# INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is an inflammatory syndrome consisting of fever, pancytopenia, hyperferritinemia, multiorgan dysfunction, and the pathologic finding of hemophagocytosis. Classically, primary HLH is defined as an inherited genetic disorder manifesting in the first few years of life, while secondary HLH, which presents in older children and adults, occurs in the context of infection, autoimmune disease, or malignancy ***(Olin et al., 2008).*** The diagnosis of familial HLH or secondary HLH is based on a number of clinical signs and laboratory findings. Due to the relatively non-specific nature of the clinical signs and symptoms, and significant overlap with other illnesses, diagnosis is often delayed ***(Henter et al., 2007).***

The term sepsis signifies infection giving rise to a systemic inflammatory response in the host. The varying degrees of sepsis are defined by the host response to infection, ranging from uncomplicated fever and leukocytosis to refractory hypotension and multi-organ failure ***(Gantner and Mason, 2015).***

HLH has common clinical and laboratory features with systemic inflammatory response syndrome (SIRS), sepsis and severe sepsis ***(Burcin et al., 2016).*** According to diagnostic criteria published by Histiocyte Society for HLH, fever, splenomegaly, at least bicytopenia, hypertriglyceridemia and/or

hypofibrinogenemia, hemophagocytosis, high serum ferritin level, low or absence of natural killer (NK) cell activity and increased soluble interleukin-2 (IL-2) receptor (sIL- 2R or sCD25) level are accepted parameters for evaluation which also appear to be consistent with sepsis ***(Schneider et al., 2002).*** It is thought that underlying cause of HLH in septic patients may be severe uncontrolled inflammatory response. Therefore, we cannot view HLH as a separate disease from severe sepsis but rather as a consequence of a severe uncontrolled inflammatory response heralding the inability to control the infectious trigger. Pancytopenia associated with severe sepsis was the best warning feature for secondary HLH, although it can occur as well in septic patients without underlying hemophagocytosis ***(Burcin et al., 2016).***

In addition to difficulties in diagnosis of HLH in critically-ill patients, its treatment is a challenge, as well. There is no certain evidence about the benefit of HLH-2004 treatment protocol in critically-ill patients. However, initiation of immunosuppressive treatment without delay in cases with diagnosed or highly suspected HLH is recommended ***(Raschke and Garcia-Orr, 2011).***

# AIM OF THE WORK

The aim of this study is to screen pediatric patients with severe sepsis for the possible presence of underlying unrecognized HLH. This is intended to examine its frequency among septic patients and its effect on the prognosis and have the chance to give those patients the proper management.

Chapter One

# SEPSIS

Sepsis, is a clinical syndrome that complicates severe infection and is characterized by the systemic inflammatory response syndrome (SIRS), immune dysregulation, microcirculatory derangements, and end-organ dysfunction. In this syndrome, tissues remote from the original insult display the cardinal signs of inflammation, including vasodilation, increased microvascular permeability, and leukocyte accumulation. Early recognition of sepsis is crucial to ensuring the best outcomes in children and is aided by a working knowledge of the children at particular risk, the common pathogens, and the clinical manifestations ***(Hall et al., 2011).***

## Definitions:

### Sepsis:

The systemic inflammatory response syndrome (SIRS) in the presence of suspected or proven infection constitutes sepsis. Several definitions further describe sepsis in terms of severity and response to therapy ***(Weiss et al., 2012).***

### Infection:

Infection is defined as a group of symptoms and/or signs caused by a pathogen. Infections can be proven by positive culture, tissue stain, or polymerase chain reaction test. The

definition also includes clinical syndromes associated with a high probability of infection, such as petechiae and purpura in a child with hemodynamic instability, or fever, cough, and hypoxemia in a patient with leukocytosis and pulmonary infiltrates on chest radiograph ***(Goldstein et al., 2005).***

**Systemic inflammatory response syndrome (SIRS):** This syndrome can be diagnosed by 2 out of 4 criteria, 1 of which must be abnormal temperature or abnormal leukocyte count:

1. Core temperature >38.5°C or <36°C (rectal, bladder, oral, or central catheter.

2. Tachycardia:

   - Mean heart rate >2 SD above normal for age in absence of external stimuli، chronic drugs or painful stimuli

   OR

   - Unexplained persistent elevation over 0.5-4 hour

   OR

   - In children <1-year-old, persistent bradycardia over 0.5 hour (mean heart rate <10$^{th}$ percentile for age in absence of vagal stimuli, β-blocker drugs، or congenital heart disease).

