Sepsis is defined as a systemic inflammatory response to infection. The clinical definition of systemic inflammatory response syndrome (SIRS) is broad and may be present in many conditions other than infection, such as acute pancreatitis, trauma, burns injuries and autoimmune diseases [Figure 1]. Systemic inflammation is a consequence of activation of the innate immune system. It is characterised by the presence of proinflammatory cytokines and other vasoactive mediators in the circulation, and concurrent activation of innate immune cells in the blood, such as neutrophils and monocytes. The mediators cause circulatory collapse and panendothelial injury, which increases microvascular permeability everywhere in the body. The mortality of patients with septic shock is high, 50-90%. Death may occur early in the course of disease due to irreversible shock, or later due to development of multiple organ dysfunction [Figure 1]. The vulnerability of an individual patient to consequences of sepsis and systemic inflammation is determined to a great extent by genetic factors.

The strong proinflammatory response is counteracted by an immediate activation of anti-inflammatory mechanisms of the immune system. Not so infrequently, an advanced state of immune suppression predominates in critically ill patients. Monocytes of such a patient are unable to generate proinflammatory cytokines in response to bacterial cell wall structures and express on their plasma membrane low densities of class II human leukocyte antigens (HLA-DR). The patient is prone to develop secondary infections, which exacerbates microvascular coagulation and vital organ dysfunction. The evaluation of the intensity of systemic inflammation in acutely ill patients at an early stage of the disease may be helpful in identifying the patients at risk from a complicated course of sepsis. The determination of the quality of the inflammatory reaction that predominates in the patient could suggest the most beneficial mode of therapy, i.e. anti-inflammatory treatment or immune stimulation. The use of a single marker can hardly provide the physician with the information needed. Instead, a set of markers reflecting both pro- and anti-inflammatory activation profiles that is simple enough to be provided by the hospital laboratory as a 24 hour service would be ideal.

Pathophysiological events of sepsis

Mononuclear phagocytes have the key role in provoking the innate immune response against microbes in co-operation with adaptive immune mechanisms [Figure 1]. Mononuclear phagocytes recognise microbial structures such as endotoxins (lipopolysaccharides) by using CD14 receptors. The CD14 receptor has no intracellular domain, and thus needs the help of adjacent Toll-like receptors. Toll-like receptors are able to mediate signalling into the cell. This results in activation of nuclear factor kappaB, a transcription factor, which promotes synthesis and release of a variety of pro- and anti-inflammatory cytokines by activated mononuclear phagocytes. These events are associated with only minor clinical symptoms, such as somnolence and fatigue. Subsequently, the cytokines induce fever, and contribute to the changes in heart rate, respiratory rate and white blood cell counts. The optimal innate immune response is characterised by fast and efficient recruitment of polymorphonuclear phagocytes to the site of invasion, and when the pathogen is

---

**Markers for the clinical diagnosis of sepsis**

by Dr. Annika Takala and Dr. Heikki Repo

Sepsis is defined as a systemic inflammatory reaction response to infection. During sepsis, circulating phagocytes are activated and multiple mediators are released into the blood. The balance between proinflammatory and anti-inflammatory responses affects the outcome of a sepsis patient. In predicting the outcome, the markers of systemic inflammation may help to identify patients at risk of severe sepsis or septic shock at an early stage of the disease.

---

**Figure 1. Clinical events in the evolution of a complicated course of sepsis and concurrent steps in activation of the innate immune system.** Microbial structures activate mononuclear phagocytes by binding to CD14 receptors. A signal is transmitted into cells via Toll-like receptors and results in activation of nuclear factor-kappaB. Transcription of pro- and anti-inflammatory genes and the release of cytokines into circulation occurs. Circulating neutrophils and monocytes become activated and express increased surface density of CD11b/CD18 molecules. Procalcitonin levels start to increase. Genetic factors determine the magnitude of proinflammatory response and the patient may develop shock. Deep immune suppression further complicates the course of sepsis. The chronological schedule should be considered as a rough estimate.
destroyed, the proinflammatory response is down-regulated by anti-inflammatory cytokines.

The loss of the control that limits the inflammatory response at the site of microbial invasion, or an exaggerated intensity of the inflammatory response, causes the spill-over of the proinflammatory cytokines into the circulation. Tumour necrosis factor α (TNFα) and interleukin (IL)-1 are associated with the early pathophysiological events of septic shock. The administration of exogenous TNFα results in rigors, fever, tachycardia and hypotension in a dose dependent manner. In animal experiments, TNFα has been shown to induce shock and tissue injury, which resembles the mimic septic shock in terms of haemodynamic, haematological and histological findings.

**Soluble markers**

Multiple cytokines, including TNFα and IL-1, can be assayed by an automated analyser (Immuli). Although TNFα and IL-1 are important mediators in the pathophysiology of sepsis, they have only limited value when used as markers of systemic inflammation in clinical practice. TNFα has an extremely short half-life, and, furthermore, immunoreactive methods are not accurate enough to estimate the actual biological activity of TNFα and IL-1. However, TNFα and IL-1 stimulate the synthesis of other cytokines, such as IL-6, IL-8 and IL-10 that can be used as estimates of previous TNFα and IL-1 activities. IL-8 is a proinflammatory chemokine molecule for neutrophils. IL-10 is the most important anti-inflammatory cytokine, which inhibits macrophage production of TNFα, IL-1, IL-8 and IL-6. It is clear that already at the early stage of sepsis, pro- and anti-inflammatory responses co-occur. IL-6 has both pro- and anti-inflammatory properties and induces the acute phase protein synthesis in the liver.

