

# Neutrophil Dysfunction in Sepsis

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## Abstract

**Objective:** Sepsis is defined as life-threatening organ dysfunction due to a dysregulated host response to infection. In this article, we reviewed the correlation between neutrophil dysfunction and sepsis.

**Data Sources:** Articles published up to May 31, 2016, were selected from the PubMed databases, with the keywords of “neutrophil function”, “neutrophil dysfunction”, and “sepsis”.

**Study Selection:** Articles were obtained and reviewed to analyze the neutrophil function in infection and neutrophil dysfunction in sepsis.

**Results:** We emphasized the diagnosis of sepsis and its limitations. Pathophysiological mechanisms involve a generalized circulatory, immune, coagulopathic, and/or neuroendocrine response to infection. Many studies focused on neutrophil burst or cytokines. Complement activation, impairment of neutrophil migration, and endothelial lesions are involved in this progress. Alterations of cytokines, chemokines, and other mediators contribute to neutrophil dysfunction in sepsis.

**Conclusions:** Sepsis represents a severe derangement of the immune response to infection, resulting in neutrophil dysfunction. Neutrophil dysfunction promotes sepsis and even leads to organ failure. Mechanism studies, clinical practice, and strategies to interrupt dysregulated neutrophil function in sepsis are desperately needed.

**Key words:** Migration; Neutrophil Dysfunction; Neutrophil Function; Sepsis

## INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction due to a dysregulated host response to infection,<sup>[1]</sup> which is still a leading cause of deaths in the critical illness. Sepsis is currently defined using clinical parameters, rather than biologic and/or molecular criteria.<sup>[2]</sup> Neutrophils are the most abundant of all white blood cells in the human circulation and play a key role in host protection against microbial infections and in inflammation.<sup>[3]</sup> In this article, we reviewed the correlation between neutrophil dysfunction and sepsis.

## DEFINITION OF SEPSIS

The Third International Consensus Definitions Task Force updated the definition of sepsis as “life-threatening organ dysfunction due to a dysregulated host response to infection (sepsis-3)”.<sup>[4]</sup> Sepsis is still a leading cause of deaths in the critical illness.

Although the recognition and interest of human’s response to an invasive pathogen have existed for centuries, the

first standard definition of sepsis dated back to 1992.<sup>[5]</sup> Participants of the American College of Chest Physicians and Society of Critical Care Medicine Consensus Conference first derived what was the most widely accepted definition for sepsis and its severity until sepsis-3 came out. They described systemic inflammatory response syndrome (SIRS) as the clinical response to an inflammatory process, and at least two of the following criteria were required for the diagnosis: body temperature  $>38^{\circ}\text{C}$  or  $<36^{\circ}\text{C}$ ; heart rate  $>90$  beats/min; respiratory rate  $>20$  breaths/min or arterial partial pressure of carbon dioxide ( $\text{PaCO}_2$ )  $<32$  torr ( $<4.3$  kPa); or white blood cell count  $>12,000$  cells/ $\text{mm}^3$  or  $<4000$  cells/ $\text{mm}^3$ . Moreover, sepsis was defined as a subgroup of SIRS when

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infection was determined to be the cause of the inflammatory process. What's more, severe sepsis was defined as organ dysfunction in the setting of sepsis.<sup>[5]</sup> Definitions of sepsis and septic shock were last revised in 2001. Limitations of previous definitions included an excessive focus on inflammation, the misleading model that sepsis follows a continuum through severe sepsis to shock, and inadequate specificity and sensitivity of the SIRS criteria.<sup>[1]</sup>

During the past twenty years, we have witnessed an in-depth understanding of sepsis, especially of its pathophysiological mechanisms. Sepsis is a complex process involving a generalized circulatory, immune, coagulopathic, and/or neuroendocrine response to infection.<sup>[6,7]</sup> Both pro-inflammatory and anti-inflammatory progresses play important roles in the immune response.<sup>[8]</sup> Many studies focus on neutrophil burst or cytokines. The immunologic progress and neutrophil activity vary within an individual throughout the course of their illness.<sup>[9]</sup> However, definition of sepsis has not been replaced according to mechanism researches. Up to now, sepsis is still defined using clinical parameters, rather than using biologic and/or molecular criteria. It remains unclear whether there are biologically relevant differences among clinically defined subtypes of sepsis.<sup>[2]</sup>