3. Respiratory rate >2 SD above normal for age or acute need for mechanical ventilation not related to neuromuscular disease or general anesthesia.

4. Leukocyte count elevated or depressed for age (not secondary to chemotherapy) or >10% immature neutrophils.

***(Turner and Cheifetz, 2015)***

**Severe sepsis:**

Sepsis is considered severe when it is associated with cardiovascular dysfunction, acute respiratory distress syndrome (ARDS), or dysfunction in two or more other organ systems.

**Septic shock:**

It is a subset of sepsis in which underlying circulatory and cellular metabolic abnormalities (cardiovascular dysfunction) are profound enough to substantially increase mortality. Patients with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain mean arterial pressure (MAP) 65 mm Hg and having a serum lactate level >2 mmol/L (18 mg/dL) despite adequate volume resuscitation ***(Goldstein et al., 2005).***

**Refractory septic shock**:

There are two types of refractory septic shock: Fluid-refractory septic shock exists when cardiovascular dysfunction

persists despite at least 60 mL/kg of fluid resuscitation; and *catecholamine-resistant septic shock* exists when shock persists despite therapy with dopamine ≥10 ug/kg per min and/or direct-acting catecholamines (epinephrine, norepinephrine) ***(Goldstein et al., 2005).***

## Epidemiology:

The overall burden of illness from pediatric sepsis is globally high with important regional differences ***(Hartman et al., 2013).*** For example, in the United States approximately 75,000 children are hospitalized for severe sepsis each year with an annual incidence of about 1 case per 1000 population. The occurrence of pediatric severe sepsis has been steadily rising since the mid-1990's and now accounts for 4.4 percent of admissions to children's hospitals and 7 percent of patients treated in pediatric intensive care units (PICUs) in the United States ***(Ruth et al., 2014).*** In China, the incidence of sepsis is estimated at 1.8 cases per 1000 population or more than 360,000 cases annually ***(Wang et al., 2014).***

Respiratory infection and bloodstream infections are found in almost two-thirds of cases of severe sepsis worldwide. Many of these illnesses are caused by vaccine-preventable pathogens ***(Weiss et al., 2015).***

Since 1960, mortality from pediatric severe sepsis among patients managed in resource-rich regions has decreased from 97 percent to approximately 4 to 10 percent in patients treated

with severe sepsis and 13 to 34 percent in patients with septic shock ***(Ruth et al., 2014).***

## Pathophysiology:

Sepsis occurs when the release of proinflammatory mediators in response to an infection exceeds the boundaries of the local environment, leading to a more generalized response (SIRS) ***(Mermutluoglu et al., 2016).*** After SIRS, i.e., an excessive proinflammatory condition, occurs at the beginning, compensatory anti-inflammatory response syndrome (CARS), i.e., an excessive anti-inflammatory condition, occurs. While SIRS results in shock-based mortality, immunosuppression in the advanced phase of CARS and sepsis lead to mortality due to secondary lethal infections. For many years, it was believed that pathogen invasion is responsible for the damage seen in sepsis. However, today, it is obvious that damage is substantially caused by an excessive uncontrolled host response ***(Schouten et al., 2008).***

### Pathophysiological responses are summarized as follows:

#### *Host response in sepsis*

It was found that the host is not passive in sepsis. The roles of indigenous inflammatory mediators in organ damage and non-infectious triggers also lead to the same inflammatory response, and the clinical response can be maintained even though the infection can be eradicated ***(Bone et al., 1997).***

The most important factor in sepsis formation originates from the insufficiency of non-adaptive host factors. The deterioration in defense mechanisms protecting the host against infection paves the way for local or systemic infections. Host defense mechanisms include anatomic barriers, cellular immunity (phagocytic cells or lymphocytes), and specific and nonspecific humoral defenses ***(Polat et al., 2017).***

Macrophages, bacterial toxins such as lipopolysaccharides (LPSs), and proinflammatory cytokines are activated through the release of other mediators ***(Polat et al., 2017).*** Pathogen-related molecular patterns (PAMPs) in microorganisms, are recognized by receptors, which are called pattern recognition receptors (PRRs), found on the surface of the cells of the natural immune system. As pathogen-related molecular patterns of microorganisms are recognized by PRRs in natural immune system cells, the natural immune response occurs. PRRs are proteins with different structures, and they constitute many receptor families [toll-like receptor (TLR) or collagenous lectins] (figure 1) ***(Russell, 2006).***

