-30-kd widely produced by the cells, including monocytes and macrophages, T cells, endothelial cells, fibroblasts and keratinocytes. This molecule is the biggest cause of the acute phase response, causing the growth and differentiation of T cells, NK cell activity and promote the maturation of megakaryocytes. IL-6 can inhibit endotoxin -induced TNF- $\alpha$  and IL-1 and increase the degree of soluble TNF- $\alpha$  receptor type I and IL-1ra.<sup>1</sup>

IL-6 is a cytokine with important prognostic value in sepsis. Although the role of IL-6 in this syndrome remains controversial, IL-6 cytokine proposed as an important

biomarker in sepsis due to the slow kinetic plasma, stable and easily detected in blood samples and correlated well with the intensity of the inflammatory response. Persistent increases in levels of IL-6 associated with organ failure and death.<sup>2,6</sup>

Such as TNF- $\alpha$ , IL-6 plays a role in the immune response at the beginning. Value for adults in sepsis reported to range from 300–2700 ng/L, above 100 ng/L for SIRS. However, there are reports that say that no significant difference between the concentrations in SIRS and sepsis, and between sepsis and trauma patients. This contradiction has been confirmed by the lack of sensitivity and specificity based on ROC analysis.<sup>8,9</sup>

### Interleukin-8 (IL-8)

Interleukin-8 (IL-8) is a chemokine, an agency that recruits inflammatory cells to the site of injury. IL-8 is synthesized by monocytes, macrophages, neutrophils and endothelial cells. TNF- $\alpha$ , IL-1 $\beta$  and IL-2 stimulates the release of IL-8. Following stimulation of IL-8, also stimulated neutrophil function, promote chemotaxis, adhesion molecule expression and regulation of activity of respiration changes and degranulation.<sup>1</sup>

Among other biomarkers associated with sepsis, IL-8 were higher in adult studies, although the main focus is the diagnosis of IL-8 in neonatal research. Concentrations of IL-8 in septic neonates was 94–4335 ng/L compared with 2–42 ng/L in healthy neonates. Although in one study said that is not useful for the diagnosis, the majority of studies reported a consistent increase of the concentration of IL-8. ROC analysis mentioned sensitivity 92 % and specificity of 70%.<sup>8</sup>

The degree of IL-8 correlated with lactic acid, the presence of DIC, severe hypoxemia and mortality in patients with severe infection or septic shock (Balk, 2004). IL-8 together with IL-1 cytokines are the best in terms of prediction output.<sup>2</sup>

### C-reactive protein (CRP)

C-reactive protein is a member of the pentraxin family of proteins decomposed during acute inflammation, causing the immune response to the antigen, activates the complement and enhance the production of monocyte tissue factor. C-reactive protein binds phosphoryl choloin on the surface of bacteria, acts as opsonin for gram-positive bacteria and play a role in the body's defenses. C-reactive protein also binds low density lipoprotein cholesterol (LDL-C) in vitro, suggesting a direct interaction with atherogenic lipids.<sup>9</sup>

CRP is often used as a marker of bacterial infection, however CRP may also be released because of non bacterial stimuli such as state after surgery, autoimmune diseases and rheumatic even on myocardial infarction and malignancy.<sup>10</sup>

CRP is an acute -phase proteins, which are in a state of acute phase plasma levels were varied. CRP is an additional biomarker in the diagnosis of sepsis. CRP has a plasma

half-life that is constant in almost all circumstances. Levels in the plasma is determined by the speed of synthesis, which reflects the presence and spread of disease activity. Induction of CRP requires a minimum of 12–18 hours and CRP increased late during sepsis also decline takes several days. CRP is not useful to distinguish the evolution of sepsis in severe sepsis and septic shock and septic complications in patients with trauma, the slow period after the trauma of high CRP values. Patients with SIRS also have elevated levels of CRP. Opinions on the usefulness of CRP as a diagnostic tool varies, on the one hand claim to have high value and low on the other side. Concentrations were reported in patients with sepsis is between 12–159 mg/L, showing overlap with SIRS patients who are between 13–119 mg/L. ROC analysis showed low sensitivity and specificity.<sup>8,11,12</sup>