## NEUTROPHIL FUNCTION IN INFECTION

Neutrophils are the body's first line of defense against foreign invaders and constitute the major cell type involved in acute and some forms of chronic inflammation. The most important roles of neutrophils are release, migration, and phagocytosis. During sepsis, there are significant alterations in a multitude of neutrophil functions, which not only help to resist inflammation but also contribute to the development of secondary complications.<sup>[3]</sup>

Neutrophils are originated from bone marrow stem cells and released into circulation under the influence of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF) under baseline conditions. In the absence of inflammation, neutrophils circulate for a brief period (approximately 6 h) before becoming senescent and being cleared by liver, spleen, or bone marrow. When infection occurs, neutrophils react as a rapid and selective mobilization to insure abundant amount of neutrophils at the site of infection.<sup>[10,11]</sup> Once neutrophils arrive at their destination, their short lifetime is extended.<sup>[12]</sup> The identity of the factors mediating this response is still unclear, while four distinct phases of neutrophil migration have been described: mobilization, margination and rolling, adherence, and transmigration through the vessel wall. Interestingly, all of the above are impacted during sepsis.<sup>[13]</sup>

Neutrophils egress from bone marrow into the peripheral blood and are antagonistically regulated by CXC chemokine receptor 2 (CXCR2) and CXCR4, both of which are expressed on neutrophils.<sup>[14]</sup> CXCR2 promotes neutrophils' egress while CXCR4 plays a contrary role in this

progress.<sup>[15]</sup> In acute inflammation, G-CSF shifts this balance so that CXCR2 gains the upper hand.<sup>[16]</sup> After released into circulation, neutrophils start their way to the site of infection. Movement of neutrophils is guided by pro-inflammatory mediators (e.g., interleukin [IL]-1 $\beta$  and tumor necrosis factor [TNF]) and neutrophil-active chemoattractants (e.g., chemokines and lipid mediators). They are released by sentinel cells at the infection site while activated by pathogen-associated molecular pattern.<sup>[17]</sup> L-selectin is constitutively expressed on circulating leukocytes, whereas E-selectin and P-selectin are expressed on endothelial cells after activation by chemokines and other inflammatory mediators, leading to the tethering of free-flowing neutrophils to the surface of endothelium and their subsequent rolling along the vessel in the direction of blood flow.<sup>[18,19]</sup> Approaching the destination, endothelial adhesion molecules mediate high-affinity adhesion between neutrophils and endothelium.<sup>[17]</sup> Finally, neutrophils leave the vasculature based on the concentration gradients of chemoattractants.<sup>[20]</sup>

## NEUTROPHIL DYSFUNCTION IN SEPSIS

In the early phase of sepsis, neutrophils are released from bone marrow in response to a variety of cytokines, bacterial products, and other inflammatory mediators. However, it is also possible that the cells entering circulation could disseminate inflammation into other organs, eventually leading to damage.<sup>[12]</sup> Indeed, in the later stages of sepsis, many patients are in a state of immune refractoriness with undetectable levels of pro-inflammatory cytokines but reasonably high quantities of anti-inflammatory cytokines and specific cytokine inhibitors.<sup>[21]</sup> The most important character of sepsis is proved to be the failure to maintain balance between excessive and inadequate inflammation.<sup>[22]</sup>

### Complement activation and its correlation with neutrophils in sepsis

Overactivation of the innate immune response and the complement system is generally associated with the excessive inflammatory response that characterizes sepsis. Both rodents and human sepsis show complex interactions between the neutrophils and complement system, which cause poor outcomes of septic patients.<sup>[23,24]</sup> Increased production of complement fragment 5a (C5a) and increased expression of C5a receptor (C5aR) enhanced neutrophil trafficking.<sup>[25,26]</sup> Riedemann *et al.*<sup>[27]</sup> demonstrated that C5a caused decreased gene transcription for TNF- $\alpha$  in the presence of lipopolysaccharides (LPSs) *in vitro*. In human sepsis, LPS is known to induce TNF- $\alpha$  production by activating various kinases, leading to NF- $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells) activation. I $\kappa$ B $\alpha$  (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) degradation has been considered to be crucial in permitting activation and nuclear translocation of NF- $\kappa$ B.<sup>[28]</sup> C5a induces elevated levels of I $\kappa$ B $\alpha$  in neutrophils, causing greatly reduced LPS-induced expression of TNF- $\alpha$ . Another study focused

on the dynamics of C5aRs expression in septic patients and the crosstalk between C5a and C5aRs on neutrophil function. It was revealed that septic patients had low expression levels of C5aR and C5L2 on neutrophils compared to healthy and SIRS cases, and this expression pattern was correlated with disease severity.<sup>[29]</sup>