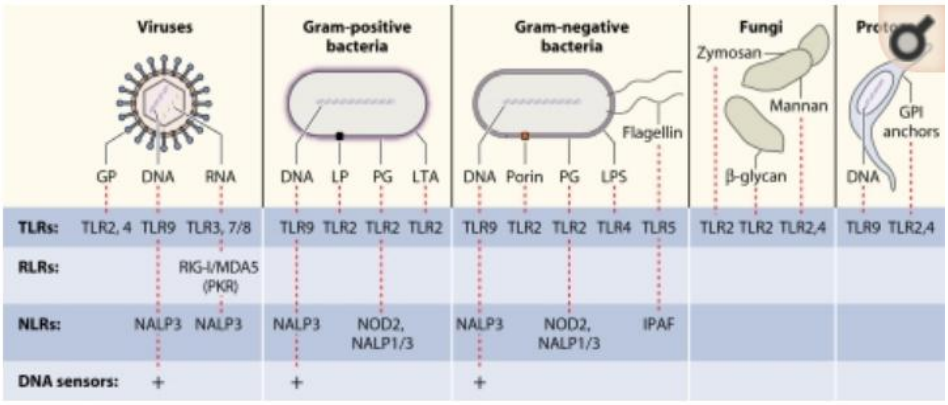


**Figure 1**: Recognition of PAMPs from different classes of microbial pathogens. Viruses, bacteria, fungi, and protozoa display several different PAMPs, some of which are shared between different classes of pathogens. Major PAMPs are nucleic acids, including DNA, dsRNA, ssRNA, and 5′-triphosphate RNA, as well as surface glycoproteins (GP), lipoproteins (LP), and membrane components (peptidoglycans [PG], lipoteichoic acid [LTA], LPS, and GPI anchors). These PAMPs are recognized by different families of PRRs ***(Russell, 2006).***

In sepsis, bacterial products such as LPS from gram-negative bacteria, peptidoglycan and lipoteichoic acid from gram-positive bacteria, lipoarabinomannan from mycobacteria, fungal antigens, and prokaryotic DNA enter the circulation and start the immune response by means of the LPS binding protein, soluble CD14, membrane CD14, CD11/CD18 complex, and TLRs ***(Medzhitov and Janeway, 2000).***

The hemodynamic, metabolic, and immune changes seen in sepsis occur through mediators and cytokines that play a role in intercellular signal transmission. Cytokines show their effects not only by entering the systemic circulation but also by their direct cell-to-cell relationship and at very small concentrations ***(Cheng et al., 2015)***.

With the uncontrolled activation of the natural immune response in sepsis, the recognition by macrophages and endothelial and epithelial cells of bacterial products such as LPSs or non-methylated CpG DNA fragments with their specific receptors results in the trigger of the cytokine cascade [such as the release of tumor necrosis factor-alpha (TNF)-α; interleukin (IL)-1, IL-6, IL-8, IL-12, and IL-18; and interferon (IFN)-γ]. High-mobility group B1 (HMGB1) is a cytokine-like structure produced in macrophages, and it occurs in the later phases of sepsis when compared to TNF and IL-1 ***(Wang et al., 2014)***.

## Coagulation abnormalities in sepsis:

The function of coagulation in infection is to surround the infection and keep the inflammatory response local. However, its excessive activation leads to negative effects ***(Yuzbasioglu et al., 2016).***

Most of the cytokines released from the cells in sepsis stimulate thrombin formation. First, the extrinsic pathway, and then, the intrinsic coagulation system are activated with factor XII activation. Fibrin thrombi occur in the microvascular bed and contribute to organ failure. The consumption coagulation proteins leads to bleeding, and this is seen with both bleeding and thrombus development in the patients ***(Reinhart et al., 2005)***.

Under normal conditions, coagulation is prevented by some natural anticoagulants, such as antithrombin (AT III), thrombomodulin, protein C, protein S, and tissue factor pathway inhibitor (TFPI). Beside the thrombin formation due to endothelium damage in sepsis, the thrombomodulin and endothelium protein C receptor functions are disrupted, and the anticoagulant system is affected ***(Reinhart et al., 2005)***, these natural anticoagulants also attract attention with their anti-inflammatory properties. Protein C is important among the mentioned natural anticoagulants. It is activated as thrombomodulin, an endothelium membrane glycoprotein, and thrombin forms a common complex ***(Mermutluoglu et al., 2016).***

While coagulation is activated, fibrinolysis is inhibited. The reason is the increase in the two fibrinolysis inhibitors, PAI-1 (plasminogen activator inhibitor) and thrombin activated fibrinolysis inhibitor in sepsis. Protein C and AT III levels decrease due to the increase in consumption in sepsis and the reduction in the formation. Thus, the procoagulant and anticoagulant balance is disrupted, in favour of the procoagulant activity ***(Wang et al., 2014)***.