### Procalcitonin

Procalcitonin (PCT) is a peptide with 116 amino acids with a sequence that is identical to the prohormone of calcitonin, but PCT itself has no activity as a hormone. In normal metabolic conditions, PCT only in thyroid gland C cells. In bacterial infection and sepsis, intact PCT is found in the blood and more importantly PCT levels associated with severe sepsis.<sup>12</sup>

During severe systemic infection, procalcitonin allegedly generated by the extra thyroid tissue. Patients who previously underwent total thyroidectomy procalcitonin still produce at a high level during severe sepsis. Procalcitonin for sepsis pathophysiology is unclear.<sup>10</sup>

In normal physiology PCT is a precursor of calcitonin. Calcitonin is known to regulate the function of bone and calcium metabolism and inhibits osteoclast resorption. Regulation of the release of calcitonin was first influenced by the concentration of ionized calcium in plasma. Whether this can be attributed to a condition with hypocalcemia in patients with sepsis, remains unclear.<sup>13</sup>

Serum procalcitonin levels increased during bacterial infections, parasites or fungi with systemic manifestations. In severe viral infections or inflammatory reactions of non-infectious cases, procalcitonin levels are not increased or only a modest increase. In patients without the presence of infection is very low procalcitonin levels (<0.1 ng/L) or very high (6–53 ng/L) in severe infections. Resolution of infection with antibiotic therapy reduce levels of procalcitonin. Local bacterial infection and viral infection causes only mild and moderate increase (0,3–1,5 ng/L). That's why the proposed procalcitonin as an indicator of severe infection or sepsis.<sup>12,14</sup>

For the record, procalcitonin levels may be elevated in the first days of life in the absence of infection. Patients with C-cell carcinoma of the thyroid gland may also be there is an increase in the level of procalcitonin in the absence of underlying infection.<sup>14</sup>

In vivo studies showed increased LPS stimulation after a period of 2–6 hours after injection, with a plateau curve from 8–24 hours. PCT as a biomarker measurement is



preferred because it has a half-life 22–29 hours and this increase will be long during sepsis. Positive and gram negative organisms causing an increase in the concentration of PCT in the absence of a significant difference.<sup>8</sup>

Procalcitonin levels increased with increasing degree of inflammatory response in response to infection. When patients were categorized into SIRS, sepsis, severe sepsis and septic shock, particularly increased procalcitonin levels in patients with severe sepsis and septic shock. In a recent study, the levels of TNF- $\alpha$ , IL-6, C-reactive protein and procalcitonin were followed for 14 days after the diagnosis of sepsis. Procalcitonin levels were consistently lower in patients compared to survivors who did not over a period of 14 days. While TNF- $\alpha$  and IL-6 are not consistent and not significantly increased in patients who can not be saved, possibly because of a too high variability from day to day. C-reactive protein is increased in both, patients who survived and did not survive. Procalcitonin levels associated with the severity of the inflammatory response to infection, efficient therapy may be adjusted by a decrease in the levels of procalcitonin. Instead procalcitonin levels indicated poor prognosis. So, procalcitonin can be used as an important indicator for the severity of infection and prognosis of infection and can determine the wisdom of therapy efficacy measurements.<sup>10</sup>

Castelli et al, reported differences in CRP and PCT picture. CRP concentrations increased immediately during severe organ dysfunction and systemic inflammation, but the value is not increased during stage organ dysfunction gain weight. While increased levels of PCT, especially in patients with organ dysfunction, severe sepsis and septic shock.<sup>11</sup>

## SUMMARY

Sepsis has been diagnosed according to the consensus guidelines established in 1991 as an infection in addition to the symptoms of systemic inflammatory response syndrome. It is frequently fatal infectious condition. The incidence continues to increase despite the use of specific antibiotics. We are at urgent need for biomarkers and reliable measurements that can be applied to risk stratification of septic patients and that would easily identify those patients at the highest risk of a poor outcome. Such markers would be of fundamental importance to decision making for early

intervention therapy. Pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukins-1, -6, -8 (IL-1, IL-6, IL-8) are postulated to play a major role in the pathogenesis of the syndrome. C-reactive protein (CRP) and procalcitonin (PCT) are among a few biomarkers that incorporated into clinical practice although their precise role in the pathophysiology of sepsis and organ dysfunction still unclear.

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