IL-2 is the most powerful growth factor for T-cells. The receptor of IL-2 (IL-2R) is presented on T-cells as a high affinity variant upon antigen-stimulation. A subunit of this receptor (sIL-2R) can be detected as a soluble form and reflects the activation state of T-cells.

The level of procalcitonin, a calcitonin hormone precursor, is increased in bacterial infections. Procalcitonin can be detected earlier than C-reactive protein (CRP), a more commonly used marker of systemic inflammation in patients with sepsis. Procalcitonin and CRP are both synthesised in the liver upon cytokine stimulation.

**Cellular markers**

The measurement of cell-bound receptor molecules involved with inflammation has some advantages. The volume of blood needed to quantitate the cell surface markers, using whole-blood flow cytometry, is minimal compared to that needed to determine soluble markers. The sample size may be critical in some patient groups such as small babies. The CD11b/CD18 receptor, that mediates the irreversible adhesion and transmigration of phagocytes from the circulation into tissues, is stored in the cytoplasmic granules and quickly multiplies its expression on neutrophils and monocytes upon stimulation with mediators such as TNFα.

The cell surface expression of HLA-DR molecules is associated with the ability of monocytes to present antigens to T-cells. IL-10 decreases monocyte HLA-DR expression and the proportion of HLA-DR positive monocytes (HLA-DR%).

**Early detection of infection**

In febrile patients admitted to the hospital medical emergency department, clinical examination, radiological and routine laboratory tests usually reveal a focus of infection. Sometimes, however, infection does not cause fever or the focus cannot be verified. In these cases, the elevated serum CRP level denotes the presence of systemic inflammation but may not distinguish between an infectious or non-infectious cause of symptoms. Indeed, CRP is not a specific marker of infection, but is increased in a wide range of diseases characterised by the presence of systemic inflammation. Furthermore, CRP levels increase slowly, and are not necessarily elevated at an early stage of infection. Although the levels of IL-6 and procalcitonin and CD11b/CD18 expression increase earlier than do CRP levels, none of them is specific for infection either. Finally, critically ill patients with secondary infections are problematic due to their suppressed innate immune responsiveness. Indeed, they frequently fail to increase the levels of proinflammatory cytokines in response to microbiologically verified secondary infections.

**Prediction of organ dysfunction in patients with sepsis**

Shock is the major epidemiological predictor for mortality in patients with sepsis. Inadequate perfusion supply to tissues is a risk factor for the development of organ dysfunction. The patients with a complicated course of the disease exhibit stronger proinflammatory responses, determined on admission to the hospital, than do the patients who subsequently recover without complications. Strongly enhanced levels of CD11b/CD18 expression and high serum concentrations of IL-6, IL-8, sIL-2R and procalcitonin on admission have been reported to be associated with poor outcome of sepsis. Unlike these markers, the serum levels of CRP do not distinguish between the patients who are bound to develop organ dysfunction and those who are not.

In sepsis patients with poor prognosis, the proportion of HLA-DR% is persistently low. These patients are prone to secondary infections. Although in early studies, the fall of HLA-DR% below 30% was predictive for death, in recent studies HLA-DR% on admission is inferior to IL-10 level in predicting the patient’s outcome. Evidence has accumulated to show that immune suppression occurs mainly in the circulation whereas in other organs of the body, such as the lungs, inflammatory reaction is predominant.

Because considerable overlap can be detected between the patient groups, the use of a combination of markers might identify the patients at risk of complications more accurately than does any single marker. Indeed, the risk of death was highest in febrile patients with high IL-10 concentration related to low TNF level.

**Conclusions**

The mortality due to septic shock is high. Intense systemic inflammatory response, and anti-inflammatory reaction induced by it, co-occur and contribute to poor prognosis of patients with septic shock. The use of markers of systemic inflammation may help to identify patients at risk of a complicated course of sepsis at an early stage of disease. A combination that includes markers of both systemic inflammation and immune suppression would be useful when choosing between suppressive and stimulatory immunotherapies. Although none of the markers is specific for infection, in selected clinical settings markers may improve the diagnosis of sepsis. In the future, the profiles of inflammatory and anti-inflammatory reactions may be determined at the levels of the transcriptome and proteome using appropriate microarray technology.

**The authors**

Anniko Takala, M.D., Ph.D., Consultant in anaestheiology and intensive care medicine, Department of Bacteriology and Immunology, Haartman Institute, P.O.Box 21, FIN-00014 University of Helsinki Helsinki, Finland.

Tel. +358-50-5203467
Fax. +358-9-57135500
Email annika.takala@helsinki.fi

Heikki Repo, M.D., Ph.D., Consultant in anaestheiology and intensive care medicine, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland.

Tel. +358-50-5203467
Fax. +358-9-57135500
Email heikki.repo@helsinki.fi

**Correspondence** Dr Takala

**as published in CLI April 2004**