In particular, proinflammatory cytokines such as IL-1 and IL-6 strongly trigger coagulation. IL-10 inhibits the tissue factor release from monocytes and regulates coagulation ***(Mermutluoglu et al., 2016).***

**Role of anti-inflammatory mechanism response and immunosuppression in sepsis:**

The excessive inflammatory response occurring in sepsis must be balanced, and is regulated by the molecules, mediators, and cytokines showing the opposite effect. This immune suppression state is defined as CARS ***(Yuzbasioglu et al., 2016).*** Soluble TNF receptors and IL-1 receptor antagonists can be given as examples of counter-inflammatory cytokines. IL-10 is the prototype of anti-inflammatory cytokines. In addition to these responses, an obvious increase in the metabolic activity (increase in cortisol production, increase in catecholamine release), induction of acute phase proteins, endothelium activation, increase in the adhesion molecules, prostanoids, and thrombocyte activation factor releasing also occur ***(Mermutluoglu et al., 2016)***. Lymphocyte apoptosis is an important reason for the suppression of immunity in septic patients ***(Bone et al., 1997).***

Septic patients are usually lymphopenic. Additionally, a reduction is seen in the B and CD4 lymphocyte subgroups in these patients. The decrease in the T-cell response and anergia seen in most of the septic patients is an excessive counter response to balance the proinflammatory response that occurs at the beginning. This can also lead to the development of organ failure, which can later occur ***(Mermutluoglu et al., 2016)***.

**Organ dysfunction in sepsis:**

Usually, multiple organ failures develop in sepsis patients, and patients die. While firstly single organ failure develops in the patients, unless the sepsis origin is eliminated, multiple organ failure develops in the later phase. The death risk increases by 15-20% for each organ failure. If there are four or five organ failures, the death rate goes above 90% ***(Polat et al., 2017).***

Even though the pathogenesis of multiple organ failure is not exactly known, the main factors for its occurrence are the microvascular occlusion caused by fibrin accumulation, platelet activating factor, disruption of the microvascular homeostasis by vasoactive substances such as histamine and prostanoids, and further disruption of oxygenation with tissue exudate. Lysosomal enzymes released from neutrophils and reactive oxygen radicals directly damage the tissue. The inducible nitric oxide synthase enzyme excessively increases nitric oxide (NO) synthesis. The excessive increase in the NO amount leads to both vascular instability and myocardial depression ***(Mermutluoglu et al., 2016)***.

In some patients, the mitochondria are not capable of using oxygen, and in those patients, cells are not able to use oxygen even if the oxygenation is normal ***(Reinhart et al., 2005).*** The highest organ damage in sepsis is seen in the lungs, liver, kidneys, heart, and intestines. Histopathological changes are

characterized with the lesions including congestion, oedema, fibrin thrombi, haemorrhage, and necrosis ***(Polat et al., 2017).***

## Etiology of sepsis:

Sepsis can result from bacteria, viruses, fungi, or parasites, or it can develop in non-infectious intraabdominal incidents such as severe trauma, pneumonia, pancreatitis, and other incidents such as urinary system infection ***(Bone et al., 1997).***

In different studies, it was reported that gram-negative bacteria were isolated 20-64% of the time in sepsis patients, while gram-positive bacteria were isolated 27-74% of the time. The most frequent causes isolated in gram-negative bacterial sepsis are E. coli, Enterobacter, Pseudomonas, Proteus, Acinetobacter, Klebsiella, and other rare gram-negative bacteria, in order of their frequency. On the other hand, in gram-positive bacterial sepsis; coagulase negative staphylococcus, S. aureus, and Enterococcus were isolated as the most frequent causes. Microorganisms do not need to pass into the blood for the development of sepsis. The local or systemic extension of signal products and toxins of the pathogen might initiate sepsis ***(Zarakolu and Akova, 2005)***. Multiple bacteria can be responsible in some sepsis patients ***(Rivers et al., 2005).***