### Impairment of neutrophil migration in sepsis

Neutrophil migration to the infectious focus is extremely important for the local control of bacterial growth and consequently for the prevention of bacterial dissemination.<sup>[30]</sup> Impairment of neutrophil migration has been described in sepsis, suggesting that in human sepsis, failure of neutrophil migration is associated with a poor prognosis.<sup>[31]</sup> The mechanism involved in the impairment of neutrophil migration is still elusive. An excessive release of pro-inflammatory mediators is supposed to account for this effect.<sup>[32]</sup> Neutrophils adhere to the endothelium in response to chemokines, including chemokine (C-X-C motif) ligand 8 (CXCL8 or IL-8). CXCL8 binds to the high-affinity receptors CXCR1 and CXCR2 and evokes neutrophil chemotaxis via activation of the phosphoinositide 3-kinase – phosphatase and tensin homolog pathway.<sup>[33]</sup> A prospective cohort clinical study revealed that surface expression of the chemokine receptor CXCR2 and the beta-integrin CD11b, but not CXCR1, was reduced on neutrophils isolated from patients with septic shock compared with healthy controls. Chemotaxis to IL-8 was also reduced in neutrophils from septic patients compared with healthy controls. The changes in receptor expression correlated with measures of disease severity.<sup>[34]</sup> A prospective experimental study suggests that the poor outcome of severe sepsis is associated with toll-like receptor (TLR) 9 activation in neutrophils, which triggers G-protein-coupled receptor kinase 2 expression and CXCR2 downregulation.<sup>[31,34]</sup>

TLRs are a family of cell surface and intracellular pathogen recognition receptors that are required for the generation of immune responses to microbial pathogens. TLR4 is the major recognition receptor for LPS, a component of the Gram-negative bacteria cell wall. It is indicated that TLR4-normal but not TLR4-deficient mice exhibit an impaired neutrophil migration during lethal polymicrobial infection. A harmful role of TLR4 is also indicated in the development of septic shock induced by polymicrobial infections.<sup>[35]</sup>

Sepsis results in a dramatic increase in the elaboration of nitric oxide (NO), which is largely attributed to inflammatory cytokine and endotoxin-mediated upregulation of inducible NO synthase (iNOS).<sup>[36]</sup> Neutrophil paralysis and reduction of rolling/adhesion found in lethal sepsis were not observed in iNOS-deficient mice or in animals treated with aminoguanidine, a selective iNOS inhibitor. The failure of neutrophil migration caused by TNF- $\alpha$ , IL-8, and macrophage-derived neutrophil chemotactic factor is mediated by NO. This phenomenon is important in sepsis.<sup>[37]</sup>

The mechanisms governing neutrophil chemotactic function in sepsis is complex. Taken together, these data suggest that

an overproduction of cytokines, chemokines, and NO is a critical event that might contribute to the impairment of neutrophil migration to the infectious site observed in lethal sepsis induced by microbial infections.

### Endothelial lesions and polymorphonuclear leukocytes activation in sepsis

Significant derangement in metabolic autoregulation, the process that matches oxygen availability to the changed tissue oxygen demand, is typical of sepsis. The endothelial lesions may be a consequence of interactions between endothelial cells and activated polymorphonuclear leukocytes. The increase in receptor-mediated neutrophil-endothelial cell adherence induces the secretion of reactive oxygen species, lytic enzymes, and vasoactive substances (NO, endothelin, platelet-derived growth factor, and platelet-activating factor) into the extracellular milieu, which may injure the endothelial cells. LPSs may also induce cytoskeleton disruption and microvascular endothelial barrier integrity, in part, through NOS, RhoA, and NF- $\kappa$ B activation.<sup>[38]</sup>

### CONCLUSIONS

Sepsis represents a severe derangement of the immune response to infection, resulting in neutrophil dysfunction. The mechanism of this phenomenon has not been indicated clearly. Complement activation, impairment of neutrophil migration, and endothelial lesions are involved in this progress. Alterations of cytokines, chemokines, and other mediators contribute to neutrophil dysfunction in sepsis. At the mean time, neutrophil dysfunction promotes sepsis and even leads to organ failure. Mechanism studies, clinical practice, and strategies to interrupt dysregulated neutrophil function in sepsis are desperately needed.

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